



Behavioral and morphological adaptations to locomotion on water surface in large water striders, Gerridae

소금쟁이과 거대 소금쟁이의 수면 위 거동에 대한 행동 및 형태학적 적응

August 2023

Graduate School of Biological Sciences Seoul National University Biological Sciences Major

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Behavioral and morphological adaptations to locomotion on water surface in large water striders, Gerridae

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Submitting a Ph.D. Dissertation of Biological Sciences

August 2023

Graduate School of Biological Sciences

Seoul National University

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August 2023

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Abstract

Allometry is a study of the relationships between body size and other morphological and behavioral characteristics of an organism that result from the physics of the habitat and the biology of the organism living in its typical habitat. Water striders, Gerridae, is a good model taxon to study the locomotion and morphological adaptations to the laws of physics of their semiaquatic habitat: the water surface. The hydrodynamics and biomechanics of jumping and striding by water striders are well-understood in certain genera such as Gerris and Aquarius. Also, the hydrodynamic functions of micro hair structures on insect bodies have been studied in a relatively narrow range of water strider species. I studied two large-sized subtropical SE Asian species: Gigantometra gigas and Ptilomera tigrina. The body sizes of these species are approximately 2-10 times heavier than those of the typically studied species. The existing theory of jump of water striders predicts water striders use surface tension-dominant jump without surface breaking, which improves take-off velocity and reduces take-off delay. However, I observed that two large-sized species jump with surface-breaking and do not follow the existing theory of jump. I corrected the previous model without concerning drag to a model that includes drag calculation. The model shows that heavy species should break the water surface and utilize drag for thrust to achieve enough jump performance to escape from underwater predators. I developed another model that simulates floating conditions and sliding resistance of striding water striders. The model reveals that in order to float on the water surface, heavy species should have developed long forelegs to support the anterior part of the body with symmetric striding (two forelegs support the anterior body and two midlegs thrust simultaneously), or use asymmetric striding (one stretched forward midleg support the anterior body and another midleg and a contralateral hindleg thrust). The data on behavior observations and morphological measurements were consistent with the results of the model simulations. I explored the detailed micro-morphology of hair structures of the two species and observed how these structures are used by insects, by using scanning electron microscopy, optical microscopy, x-ray microscopy, and high-speed videography. The feasible match between the locomotive behavior of using legs and morphological characteristics of hairs implied hypothetical adaptive functions of these distinct hair structures of the two large species in comparison to the typical medium-sized water strider, A. paludum, that lives on stagnant water. Special hair brushes on the thrusting legs of P. tigrina were linked with their extremely fast striding behavior and fast-flowing habitat preference proven in this thesis. The theoretical modeling, observations, and experiments show how Gerridae illustrate adaptative links between the behavior, morphology, and habitat characteristics of organisms.

Keywords: Water strider, Gerridae, Locomotion, Behavior, Hydrodynamics, Micro-morphology, Allometry, Habitat

Student Number: 2016-20379

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Chapter 1. General introduction

Laws of physics apply to the animals moving in various environments and thus can shape biomechanical mechanisms of locomotion and morphological traits of the animals^{1,2,11–13,3–10} of different body sizes. Semiaquatic habitats present unique physical constraints resulting from the physics of the air-water interface. Various semiaquatic animals develop specific morphological and behavioral adaptations to life in these habitats, and these adaptations may depend on body size^{14–16}. Water striders, Gerridae, that live on the surface of water¹⁷ provide a convenient model to study various aspects of evolutionary processes that produce these adaptations ^{18,19}. Water striders vary in body size ranging 5²⁰–500²¹ mg, and have adapted to various semi-aquatic niches^{17,18,22–26}. I asked how relatively simple physical constraints on locomotion affect water striders' behavioral and morphological diversity resulted from the evolution in diverse water-surface habitats.

1.1. Locomotion of water striders: the necessity of extended theoretical models of jumping and striding *1.1.1. Jumping on the water*

Water striders' locomotion has been studied with respect to its surface tension-dominant mechanisms^{27–33}. Jumping on the water surface is one of the anti-predatory locomotion of water striders. Theoretical model³⁴ predicts that the water striders can optimize jump performance by controlling the leg angular velocity of midlegs during a jump to prevent the surface from breaking (Fig. 1-1). If the surface is broken when a water strider jumps, the take-off velocity becomes slow and the jump is delayed³⁴. Empirical data also confirmed that water striders actually control their leg angular velocity to jump without surface breaking^{33,34}. However, the studies so far only focused on several Palearctic/Nearctic water strider species with the body mass range of 10-50 mg. I was interested in whether this theory can be applied to large-sized water striders: Ptilomera tigrina and Gigantometra gigas. P. tigrina is a species belonging to the subfamily Ptilomerinae^{17,35–37}, and its body mass is about 100 mg. G. gigas is the world's largest semiaquatic insect, its body mass can reach up to 500 mg, which is about 10 times the body mass of typical water strider species studied so far. Therefore, I considered two feasible mechanisms of jumping for these large-sized water striders; either they follow the current theory and jump without surface breaking, or they use a different mechanism of jumping with breaking the water surface. Starting from the existing model³⁴, I built a theoretical model of jumping that generates drag after surface breaking. The goal was to predict optimal jumping behavior for a range of body sizes considering the antipredatory function of the jump, and to test the theoretical predictions in empirical observations and experiments on the large-sized species.



Figure 1-1. Jumping of water striders. A water strider jumps on the water surface to escape from underwater predators, utilizing surface tension. Surface tension contributes to upward thrust from unbroken dimples under the legs.

1.1.2. Striding on the water

The striding of the water striders has been studied to understand the underlying physical mechanisms^{16,28,40–48,29–34,38,39}. These studies mainly focused on the typical Palearctic/Nearctic water striders (~10 to ~ 50 mg)^{18,30,34}, except for a kinematic description²⁰ of small-sized water striders, *Halobates* of ~5 mg body mass. However, Gerridae, represent a wide range of body mass across water strider species. In addition, these studies were relatively narrow in focus: they concerned the mechanism of thrust^{16,30,49} only, and considered symmetric striding (Fig. 1-2; midlegs symmetrically push backward to create a forward movement of the water strider body while two forelegs and hindlegs slide on the water surface and support the body) only. Symmetric striding appears to be the typical locomotion mode of Gerridae of small and medium body size^{17,26,50}. In this striding type, the anterior body should be temporarily supported on the forelegs only, and the heavier the body the stronger the surface tension from the forelegs is needed. However, the supporting force of surface tension is constrained by the limited dimple depth⁵¹ and is correlated with the size of the dimple correlated by the wetted leg length. Therefore, in large water striders, the anterior body may be heavier than the maximal upward capillary force created by proportionally short forelegs⁵² during symmetric striding when midlegs are moving backward. I aimed at understanding how these hydrodynamic processes affect the locomotive behavior of large-bodied water striders.

It has been reported by Tseng²¹ in 1999, that the giant water striders, *G. gigas*, use a different type of gait, asymmetric striding: one midleg extended forward to support the anterior body part while the other midleg and a contralateral hindleg provide thrust (Fig. 1-2). A long midleg is expected to provide better support for the anterior heavy body than a pair of short forelegs do, and this different striding type may be a behavioral adaptation to the heavy body mass of *G. gigas*. The proportionally long forelegs of another heavy genus *Ptilomera*^{17,52,53} suggest an alternative solution to the problem of supporting a heavy anterior body while both midlegs move backward during a stroke: it appears that the relatively long forelegs characteristics for this genus may be an adaptation to produce the large supporting force needed in this situation. In addition, according to general physics for water sliders^{54,55}, the body mass, leg wetted length, and sliding velocity are expected to determine the resistance from the water during sliding on its surface. The sliding resistance will affect the energy efficiency of striding which can be important in natural situations that require long sliding distance (e.g.,

escape from danger) or very frequent striding in fast-flowing water¹⁷. However, no study (to my knowledge) has addressed these issues relevant to the large body size of water striders.

Considering that these two aspects: the support for the anterior part of the body and the resistance on the legs during the sliding, were relatively less studied than thrust generation, especially for large size species of water striders, I decided to explore these issues in a collaborative multidisciplinary approach that focuses on the sliding stage of locomotion and involves theoretical modeling, direct observations in natural habitats of the rarely studied large water strider species, and morphological comparisons to determine morphological adaptations (considering leg length proportions) to the locomotion on the water surface by large size species.



Figure 1-2. Two types of striding. Symmetric and asymmetric striding are shown in the left and right columns, respectively. In symmetric striding, two midlegs provide thrust, while each pair of fore and hindlegs provide support during sliding on the water surface. In asymmetric striding, a midleg and contralateral hind leg provide thrust, while another midleg and two midlegs provide support. Thrusting and sliding are colored as purple and blue, respectively.

1.2. Micro-morphology of water striders: the necessity to describe and evaluate detailed characteristics of hair structures on legs of large-sized water striders

The anti-wetting surfaces of various organisms have been studied^{56–61} and applied to bio-inspired technologies^{62–64}. Gerridae⁴⁷ is one of the typical taxa of semiaquatic insects that have been studied for their various types of hairs that provide hydrophobicity to them. In the previous studies, the cuticular protuberances (or hairs) on the body of insects (including Gerridae), were divided into four major categories: multicellular spines of similar cells, differentiated multicellular setae, unicellular acanthae, subcellular microtrichia⁶⁵. Heteropteran hairs on their bodies were reported to be divided into macro hairs (or macrotrichia) and micro hairs

(or microtrichia: minute cuticular outgrowths)⁶⁶. They are equivalent to setae and microtrichia by the categories of Richards⁶⁵, respectively. These setae and microtrichia are the structures for the anti-wetting mechanisms and hairs (or setae) are important parts of morphological adaptations of Gerridae to their lifestyle on the water surface^{28,34,41,42} and occasionally under water^{20,67}.

It should be noted that the anti-wetting properties of insect cuticle include two different general phenomena^{68,69}: "water protecting" and "water repellency". Water protecting refers to maintaining an air bubble and preventing water penetration under equilibrium pressure when the insect is in the water. For water protecting, it is advantageous when hairs are inclined horizontally to the surface and in contact with the water surface, and spaces between hairs are small⁶⁸. Water repellency ("waterproofing", also called "rainproofing")⁶⁹ refers to the shedding of liquid droplets such as raindrops on surfaces exposed to the air. Water droplets on the hair structures deform the hairs into their lateral direction by the adhesion between the hairs and droplets. This deformation increases the penetration of water into the hair structure. To avoid this hairs' lateral deformation, waterproofing requires the stiffness of hairs against the adhesion to the hairs' lateral direction⁶⁹, a small hairwater interface, and a large air-water interface ratio⁷⁰. The hairs, therefore, should be thick or large for stiffness, and have a low density and perpendicular inclination for the small contact areas. Consequentially, there is a tradeoff between the two anti-wetting properties: water protection and water repellency. This leads to the optimization of the hair structure between "water protecting" and "waterproofing" with compatible characteristics.

These two originally discussed basic functions of the surface layer of hairs on insects' cuticles are unlikely to heavily depend on the body size of an insect. However, if setae are importantly involved in functions related to locomotion on the water surface, e.g., in Gerridae, then the setae on legs are expected to vary with body size and habitat type to optimally serve various locomotive functions of water striders, including rowing^{16,17,30,38,40,46} and jumping^{33,34} in habitats that vary from stagnant waters overgrown with plants to fast-flowing and/or turbulent waters. The basic morphological hair types of Gerridae were described in the Palearctic and Nearctic genera *Aquarius* and *Gerris*^{24,40,66}. The recent hydrodynamic studies about hydrophobicity^{71–76} were also conducted with the underlying assumption of generalization of the shape and distribution of hairs structures from the few species of *Gerris* and *Aquarius* to all water striders (Gerridae) or even semiaquatic bugs (Gerromorpha).

However, with an exception of the genus *Halobates*, the functional micro-morphology of setae in other genera was largely not explored. I expect that larger species have special morphological adaptations related to their body size and possibly a different locomotory behavior than typical medium-sized water striders. For example, ventral and posterior surfaces of midleg tibiae and tarsi interact with the water surface to thrust in symmetric striding of typical water striders, while ventral and posterior surfaces of a hindleg additionally interact with the water surface in asymmetric striding²¹ of *G. gigas*; only ventral and lateral surfaces of legs interact with the water surface in jumping without surface breaking³⁴, while dorsal surfaces of legs are needed to interact with the water surface if *G. gigas* jump with surface breaking. Another example of less studied hair structures is the midleg hair brushes of Ptilomerinae. These hair brushes have been described by Andersen about 40–50 years ago^{17,40}. They are known in a small oceanic genus, *Halobates*²⁰, and in the subfamily Ptilomerinae,

which includes large-size species. It has been suggested that the brushes are used for rowing. The morphology of Ptilomerinae has been well studied for its importance in taxonomy^{37,77,78} but not for its detailed functions.

The exploration of the role of those various hair structures linked with water striders' locomotion can provide insights into processes responsible for adaptations to locomotion and how they may be affected by body size and habitat characteristics. Therefore, I chose the less studied large-sized species, *G. gigas* and *P. tigrina*, as my main study subjects to explore their micromorphological adaptations.

1.3. Habitats of water striders: the necessity to study how habitat preferences by water striders may be linked to their behavioral and micro-morphological characteristics

Water striders have adapted to a variety of their semiaquatic habitats such as freshwater, intertidal, marine, and marginal aquatic habitats^{17,18,23–25,40}. In their microhabitats, water striders maintain their preferred position on the water (e.g., middle, margin, edge of the water^{79–81}, certain temperature⁸², or shade/vegetation/cover^{82–85}) by using visual cues from the surroundings^{86,87}. Most precedent studies did not consider the flow speed as a characteristic of habitat types^{79–85}. However, the flow speed is one of the important characteristics of habitats, since it affects the inflow of food items available to water striders on the water surface and the costs of maintaining the insect's position in the flow (i.e., costs from striding rate to maintain location against the current, and energy income from food items delivery rate by the water current⁸⁸). One rare example of research on flow speed preference was conducted by Fairbairn & Brassard⁸⁹. In their study, the flow speed preference by *Aquarius remigis* was tested through detailed quantitative experiments.

Taking a broader perspective, it is notable that most species of Veliinae live in lotic habitats, as do many subfamilies within the Gerridae (Fig. 1-3). This suggests that the common ancestral habitat characteristics of Gerridae and Veliidae might have been predominantly lotic, even though it was suggested that ancestral Gerrinae might have lived in lentic habitats⁹⁰. This highlights the importance of studying species in lotic habitats, which challenges the current paradigm of locomotive studies that primarily focus on species in lentic habitats^{16,17,72–76,24,30,34,38,40,46,66,71}, with the exception of *Halobates*²⁰. The extensively studied species for functional morphology and locomotion, such as *Aquarius paludum* and *Gerris latiabdominis*, which belong to the subfamily Gerrinae (Fig. 1-3,4), do not fully represent the habitat characteristics of the family Gerridae. This is because many genera within Gerridae, and even some of species included in Gerrinae such as *Gigantometra* and *Aquarius elongatus* within the genus *Aquarius* (Fig. 1-5), occupy lotic or even oceanic habitats (Fig. 1-4).

Genus *Ptilomera* lives on the relatively fast-flowing streams and creeks of S.E. Asian regions^{17,21,37} and it has 'hair brushes¹⁷' for rowing (though the role of the brushes in creating thrust was never determined). *Ptilomera* may prefer fast-flowing current to take advantage of high energy income to maintain their large body size (about 100 mg), while the hairbrushes on their midlegs may help *Ptilomera* in maintaining optimal position in fast current by improving the thrust of striding. My goal was to test quantitatively their preferences for flow speed by observations and experiments, and to determine the role of hairbrushes in producing the thrust.



Figure 1-3. Cladogram of Gerromorphan subfamilies (A), genera (B), and species (C) relevant to the study. A) The main study species are *Gigantometra gigas, Ptilomera tigrina, Aquarius paludum*, and *Gerris latiabdominis. P. tigrina* belongs to Ptilomerinae, and the others belong to Gerrinae. Halobatinae represents a species that lives in fast-flowing water bodies, and is smaller in size compared to Ptilomerinae. Veliinae represents a close outgroup. The cladogram and habitat of each subfamily/tribe are based on previous studies^{24,90–94}. B) *P. tigrina* belongs to Ptilomerinae, and the others belong to Gerrinae. *Metrocoris, Asclepios, Halobates* represent species that lives in fast-flowing water bodies and are much smaller in size compared to *Ptilomera*. Veliidae represents an outgroup. The cladogram and habitat of each genus are based on previous studies^{90,92,94–99}. C) The cladogram and habitat of each species are based on previous studies^{90,92,94–99}. C) The cladogram and habitat of each species are based on previous studies^{90,92,94–99}. C) The cladogram and habitat of each species are based on previous studies^{90,92,94–99}. C) The cladogram and habitat of each species are based on previous studies^{90,92,94–99}. C) The cladogram and habitat of each species are based on previous studies^{90,92,94–99}. C) The cladogram and habitat of each species are based on previous studies^{90,92,94–99}. C) The cladogram and habitat of each species are based on previous studies^{90,94,100–106} and personal observation. Each characteristic of aquatic habitat is represented by a symbol for lentic, lotic, and oceanic habitats.

1.4. Overview of the thesis

In Chapters 2 – 6, I describe the research that addresses the above-mentioned goals (Fig. 1-1).

Chapters 2 and 3 concern the locomotion of water striders and how it depends on the body size and habitat characteristics. Here, I develop theoretical models of jumping and sliding by water striders concerning the size and leg geometry of species, and I present results of analyses of slow-motion videos of water striders' locomotion in their natural habitats and in artificial containers to test the model predictions.

Chapters 4 and 5 concern the micro-hair structures of large-sized water striders in functional (mainly on locomotive functions) aspects. Using scanning electron microscopy and optical microscopy, as well as high-speed videography, to provide detailed descriptions of the hairs, how they interact with water, and how they are used during the locomotion by water striders, I aim to describe the detailed characteristics of hair structures on legs, categorize them and propose their functions.

In Chapter 6, I present the preferences of high flow speeds of habitats by large-sized water striders, *P. tigrina*. I also described their rowing behavior in the stagnant and flowing water.

Chapter 7 contains a general overview, discussion, and conclusions from my research and my further research plans.



Figure 1-6. Summary of the chapters. The content of each Chapter is characterized in terms of study subject and phenomenon studied.

Chapter 2. Two different jumping mechanisms of water striders are determined by body size

Abstract

Current theory for surface tension-dominant jumps on water, created for small and medium size water strider species and used in bio-inspired engineering, predicts that jumping individuals are able to match their downward leg movement speed to their size and morphology such that they maximize the takeoff speed and minimize the takeoff delay without breaking the water surface. Here, we use empirical observations and theoretical modeling to show that large species (heavier than ~80 mg) could theoretically perform the surface-dominated jumps according to the existing model, but they do not conform to its predictions, and switch to using surface-breaking jumps in order to achieve jumping performance sufficient for evading attacks from underwater predators. This illustrates how natural selection for avoiding predators may break the theoretical scaling relationship between prey size and its jumping performance within one physical mechanism, leading to an evolutionary shift to another mechanism that provides protection from attacking predators. Hence, the results are consistent with a general idea: natural selection for the maintenance of adaptive function of a specific behavior performed within environmental physical constraints leads to size-specific shift to behaviors that use a new physical mechanism that secure the adaptive function.

Keywords: water strider, surface tension, jumps, antipredatory, water surface, Gerridae, drag, biomechanics, hydrodynamics, allometry

2.1. Introduction

Scaling relationships among morphological traits, the biomechanical mechanisms in which they are used, and the adaptive behaviors they serve, are the outcomes of combinations of organism's biological features, physical constraints from the organism's environment, and the nature of traits' functions^{1,2,11–13,3–10}. Compared to the allometry among morphology and structural components^{7,9,11}, the allometric relationship between the morphology and behavioral/biomechanical mechanisms is relatively less studied. Surface tension-dominant locomotion of water striders^{27–32,34} provides a unique opportunity to study the relationship between morphology and behavior that clearly serves an antipredatory function under the constraints imposed by the physical properties of water surface.

Water striders (Gerridae) are true bugs (Insecta: Hemiptera) that live on the surface of water¹⁷. They experience physical constraints on locomotion as water surface can break when the load exceeds the force resulting from surface tension of water^{30,31,34}. Studies on several water strider species have shown that they are able to jump up vertically from the unbroken water surface^{20,31,33,34} in response to predatory attacks from below^{80,107}. These species are known to have a Weber number around $0.1^{16,29,108}$, indicating that their jumping thrust is mainly derived from surface tension rather than drag force. The theoretical model³⁴ allows us to understand how water striders optimize their jumping performance within the physical constraints of water surface properties. It predicts that water surface breaks during a jump if the value of $\Omega M^{1/2}$ exceeds 4/L + 0.1(a threshold indicated with the black broken line in Fig. 2-1; mathematical symbols are explained in Table 2-1 and the basic formulae are explained in the caption of Fig. 2-1). The function involves three dimensionless variables³⁴: downward stroke (L; an indication of how far the leg can reach downward during a jump), angular leg velocity (Ω), and body mass (M). Water striders adjust the angular velocity of their downward leg movements (Ω) to the species-specific downward stroke, L, that largely depends on the midleg length, and to the species-specific body mass (M) such that they maximize the takeoff speed and minimize the takeoff delay without breaking the water surface. This optimal behavior Ω observed in small and medium water strider species is marked as the green shaded "observed" area of jumps located just under the theoretical threshold in Fig. 2-1³⁴.

The jumping behavior was studied in only several Palearctic/Nearctic water strider species with body weights below 50 mg^{20,31,33,34}, which corresponds to midleg lengths smaller than L = 10 (referred to as "typical" mid-size water striders; Fig. 2-S1). They represent a fraction of the morphological diversity among Gerridae including large species in subfamilies Gerrinae and Ptilomerinae (Fig. 2-S1b). We were interested in the applicability of this theory to the jumps of the larger-sized water striders ("unexplored range" shaded in violet in Fig. 2-1; see also Fig. 2-S1). We considered two feasible mechanisms involved in jumps of the large water striders (Fig. 2-1): (a) according to the current theory, the large water striders do not break the water surface when they jump, or (b) the large water striders break the water surface resulting in different biomechanics, perhaps similar to the basilisk lizards running on water^{14,109} or fishing spider galloping and jumping on water¹¹⁰. We suspected that the second mechanism is possible because the large body size may cause a shift in the jumping mechanism towards a relatively higher role of drag forces (i.e., mechanisms characterized by the higher Weber number²⁹).

We first focused on the world's largest water strider, *Gigantometra gigas* (Fig. 2-2a; ^{21,111}), to study their jumping in natural habitats and to provide a theoretical model of the biomechanics of jumping on water by these heavy water striders. After confirming that the giant water striders break the water surface during jumping (second mechanism), we built a theoretical model to predict the water strider's body size at which the allometric switch (from the first mechanism to the second mechanism) is expected, and we tested the predictions using observations of jumps in another previously unstudied large species, *Ptilomera tigrina*, with body mass of 83-144 mg, as well as in the previously studied "typical" medium size water strider *Aquarius paludum* with body mass of 37-52 mg.

2.2. Results and discussion

2.2.1. Empirical observation and kinematics

The detailed research on jumping behavior was carried out on the giant water striders, *Gigantometra gigas* (Fig. 2-2a), from the population in Pu Mat National Park, Vietnam (see Table 2-S1, S2, S4 for morphological data). We were able to trigger vertical jumps in freely skating giant water striders in their natural habitat (Supplementary Materials PARTS 2, 3) by imitating predator attacks from under the water surface or by creating quick movements in their visual field. We observed that *G. gigas* as well as the other large-sized water strider, *Ptilomera tigrina*, broke the water surface when they jumped on the water surface (Table 2-S5, S6). The insects jumped upward to the height of about 10-30 cm (2.5 to 10 times their body length). Next, we filmed 57 upward jumps from a stationary position by 17 individuals in an experimental basin set up in the field. We analyzed in full detail the three best clips with male water striders (we chose males in order to test the world-largest water striders; males are larger than females, Table 2-S1) facing the camera and performing relatively symmetrical (left and right) coordinated leg movements (Fig. 2-3, S5, S6). The remaining non-digitized jumps showed generally similar characteristics comprising three phases: surface tension phase, transition phase, and drag phase (see below).

From the detailed analysis of jumps it was evident that a jump starts with the pure surface tension phase (Fig. 2-2b1), which ends at the moment when the surface starts breaking under at least one of the midlegs. The surface tension phase is then followed by a transition phase, during which the midlegs' tarsi and tibia gradually break the water surface until they are entirely immersed in the water (Fig. 2-2b2; yellow-shaded vertical bands in Fig. 2-3 and Fig. 2-S5, S6). After midlegs entirely break the water surface, the drag phase begins. During the drag phase, the midlegs' tarsi and tibia surrounded by air caught within (air sheath, Fig. 2-2f) and around (air bubble, Fig. 2-2b3, e) the layer of densely packed hairs (Fig. 2-2d2, g1) are moving downward through the water (i.e., providing upward drag; Fig. 2-2b2, b3, e) pushing the body upward until the legs themselves reach the deepest point and start moving upward. The air bubble starts detaching from the midleg usually after the moment when midleg reaches the deepest point (except for only 2 cases in Table 2-S7). The hindlegs usually do not break the water surface, but maintain the dimple and provide the thrusting force stemming from surface tension (Fig. 2-S15).

A volume of air was captured by a midleg during and after surface breaking. We differentiated this volume of air into the portion caught in the 'air sheath' and the portion caught in the 'air bubble'. The former is the air captured inside the hair layer which remains attached during the leg movement and the latter is the air surrounding the leg that is detached from the leg and slowly floats upwards to the surface (Fig. 2-2b4; see more details in Fig. 2-S10). Based on the size of the detached bubbles, we evaluated that the volume of air bubble around one midleg ranges from 10 to 80 mm³ (Table 2-S8).

Finally, after the downward midleg movement stops and the leg reaches the deepest point, an additional small increase in momentum (hence, in body speed) may occur (present in Fig. 2-3, Fig. 2-S5 but not in Fig. 2-S6) for several milliseconds (<10 ms). It appears that during this time hindlegs create a dimple of constant depth (Fig. 2-S15a, c), and the wetted hindleg length gradually decreases. The abrupt and short increase of the angular downward velocity by hindleg's femur (Fig. 2-Sb1, c1; Fig. 2-S5b1, c1) is a consequence of body pitch change (head-upwards / abdomen-downwards; Fig. 2-S15c).

The momentum gained in the surface tension phase was from ~0.12 to ~0.28 g·m/s, while the momentum values gained during the transition and drag phases were 0.20-0.22 g·m/s and 0.04-0.12 g·m/s, respectively (the means from the five repeated measures in each of 3 jumps/videos; Fig. 2-3a4, Fig. 2-S5a4, Fig. 2-S6a4). Examination of Fig. 2-3 and Fig. 2-S5, S6 suggests that after midlegs reached the deepest point, the momentum gain was less than 0.05 g·m/s, if noticeable at all. The transition and drag phases together contributed to an increase in body speed by 0.6-1.1 m/s, comprising approximately 50% of the speed achieved at the end of the surface tension phase. The maximum body speed near leaving the surface was 1.1-1.6 m/s (red arrows in Fig. 2-3a2 and Fig. 2-S5, Fig. 2-S6).

2.2.2. Theoretical model based on the empirical observation

Inspired by the observations of jumps in *Gigantometra gigas*, we created a theoretical model of water strider's upward jumping. We modified the previous model³⁴ by (a) considering midlegs and hindlegs separately, (b) introducing transition and drag phases, in which midlegs are surrounded by air sheath and capture air bubbles, (c) allowing midlegs to reach deeper dimple depths before the water surface breaks depending on their length, (d) assuming that the hindlegs create only the capillary force without breaking the surface. Therefore, our model calculates upward thrust from surface tension (capillary force before breaking the surface) or/and upward drag (after completely breaking the surface) of descending midlegs while adding the surface tension from hindlegs. In the transition phase (during breaking), midlegs provide both capillary and drag force.

We assumed that the air bubble is detached from the leg after it reaches the deepest depth (see Table 2-S7). Additionally, we assumed that the left and right legs move in a synchronized manner (this synchronization makes shorter transition phase than empirically observed) with an angular velocity of leg rotation (ω) calculated according to the assumptions and formulae explained by Yang et al.³⁴. The surface tension phase was modeled according to the existing model³⁴ with an addition of the role of surface tension applied on hindlegs by assuming that their dimple depth grows in the same way as the dimple of the midlegs until it reaches its constant depth

specific for hindlegs (constant dimple depth, h_{hm} , empirically derived in Supplementary Materials PARTS 9, 10). We also permitted deeper maximum dimples for both midlegs and hindlegs (see page 38 in Supplementary Materials) owing to longer and more elastic legs in the giant water strider compared to the "typical" water striders (based on the empirical observations and measurements in Fig. 2-S12, S13).

For a given midleg length and body mass, if the angular velocity of leg downward rotation is lower than the critical angular velocity of leg rotation, ω_c , the descending midleg produces a dimple that is shallower than the critical dimple depth, h_c , at which water surface breaks. In contrast, the midleg with the angular velocity of leg rotation higher than ω_c , breaks the water surface because the dimple exceeds the critical depth, h_c , at the critical moment, t_c . The value of h_c used in the model was determined empirically and found to depend on the size of the water strider, specifically the length of the midleg tibia and tarsus (as shown in Fig. 2-S13a). When the midleg reaches the depth of h_c , the transition phase begins. In the transition phase, the water breaking happens over the duration, D_b , and midlegs experience both capillary and drag forces. The value of D_b used in the model was also determined empirically to depend on the water strider size (length of midleg tibia + tarsus; Fig. 2-S14). After the surface is completely broken (drag phase begins), the legs are fully immersed in the water and are bent such that a large portion of midleg tarsus and tibia is roughly horizontal (Fig. 2-2c2) while descending in the water and creating upward drag force for the jump. The drag phase was modeled assuming a rod, with the length equal to the vertical downward projection of the immersed section of a bent midleg and the radius equal to either the radius of midleg's tarsus and tibia covered with "air sheath" and with or without "air bubble" (Supplementary Materials PART 7), moving downward with the speed that is a byproduct of midleg's angular velocity and the ascending water strider's body velocity. In the drag phase, the role of surface tension on hindlegs was modeled by using the empirically derived constant dimple depth, h_{hm} , during the jump after the constant depth is reached, and wetted leg length, which was calculated at each moment during a jump from femur leg length and body height above the water (Fig. 2-S16; Fig. 2-S30-S32).

2.2.3. Model validation

Using empirically derived values of the angular velocity of midleg rotation (ω_e), the model reasonably predicted the insect trajectories in the specific videos of jumping *G. gigas* males (Fig. 2-4a-c). The model also provided a reasonable fit with empirically estimated upward force (Fig. 2-4d-f), including the contribution of the air bubble around midleg's tibia and tarsus during the transition and drag phases. To expand the model for the smaller species, we tested the model predictions using an extra assumption that regardless of the species/body size, the ratio of wetted midleg radius with air bubble to the radius without air bubble is equal to the average value of these ratios from the fourteen individuals of *G. gigas* analyzed in detail (Table 2-S8). The model simulations correctly predicted body center height trajectories during empirically described jumps of *G. gigas* females and *P. tigrina* individuals (Fig. 2-S19). The angular velocities of midleg rotation (ω_e) for these individuals were derived from empirical observations of midleg coordinates and velocities for *G. gigas*, *P. tigrina*, and also *A. paludum* (Fig. 2-S20-S23).

2.2.4. Model simulation results for four size classes

We used the model to predict jump outcomes (Fig. 2-5) for body weights and leg lengths corresponding to four size classes of three species of water striders (from the smallest to the largest, consistent with Fig.1): *A. paludum* female, *P. tigrina, G. gigas* females, *G. gigas* males. We used males and females of *G. gigas* separately due to the strong body size dimorphism in this species. *P. tigrina* does not show strong body size dimorphism. We observed females of *A. paludum* as the largest size class among the 'typical-sized' water striders. Those predictions were calculated for a wide range of values of the angular velocity of midleg rotation (ω ; on the horizontal axes in Fig. 2-5; See Table 2-S13 for the specific values of parameters used in each simulation), and are shown as either orange dots or dots in one of the four colors (black, dark blue, blue, and light blue) in Fig. 2-5 representing performance during surface tension and drag-involving jumps, respectively.

These results allow us to compare the theoretically predicted jumping performance (takeoff velocity, takeoff delay, and maximum height) of each size class of water striders (represented by average body size for each class) for various angular midleg velocities, including the velocities actually used by the water striders (ω_e , observed in precisely digitized jumps of multiple water striders of each species/sex classes, Table 2-S9; marked by vertical red shades in Fig. 2-5) and those that are only hypothetical/theoretical (ω_t , marked by vertical gray shades in Fig. 2-5). This hypothetical angular velocity (ω_t) is the one that results in the absence of drag force in large species and results in existence of drag force in *A. paludum*. The ranges of hypothetical angular leg velocities for each of the three large classes (who perform drag-involving jumps) were determined by using the ratio ω_e/ω_c of *A. paludum*, while those for *A. paludum* (who performs surface tension jumps mostly) were determined by using the average ratio ω_e/ω_c of the three large classes (see details in Supplementary Material PART 14).

We also calculated predictions using a range of values for Young's modulus of insect cuticle, E, (Fig. 2-5, S24, S25), as well as a range of the ratio of the wetted midleg radius with air bubble to the radius without air bubble (black, dark blue, blue, and light blue dots in Fig. 2-5, S24, S25). Young's modulus affects the critical angular velocity of the leg rotation, ω_c , but not the general results from the model (compare Fig. 2-5, S24, S25). The presence and increased size of the air bubble generally improve the performance of drag-involving jumps (as shown in Fig. 2-5, S24, S25).

2.2.5. Simulation predictions for the larger water striders

For consistency among Figures 2-1,5,6, the model results are arranged from the smallest to the largest body size class in Fig. 2-5. As we built the model based on the largest water striders, we present the results for *Gigantometra* and *Ptilomera* first, before comparing them with the smaller species (*A. paludum*). The results demonstrate that if the large water striders had used the hypothetical lower angular velocities of midlegs (ω_t) than the critical surface-breaking velocity (ω_c , ~11.3 rad/s, ~15.1 rad/s, and ~27 rad/s for *G. gigas* male and *G. gigas* female, and *P. tigrina*, respectively; marked on *x*-axis of Fig. 2-5 for E = 10 GPa) their jumping performance would have been lower than their actual jumping performance involving ω_e . Relatively higher

takeoff velocity (Fig. 2-5b-d) and greater jumping height (Fig. 2-5f-h) are likely to contribute to the success in avoiding attacks by underwater predators such as fish that snatch prey from the water surface. While, on average, the predicted takeoff delay across the gray shade appears not that different from the average predicted takeoff delay across the red-shaded band of ω_e (Fig. 2-5j-l), the hypothetical jumps by large water striders just below the critical value, ω_c , may perform better in terms of shorter takeoff delay but then the body velocity and jump height would be lower than in drag-involving jumps.

Fish, in general, can reach speeds of about 1.4 m/s (median for maximal speed from 45 studies on 14 species¹¹², Fig. 2-S27). Based on these data, we theoretically estimated that the maximum height of the hypothetical upward "jumps" (into air) by fish in pursuit of escaping (jumping) water strider would range from approximately 50 to 150 mm (lower and upper quartile in Fig. 2-S27b, c). Hence, the large water striders performing surface-breaking (i.e., drag-involving) jumps would be able to jump equal to or faster and/or higher than the fish within a presumably sufficiently short time (takeoff delay approximately up to 100 ms; Fig. 2-5j-l) to escape capture. However, if they had performed surface tension jumps, the takeoff velocities and jump heights would not likely have been sufficient to escape from the fish, especially for the heaviest class (G. gigas males; Fig. 2-5d, h). Therefore, we hypothesize that the jumps observed in large water striders produced by midlegs' angular velocities that lead to surface breaking should help the insects to escape predatory attacks, while the hypothetical surface tension jumps produced by hypothetical (not observed in nature) lower angular velocities of midlegs might put large water striders under more serious risk of predation due to relatively slow speed and low jump height. In the simulation of *P. tigrina*, we found that within a narrow range of ω , the optimal performance for surface tension jumps was equivalent to the performance of drag-involving jumps. However, since *P. tigrina* prefers fast-flowing habitats⁵³ where the maximum depth of dimple is expected to be shallower than in stagnant water¹¹⁰, we hypothesize that this peak performance for surface tension jumps may not be achievable in their natural environment.

Finally, the results show that the presence of air bubbles around midlegs improves the performance of draginvolving jumps by enlargement of projected areas of thrusting legs (Fig. 2-5, S24, S25). In our study, we assumed that this layer of air bubble enhances the drag because it increases the radius of a solid cylinder imitating the midleg in the model. However, the observed air bubble was dragged by the midleg while changing its shape (Fig. 2-2b), and we hypothesize that the air bubble of constantly changing shape may change the leg's drag coefficient and potentially enhance the drag more than just enlarging the projected area of the thrusting leg.

2.2.6. Simulation predictions for the smaller water striders

Unlike the larger water striders, the smaller water strider species such as *A. paludum* can achieve efficient escape without surface breaking (Fig. 2-5a, e, i; for E = 10 GPa), if they are able to precisely adjust the leg velocity to their individual body mass (as suggested earlier^{33,34}) such that their ω_e values lie just below the critical value, ω_c (dark red shades on the right side of red-shaded vertical band in Fig. 2-5a, e, i). If they used leg angular velocity higher than the body size-specific critical value, the jump performance would become

dramatically worse as already described by Yang et al.³⁴, and confirmed by us via considering drag calculations. This performance decrease cannot be recovered within the expected hypothetical range of (ω_t , gray vertical shade) by faster leg velocity (even with the maximum volume of air bubble; Fig. 2-5a, e). In order to achieve a performance comparable to the best performance in the surface tension jumps, this smaller species would need to use extremely fast angular leg velocities of 70-100 rad/s (Fig. 2-5a, e, i), which may not be easily achievable, or if achievable it may require more energy than the surface tension jumps. Even if they were achievable, they would not provide more protection from predatory attacks because the achievable performance of the hypothetical drag-involving jumps of *A. paludum* (Fig. 2-5a, e, i) is predicted to be lower or not higher than the best performance in the surface tension jumps with the observed leg velocity, ω_e) that is closer to the critical value, ω_c (dark red shades in Fig. 2-5a, e, i).

We also observed a range of various values of ω_e in individuals of various body sizes (Table 2-S9). Using the lower values of ω_e within this range to theoretically predict jumping performance of a female with an average body mass (48 mg; average mass for *A. paludum* females, Table 2-S3) resulted in a relatively poor performance (left side of the red-shaded vertical band in Fig. 2-5a, e, i) compared to the performance for larger ω_e values, highlighting the importance of leg rotation adjustment to body size for these water striders in performing surface-dominant jumps near the critical value, ω_c .

These model predictions allow us to understand why smaller species, who are known to perform near the threshold³⁴, would not use the drag-involving jumps. Direct empirical observations provide further explanations. In our previous empirical studies^{31,33,34}, we have occasionally observed surface breaking in the smaller species jumping in the laboratory conditions. The breaking occurred in the final moments of jump when the tibia-tarsi section was more-or-less vertically oriented (>45 deg to horizontal; example in Fig. 2-2c1) and the insect moves upward (Fig. 2-2c1) preventing the immerged leg, including its leg tips, moving downward (i.e., the leg could not create upward drag force, or even might provide downward drag force). Additionally, we noticed that the midlegs of the 'typical' smaller water strider species, such as *A. paludum*, seem not to create pronounced air sheaths in the water presumably due to shorter hairs on the legs (Fig. 2-2d, g), further diminishing the role of drag for powering the jump in these water striders.

2.2.7. Comparison of the larger and smaller water striders

Taken together, our results provide a new understanding of why jumping behaviors of the three classes of large water striders with body mass ranging from ~80 - ~500 mg and midleg downstroke (L_m) ranging from ~15 to ~38 (*G. gigas* males, *G. gigas* females, and *P. tigrina*) do not conform to the relationship between size and leg angular velocity within the surface-tension-dominated mechanism of jumping (Fig. 2-6a), while jumping of *A. paludum* females with body mass of ~40 - ~50 mg and L_m of ~6 - ~8 occurs in accordance with the theory of surface tension jumping. According to calculations based on the theoretical model³⁴, it is possible for large water striders to jump without breaking the water surface if they rotate their legs by 38-67% of their current angular velocity (Table 2-S5; using threshold line in Fig. 2-6a). However, the performance of their surface tension jumps would be worse than that of drag-involving jumps (Fig. 2-6b, c), and it would not protect them from attacking predators.

In contrast, one of the largest classes of 'typical' water striders, *A. paludum* females, does not achieve noticeably better jump performance with drag-involving jumps than with surface tension jumps (black square in Fig. 2-6b, c is located near the ratio value of 1). Hence, the shift from surface tension jumps to drag-involving jumps is predicted to occur in the species whose size lies between *A. paludum* and *P. tigrina*, (Fig. 2-S1), i.e., within the range of dimensionless midleg length (L_m) from ~8 to ~15, corresponding to the midleg length between 26 and 44 mm and body mass between ~50 and ~ 80 mg (maximal *A. paludum* = 54 mg, minimal *P. tigrina* = 83 mg).

Previous studies^{16,29,108} have determined that water strider locomotion is characterized by the Weber number of about 0.1, and our data of A. paludum female confirms this knowledge (an average value of 0.17 among individuals in Fig. 2-6a). However, our results demonstrate that Weber number can be around 2 for jumps of the large water striders (1.75, 2.91, and 1.55 for G. gigas male, G. gigas female, and P. tigrina, respectively; average values among individuals in Fig. 2-6a, Table 2-S10) indicating that drag plays an important role, similar to fishing spiders galloping and jumping on water¹¹⁰. Unlike the basilisks^{14,109}, this locomotion of large water striders does not include the fast slapping of the water surface, but it includes fast downward expansion of an already existing dimple beyond the point of breaking, leading the capture of air bubbles. Published data on several small water strider species^{20,31,33,34}, combined with our observations of A. paludum, P. tigrina, and G. gigas, match the model predictions but currently there is not enough information on jumping behavior of a variety of species within Gerridae to fully evaluate the central prediction of the model: evolutionary transitions from smaller to larger body size along branches of Gerridae phylogenetic tree will be associated by transitions from surface-tension to drag-involving jumps, especially in habitats of high predation risk where achieving sufficiently high jumping performance is important to evade predatory attacks. Future comparative studies of a variety of small and large water strider species should be able to more precisely determine the body size and midleg length at which the transitions occur. The two subfamilies of water striders, Gerrinae and Ptilomerinae, are promising study taxa because of their wide range of species body sizes (Fig. 2-S1b) and a variety of the habitats that they use.

2.2.8. General conclusion

In summary, drag-involving jumps allow large water striders to achieve performance that is comparable to the surface tension jumps of the smaller 'typical' water striders, and appears sufficient to evade predatory attacks. Hence, the results suggest that selection for sufficiently fast jumping might have led to a change in the mechanisms of jumping in the large and heavy water striders, leading to evolution of specialized hairs on their midlegs' tibiae and tarsi that capture air and enhance the drag which is important for their jumps. The results illustrate a general idea that natural selection for a specific outcome of behavior is influenced by physical constraints in certain habitats, which can break the theoretically expected scaling relationships predicted from the specific biomechanics of the behavior. As a result, a shift to a new mechanism may occur to ensure similar or better behavioral outcomes, such as escape performance from predators, and this mechanism may cause new morphological adaptations and different scaling relationships.

Many of the water strider robots developed thus far are relatively heavy (~0.5 to ~10 g^{113,114,123,115–122}; except for the ~70 mg jumping robot³¹ inspired by the theory for surface tension-dominant jumping³⁴) compared to the size range of water striders studied in nature (~10 to ~ 50 mg^{18,30,34}). In a recent study¹⁰⁸, it was shown that utilizing drag can be beneficial for large jumping robots. However, we illustrate here that in nature, adaptive pressure has already optimized the jumping behavior of large-sized water striders by shifting their behavior towards drag utilizing jumps. This highlights the importance of understanding the proximate physical mechanisms and natural selection pressures associated with animal locomotion in designing water walking robots.

2.3. Materials and Methods

2.3.1. Study species and locations

The experiments on *Gigantometra gigas* were carried out in Pu Mat National Park, Vietnam. *Ptilomera tigrina* jumps were studied at two sites: near the Me Linh Station for Biodiversity ($21^{\circ}23'01.9"N 105^{\circ}42'44.2"E =$ Google map: 21.383870, 105.712264;), Vinh Phuc Province, Vietnam, and at the "May waterfalls" (Thac May; $20^{\circ}21'51.4"N 105^{\circ}26'51.6"E=$ Google map: 20.364275, 105.447665), in the vicinity of the Cuc Phuong National Park, Vietnam. *A. paludum* individuals used in research came from water bodies located in and near Seoul, S. Korea.

2.3.2. Experiments

Water striders were filmed using Trouble Shooter camera (TS 1000 set to 500 fps) in a 30×30 cm² Plexiglas box filled with water. A second camera (Sony SR11) recorded from above simultaneously (SM PART 3: Fig. 2-S3). Each individual was photographed and weighed immediately after a test (with few exceptions of individuals that escaped before measurements). Additional colored movies were filmed using Sony RX-III camera. The photos included a ruler and were taken in a manner that allowed for body and length measurements from the photos.

2.3.3. Digitizing and analysis

We chose three best-quality videos of male *G. gigas* for detailed digitization. The videos were digitized manually using MAXTRAQ program (see details in Supplementary Material PART 6). Digitization and calculation were repeated 5 times to minimize potential human error and resolution noise. The velocities of the body and legs were based on the differences in positions of digitized points between consecutive frames. As the raw coordinates showed random fluctuations due to the errors in tracking, we used the moving average of three values of three consecutive frames: the preceding frame, the focal frame, and the following frame. The acceleration values were obtained in the same way from the velocity values (moving average of 3 consecutive values of acceleration). The momentum and force applied to the body were calculated from the velocity, acceleration, and the body mass according to standard formulas.

The jumping of *G. gigas* was divided into 3 phases. The surface tension phase lasted until the frame when the water surface started breaking under the midleg. The transition phase (marked with the yellow vertical band

in Fig. 2-3 and Fig. 2-S5, S6) lasted from the first frame with water breaking until the frame before the first frame when midlegs were entirely immersed (and surface tension did not contribute to the jump). The drag phase lasted from the first frame when midlegs were entirely immersed until the body center reached the maximum velocity. Cumulative momentum gained during each of the three jump phases was calculated in each jump. We also determined the moment when the air bubble formed and detached from the midleg. For each frame, we determined the angle between vertical line and hindleg's as well as midleg's (left and right leg separately) femur and used these values to extract angular velocity of legs (see details in Supplementary Materials PART 6). The hindleg's maximum dimple depth was also digitized (see details in Fig. 2-S9) because it is crucial in the empirical analyses and in the mathematical model (Supplementary Materials PARTS 9, 10).

The total volume of air bubbles captured around the midleg during the drag phase was calculated by adding the volumes of all air bubbles formed by air detached from the leg during the last stages of the drag phase (n = 14, Table 2-S8). In volume calculations, we used the vertical diameter of each air bubble after its shape stabilized and approximated a sphere.

The dimensionless indices crucial in the mathematical model, the maximal downward reach of legs (*L*) and the combination of leg downward angular speed with the insect mass ($\Omega M^{1/2}$) were calculated for Fig. 2-6 based on the previous study³⁴. However, unlike in the original model³⁴ that used average leg length (from four legs: two hindlegs and two midlegs), we followed the reasoning introduced in the recent model correction³³, which we further modified: we used only the empirically established midleg length (Table 2-S1-4) in calculations of those indices (Table 2-S12, S13). We did not use hindleg length in the determination of *L* because their push downward is shallower even in the surface tension jumps^{33,34}, and they do not enter deeper into the water in drag-involving jumps (i.e., do not break surface; see Fig. 2-S15).

2.3.4. Theoretical model and simulations of jumps

Supplementary Materials PART 7-11, 19 contain the detailed presentation of the core mathematical part of the model, and additional details concerning assumptions and parameters based on empirical observations. We assumed that the cuticle of water striders has Young's modulus similar to that of locusts, reported to be up to ~ 10 GPa^{124,125}. As the modulus of insect cuticles can vary widely^{126,127}, We additionally run the model using values of 5 and 15 GPa.

We used the model to theoretically simulate jumps and to predict jump outcomes for body masses and leg lengths corresponding to four size classes based on real water striders from the three study species: (from the largest to the smallest): *G. gigas* males, *G. gigas* females, *P. tigrina, A. paludum* females. We used males and females of *G. gigas* separately due to the strong body size dimorphism in this species, and we used females of *A. paludum* because they represent the largest size from among the 'typical-sized' water striders. Those predictions were calculated for wide ranges of values of the midleg angular velocity (ω) covering the surface tension-based and drag-involving jumps and were expressed as three measures of jump performance: takeoff velocity, maximum jump height, and takeoff delay. Table 2-S13 contains the specific values of parameters used in each simulation.



Fig. 2-1. Graphical explanation of the research aims. Theoretical model³⁴ proposes an optimized surface tension jumping strategy for smaller water strider species weighing up to ~50 mg (indicated by the yellow shaded area on the left side of the panel). These species have leg lengths up to ~3 cm, which corresponds to dimensionless downward strokes of up to ~10 ($L = \Delta l_l / l_c$; explanations of mathematical symbols are in Table 2-1) indicated by the green shaded area under the horizontal axis. Angular velocity of leg rotation during a jump, ω , is expressed as a non-dimensional variable, $\Omega = \omega (l_c/g)^{1/2}$ and is combined with a nondimensional measure of body mass, $M = m/(\rho l_c^2 C l_w)$, into one function, $\Omega M^{1/2}$. Yang et al.³⁴ empirically determined that the angular speeds of downward leg rotation by the "typical" water striders locate in the "Observed" green shaded area under the black broken line marking the threshold described by the formula: $\Omega M^{1/2} = 4/L + 0.1$ The pink shaded area above the threshold line represents jumps that lead to the breaking of water surface and lower jump performance³⁴. $\Omega M^{1/2}$ was treated by Yang et al.³⁴ as an index of angular speed of leg downward movement rotation because an individual water strider has control over their leg speed but not body weight. We asked whether two large subtropical water strider species, Gigantometra gigas (L up to 40; body weight 217-503 mg) and *Ptilomera tigrina* (L between 14 and 16; body weight 83-144 mg), use relatively low angular speeds of midleg rotation (green shaded area below the threshold line) and follow the same physical principles for surface tension powered jumps as the small species, or they jump with water surface breaking by using higher angular speeds of midleg rotation resulting in $\Omega M^{1/2}$ value above the threshold line.



Fig. 2-2. Photographic explanation of how the giant water strider (Gigantometra gigas) jumps on water, including morphological adaptations on midlegs to capture air during penetration of the water. a - G. gigas on the water surface. The hindleg's tibiae and tarsi press the surface downwards and create dimples during jumping; b) - The midleg's femur+tibia+tarsi functional unit moves downward while bending and deforming the surface of water to create a dimple (b1), which eventually starts to break (b2), and each midleg continues to operate as a bending rod-like functional unit pushing down in the water after complete breaking (b3) and creating upward drag force. Air sheath is caught among the long hairs on midleg's tibia and tarsus (d2, f, g1) and an additional air bubble surrounds the legs (b3), contributing to the drag force. Finally, the midlegs slide out and leave air bubbles (b4). c) – Stacked frames from a jump, starting with the moment right after surface breaking (0 ms) in A. paludum and G. gigas; in A. paludum, midlegs move upward after breaking (c1), the legs of G. gigas move downward in the water (here up to 16 ms from the moment of breaking the surface); d) - SEM image of midleg tibia of the giant water strider (d2) compared with Aquarius paludum (d1); e) - a frame from a high-speed movie of the midleg experimentally pushed downward in the water to illustrate the presence of air bubble surrounding the fast-moving leg; f) – midleg tibia in water in static situation: the layer of air sheath captured in the hairs around the leg increases the effective radius of the leg; g) – cross-section of the midleg's tibia to illustrate the distribution of hairs: relatively shorter hair on A. paludum (g2), and longer hair on G. gigas (g1). In (f) and (g), the white broken line with arrowheads indicates the actual radius of the leg while the black broken line with arrowheads indicates the effective radius that captures air sheath and creates drag force (with additional air bubble caught during leg downward). The radius of leg with hair capturing air sheath is marked as r and the radius of leg with the surrounding air bubble is marked as r_b in the model and in Fig. 2-5. Photo credits: P.G.Jablonski, J.Ha, W.Kim & S.-i.Lee.



Fig. 2-3. Empirical analysis of the kinematics and dynamics of the jumping on water by the giant water strider, Gigantometra gigas. a) - variables obtained from the body movement: changes of body height above the water surface (a1), body velocity (a2) and body acceleration (a3) during the jump. Right side axes in a2 and a3 indicate the changes of body momentum (a2) and net force (a3) during the jump calculated from the body movement and body mass. a4 shows the comparison between the values of momentum gained during the three phases of jump: the surface tension phase (green), the transition phase (yellow), and the drag phase (purple). b) and c) contain variables concerning the left (b) and right (c) midlegs (blue circles) and hindlegs (red circles in b1, b2, c1, c2): angular downward velocity (b1, c1), depth (b2, c2), downward velocity (b3, c3) and downward velocity relative to the body position (b4, c4). Yellow band across the panels indicates the transition phase for left and right separately in (b) and (c), which are overlaid on each other in (a). Blue bands across the panels indicate the bubble detaching duration for left and right leg separately. Red arrow in a2 indicates the moment of maximal body velocity. Filled circles and error bars indicate means and standard deviations, respectively, from 5 independent runs of frame-by-frame manual analysis of the same clip (EVT16). Leg depth (b2, c2), leg velocity (b3, c3), and relative leg velocity (b4, c4; relative to the body center) were measured only until the moment soon after the deepest point was reached because afterwards the detection of the deepest point was unreliable in the video. All the remaining variables are measured until the water strider loses contact with water. Results from analyses of two other jumps are in Fig. 2-S5, S6.



Fig. 2-4. Comparison of the theoretical model predictions (lines) of body height trajectory and thrust force from theoretical simulations with empirically derived values (circles) from the three specific jumps of *Gigantometra gigas*. Theoretically calculated height (black solid line) and the empirically measured height (red circles) in the jumps of *G. gigas* are represented in a, b, and c for the three analyzed videos: EVT16, EVT05 (2), and EVT41, respectively (shown in Fig. 2-3, S5, S6). Calculated forces from the model for each video are represented in d, e, and f for EVT16, EVT05 (2), and EVT41, respectively. The black dashed lines show the total generated force from two midlegs and two hindlegs. The orange and blue solid lines represent surface tension and drag, respectively. The purple circles represent the empirical force calculated from each movie by using body mass and acceleration with gravitational force added. The yellow shades represent the transition phase.



Fig. 2-5. Theoretically predicted jump performance as a function of midleg angular velocity for four classes of water striders' body size based on A. paludum females, P. tigrina, G. gigas females, and G. gigas males when E = 10 GPa. Jump performance measured by three variables calculated by the model: takeoff velocity (a-d), maximum jump height (e-h), takeoff delay(i-l). Average empirical values (mass, leg length for each leg section, leg radius, initial height of the body) for each body size class were used to simulate the jumps for each body size class across a wide range of angular velocity of leg rotation (x-axis). Orange dots represent surface tension jumps, and other dots represent drag-involving jumps. The radius ratio of 5.05, 3.5, 2.24, and 1 (i.e., no bubble situation) are represented as light blue, blue, dark blue, and black dots, respectively. The redshaded vertical bars represent the ranges of the observed leg angular velocity values (ω_e). For smaller species, known to be able to precisely adjust their leg angular velocity in order to perform just under the threshold line¹⁹, a narrow band is additionally marked with darker shade for the range of ω_e values that represent jumps in this optimal situation. The gray-shaded vertical bars represent the range of the hypothetical leg angular velocity (ω_t) for A. paludum using drag in their jumps, and for the other large species using surface tension jumps. The angular velocity of leg rotation, ω_e , values were determined from slow motion jumping videos as explained in the Supplementary Materials PART 14 and shown in Table 2-S9. The performance of drag-involving jumps was calculated for three sizes of air bubble surrounding the leg: minimal, maximal and average. Similar figures for Young's modulus of 5 and 15 GPa are shown in Fig. 2-S24, 25.



Fig. 2-6. Summary of the results. a) – empirically observed jumps in the four classes of water striders (from the smaller to the larger), *A. paludum* females, *P. tigrina*, *G. gigas* females, *G. gigas* males, in the simplified phase diagram based on the original model of surface tension jumping (Yang et al.³⁴; see Fig. 2-1) with the theoretical water surface breaking threshold line (blue solid line) to illustrate that large water striders use water surface breaking jumps that involve drag (inset photos). b-d) – comparison of the theoretically calculated performance in drag involving and surface tension dominant jumps for the four size classes of water striders: the points indicate the estimated ratios calculated by dividing the midpoint of performance (takeoff velocity in b, maximum height in c, and takeoff delay in d; all calculated based on Fig. 2-5) in the drag-involving jumps by the analogical midpoint of performance in the surface tension jumps. The black empty squares, green filled diamonds, red empty circles, and blue filled circles represent *A. paludum* females, *P. tigrina*, *G. gigas* females, and *G. gigas* males, respectively. In b-d, the red lines represent a ratio of 1, where the performances of drag-involving jumps are equal.

Table 2-1. Explanations of the symbols used in the model and present in the main text. Full list of all

symbols with descriptions is presented in Table 2-S11.

Explanations of the sym	bols appearing in the main text
$L = \Delta l_l / l_c$	Downward stroke: dimensionless maximal reach of the average of two midlegs (scaled by the capillary length, l_c (originally used in ³⁴)
$\Omega = \omega (l_c/g)^{1/2}$	Dimensionless angular velocity of the average four legs' rotation of a jump (originally used in 34)
$M = m/(\rho l_c^2 C l_w)$	Dimensionless index of insect body mass with respect to the leg; body mass with respect to maximal water mass can be displaced by the average of four legs (originally used in ³⁴)
$L_m = \Delta l_l / l_c$	Midleg downward stroke; dimensionless maximal reach of the midleg (modified <i>L</i> for midleg only)
$\Omega_{\rm m} = \omega_e (l_c/g)^{1/2}$	Dimensionless angular velocity of midleg rotation of a jump (modified Ω for midleg only)
$M_m = m/(\rho l_c^2 \tilde{C}_{m0} l_m)$	Dimensionless index of insect body mass with respect to the midleg; body mass with respect to maximal water mass can be displaced by the midleg (modified <i>M</i> for midleg only)
ω	Angular velocity of midleg rotation of a jump
ω_e	Derived angular velocity of midleg rotation of the empirical jump
ω_t	Hypothetical angular velocity of midleg rotation of the hypothetical jumps (i.e., surface
	tension jumps of G. gigas and P. tigrina; drag-involving jump of A. paludum)
ω_c	Critical angular velocity of leg rotation; For a given midleg length and body mass,
5	descending midleg can produce a dimple of the critical dimple depth, h_c , with ω_c
D_b	Duration of dimple breaking
$l_c = [\sigma/(\rho g)]^{1/2}$	Capillary length
$\Delta l_l = l_l - y_i$	Maximal downward reach of the midleg
l _w	wetted length of the reidler expectation of formula tible, and torsus
l_l	Entire length of the midleg consisting of remur, tibla, and tarsus
l_m	Constant wetted length of midleg (the length of tibla plus tarsus of the midleg)
\mathcal{Y}_i	Initial height of the body center from the undisturbed free surface
σ	Surface tension coefficient of water
ρ	Density of water
g	Gravitational acceleration
m	Mass of the water strider
С	Flexibility factor; function of wetted length of a leg, l_w , and its bending rigidity, B
C_{m0}	Midleg flexibility factor; function of wetted length of a midleg,
	l_m , and its bending rigidity, B (bending rigidity is explained in Supplementary Model
	Description file.
E	Young's modulus of insect cuticle
r	Radius of the wetted midleg as a cylinder
r_b	Radius of the wetted midleg as a cylinder surrounded by air bubble

Supplementary Materials PART 1. Morphology of the study species: *Gigantometra gigas*, *Ptilomera tigrina*, and *Aquarius paludum*.

 Table 2-S1.
 Morphology of the giant water striders, Gigantometra gigas, from the study site in Pu Mat

 National Park, Vietnam.
 Vietnam.

Variable	Males	Females
Sample size (nr of individuals)	16	9
Mass (mean ± SD, min-max; mg)	414 ± 59, 316-511	265 ± 40, 217-318
Body length (cm)	3.52 ± 0.20	3.39 ± 0.32
FRONT LEGS:		
Tibia Thickness (mm)	0.41 ± 0.05	0.39 ± 0.04
Femur length (cm)	1.11 ± 0.10	1.00 ± 0.10
Tibia length (cm)	0.89 ± 0.07	0.75 ± 0.06
Tarsus length (cm)	0.27 ± 0.03	0.23 ± 0.03
Total leg length (cm)	2.27 ± 0.17	1.99 ± 0.15
MIDLEGS:		
Tibia Thickness (mm)	0.46 ± 0.08	0.39 ± 0.04
Femur length (cm)	4.84 ± 0.45	3.32 ± 0.28
Maximum wetted leg length (Tibia + tarsus length; cm)	5.35 ± 0.60	3.85 ± 0.24
Total leg length (cm)	10.19 ± 1.04	7.17 ± 0.43
HINDLEGS:		
Tibia Thickness (mm)	0.56 ± 0.09	0.40 ± 0.05
Femur length (cm)	4.95 ± 0.69	3.21 ± 0.11
Maximum wetted leg length (Tibia + tarsus length; cm)	7.35 ± 1.16	4.06 ± 0.50
Total leg length (cm)	12.30 ± 1.25	7.26 ± 0.57

Table 2-S2. Morphology of the three individuals of *Gigantometra gigas* for whom the jumps were fully analyzed. Thickness of the tibia was measured near the femur/tibia joint as a diameter of leg measured along the horizontal line (parallel to the water surface in the normal position of a leg of a water strider standing on the water surface). The thickness is used in the model to calculate drag force after correction for the presence of air bubble that surrounds the leg moving in the water (see calculations in Supplementary Materials PART 7)

	Mass (mg)		Middle	Hind leg		
Clip name		Basal tibia thickness (mm)	Femur length (cm)	Constant wetted length: <i>tibia</i> + <i>tarsus</i> (cm)	Femur length (cm)	Constant wetted length <i>tibia</i> + <i>tarsus</i> (cm)
EVT16	483	0.455	4.809	5.460	4.742	7.948
EVT05 (2)	375	0.390	4.286	4.578	4.274	6.336
EVT41	325	0.475	4.913	5.405	4.824	7.717

Table 2-S3. Morphology of *Ptilomera tigrina* from two study sites: the Melinh Station for Biodiversity, Vinh Phuc Province, Vietnam, and at the "May waterfalls" (Thac May) of the Cuc Phuong National Park, Vietnam; and morphology of *Aquarius paludum* females from Seoul, South Korea.

Variable	Ptilomera tigrina	Aquarius paludum
Sample size (nr of individuals)	18	8
Mass (mean ± SD, min-max; mg)	115 ± 22, 83-144	48 ± 4, 43-54
Body length (cm)	1.72 ± 0.08	1.55 ± 0.06
MIDLEGS:		
Tibia thickness (mm)	0.31 ± 0.03	0.18 ± 0.02
Femur length (cm)	2.40 ± 0.16	1.15 ± 0.10
Maximum wetted leg length (Tibia + tarsus length; cm)	2.39 ± 0.16	1.34 ± 0.07
Total leg length (cm)	4.79 ± 0.30	2.50 ± 0.16
HINDLEGS:		
Femur length (cm)	2.76 ± 0.23	1.21 ± 0.08
Maximum wetted leg length (Tibia + tarsus length; cm)	1.91 ± 0.19	0.95 ± 0.07
Total leg length (cm)	4.66 ± 0.42	2.16 ± 0.14

Table 2-S4. The midleg downward stroke, L_m , the dimensionless angular velocity of middle leg rotation of a jump, Ω_m , and the dimensionless index of insect body mass with respect to the middle leg, M_m , were calculated according to the following formula from Yang et al.¹, but modified to focus on the midleg, as $L_m = \Delta l_l/l_c$, $\Omega_m = \omega_e (l_c/g)^{1/2}$, $M_m = m/(\rho l_c^2 C_{m0} l_m)$, because we observed that hindleg does not penetrate the water surface (see details in Supplementary Material PART 8, 19). The summary of these data is shown in Fig. 2-6.

Parameter/	G. gigas male		G. gigas female		P. tigrina			A. paludum female				
variable (unit)	EVT05 (2)	EVT16	EVT41	EVT28	EVT33	EVT35	C0046	C0049	C0066	P_Female _evt25	P_Female _evt32	P_Female _evt33
m (kg)	374.76e-6	483.23e-6	325.41e-6	305.67e-6	226.81e-6	226.81e-6	134e-6	134e-6	123e-6	48.5e-06	42.6e-06	42.6e-06
<i>y</i> _{<i>i</i>} (m)	0.00017	0.00165	0.00088	0.00333	0.00435	0.00274	0.00271	0.00473	0.00806	0.00271	0.00473	0.00806
<i>l</i> _{<i>l</i>} (m)	88.64e-3	102.69e-3	103.17e-3	72.59e-3	70.13e-3	70.13e-3	44.72e-3	44.72e-3	50.63e-3	0.02434	0.02413	0.02413
l_m (m)	45.78e-3	54.60e-3	54.05e-3	39.80e-3	38.87e-3	38.87e-3	22.70e-3	22.70e-3	25.56e-3	13.56e-3	13.06e-3	13.06e-3
ω_e	20	15	16	16	19	17	41	33	29	39	40	41
L_m	32.604	37.240	37.701	25.527	24.240	24.836	15.481	14.740	15.688	7.970	7.151	5.921
$\Omega_{\rm m}$	0.333	0.250	0.266	0.266	0.316	0.283	0.682	0.549	0.483	0.649	0.666	0.682
M_m	3.029	3.369	2.169	2.388	1.993	1.993	1.624	1.624	1.190	0.874	0.696	0.696
$\Omega_m M_m^{1/2}$	0.579	0.458	0.392	0.411	0.446	0.399	0.869	0.700	0.526	0.607	0.555	0.569

Table 2-S5. Calculations of theoretical threshold conditions for the large water striders, assuming that the surface-tensiondominant mechanism applies to the jumping by the large water striders (in Table 2-S4). The table shows the predictions of the theoretical critical angular leg velocity values for the empirically observed body masses.

Species	Observed mass (mg)	Observed angular velocity (rad/s)		Theoretical critical angular velocity (rad/s)	Angular velocity ratio (theoretical /observed)
G. gigas male	483		15	6.79	0.45
G. gigas male	375		20	7.69	0.38
G. gigas male	325		16	8.41	0.53
G. gigas female	306		16	9.98	0.62
G. gigas female	227		19	11.28	0.59
G. gigas female	227		17	11.11	0.65
P. tigrina	134		41	16.90	0.41
P. tigrina	134		33	17.51	0.53
P. tigrina	123		29	19.56	0.67



Fig. 2-S1. Relationship between body size and midleg length in the previously studied (yellow ranges on axes) and the unstudied (blue ranges on axes) species of Gerridae divided into 5 subfamilies. a) the absolute midleg length (mm; the variable crucial for empirical biologists and directly related to the dimensionless downward stroke L, the key variable crucial in the theoretical hydrodynamic model of jumping and shown on the horizontal axis in Fig. 2-1) and the species body mass (empirical variable related to the dimensionless body mass M contributing to the index of angular velocity of midleg rotation, $\Omega M^{1/2}$. which is the key variable the theoretical hydrodynamic model of jumping and is shown on the vertical axis in Fig. 2-1) for several species from the "typical" water striders (measured in this study: Gerris latiabdominis, G. gracilicornis, Aquarius remigis, A. paludum), and from the two large species that have rarely been studied before and were measured here (Ptilomera tigrina and Gigantometra gigas). b) Reconstructed data from Table 16 in Matsuda 1960². Matsuda states the unit converting rule on page 32: "In Table 16, 82 units are equal to 10 mm. For those values with asterisks, 173.7 units are equal to 10 mm." However, in comparison with our measurement data, the rule seems clearly to be a mistake. Therefore, we used the converting rule where '173.7 units are equal to 10 mm', which leads to results consistent with our data on leg and body lengths directly measured by us from specimens. Yellow-shaded area indicates the range of body masses and leg lengths of small and medium sized Palearctic and Nearctic water striders that have typically been studied in the past. Blue-shaded area indicates body and leg lengths that have not been studied earlier. The data point for A. paludum is highlighted to indicate the largest of the species (belongs to Gerrinae) studied that uses surface-tension jump.

Supplementary Materials PART 2: Description of the Supplementary Movies and links to additional movies deposited to Wikimedia.

Description of the content of the additional video clips available at Wikimedia and You tube (with links):

NATURAL HABITAT JUMP1.mp4. The clip shows an example of an upward jump by the giant water strider. The movements are slowed down (0.0375 normal speed). The second smaller water strider jumping belongs to the genus *Ptilomera*. *The movie* (*C0143*) *was captured in the field at 239.76fps and saved in the standard format of 29.97fps, which was additionally slowed down to 30% of playback speed. Wikimedia:*

https://commons.wikimedia.org/wiki/File:Gigantometra_gigas_upward_jump_in_Natural_Habitat_Pumat_National_Park_Jump_1.webm Youtube: https://youtu.be/2EuG5vT4YHs

NATURAL HABITAT JUMP 2.mp4. The clip shows an example of an upward jump by the giant water strider. The movements are slowed down (0.125 normal speed). The giant water strider enters the field of view from the left. The smaller water strider on the right belongs to the genus *Ptilomera*. *The movie* (*C0153*) was captured in the field at 239.76fps and saved in the standard format of 29.97fps, resulting in the slowdown of 0.125 relative to the normal speed. *Wikimedia: https://commons.wikimedia.org/wiki/File:The giant water strider* (*Gigantometra gigas: Gerridae*) *Natural Habitat Jump 2.webm Youtube: https://youtu.be/jGN1gJBlk5k*

NATURAL HABITAT JUMP3.mp4. The clip shows an example of an upward jump by the giant water strider. The movements are slowed down (0.0375 normal speed). The second smaller water strider jumping belongs to the genus *Ptilomera*. *The movie* (*C0027*) *was captured in the field at 479.52 fps and saved in the standard format of 29.97fps, resulting in the slowdown of 0.0625 relative to the normal speed.*

Wikimedia: https://commons.wikimedia.org/wiki/File:Gigantometra_gigas_(Gerridae)_Natural_Habitat_Jump_3.webm Youtube: https://youtu.be/zyW-eV9kxs8

NATURAL HABITAT JUMP5.mp4. The clip shows a close-up on the water surface under the water strider legs. The movements are slowed down (0.01875 normal speed). At the end of the clip, several small bubbles of air, which was originally wrapped around midlegs during the drag phase of the jump, "pop-up" on the water surface after being dis-attached from the legs (visible in the supplementary video "JUMP IN THE BOX.mp4"). *The movie (C0086) was captured in the field at 959.04 fps and saved in the format of 59.94 fps, which was additionally slowed down to 30%. Wikimedia: https://commons.wikimedia.org/wiki/File:Gigantometra_gigas (Gerridae) Natural Habitat Jump 5.webm* Youtube: <u>https://youtu.be/bbwkCEwDtrA</u>

JUMP IN THE TANK 1. AVI. The clip shows an example of an upward jump by the giant water strider filmed by Trouble Shooter camera (TS1000) with 500 fps. This is one of the three movies analyzed (*EVT16*). *Wikimedia:* <u>https://commons.wikimedia.org/wiki/File:Gigantometra_gigas (Gerridae) JUMP IN THE TANK 1.webm</u> <u>https://commons.wikimedia.org/wiki/File:Gigantometra_gigas (Gerridae) Jump in Tank 1 annotated EVT16 50%25.webm</u> *Youtube:* <u>https://youtu.be/wSd5EKYdPi8</u>

JUMP IN THE TANK 2.mp4. This clip shows a close up of the midlegs moving downward and surrounded by air bubble caught in the hair around the leg. Eventually, the legs are quickly mowing upward and leave the air bubble, which forms air bubbles that travel slowly upwards towards the surface. This clip (*EVT22 (2) ind20*) was filmed by Trouble Shooter camera (TS1000) at 500 fps.

Wikimedia: https://commons.wikimedia.org/wiki/File:Gigantometra_gigas (Gerridae) JUMP IN THE TANK 2_70PERC.webm Youtube: https://youtu.be/ReE8NQhjo_4

JUMP IN THE TANK 3.mp4. This clip shows nearly symmetrical movements downwards of midlegs. The movements are slowed down (0.03125 normal speed). The air released from the legs under water creates small air bubbles that travel slowly upwards towards the surface. *The movie (C0041) was captured with Sony RX10-III at 959.04 fps and saved in the format of 59.94 fps, which was additionally slowed down to 50%.*

Wikimedia: https://commons.wikimedia.org/wiki/File:Gigantometra_gigas_(Gerridae)_JUMP_IN_THE_TANK_3.webm Youtube: https://youtu.be/DagN4SMZOgc

JUMP IN THE TANK 4.mp4. This clip shows a close-up on legs seen approximately from the side to illustrate that during fast leg downward movements the air bubble around the legs is extended along the direction of leg movements (also seen in the two other clips: JUMP IN THE TANK 2 and DEAD LEG DOWNWARD MOVE. Therefore, we could imagine that a cross-section of leg including the air bubble may resemble an irregular ellipse rather than a circle. The drag force is proportional to the effective leg diameter, which is a function of the diameter of the leg plus the thickness of the air

measured during downward movement in the plane perpendicular to the leg downward movement (see Supplementary Materials PART 7 for more details on how this aspect was simplified in the theoretical model). The movements are slowed down (0.015625 normal speed). *The movie (C0046) was captured with Sony RX10-III at 959.04 fps and saved in the format of 59.94 fps, which was additionally slowed down to 25%.*

Wikimedia: https://commons.wikimedia.org/wiki/File:Gigantometra_gigas_(Gerridae)_JUMP_IN_THE_TANK_4.webm Youtube: https://youtu.be/DagN4SMZOgc

DEAD LEG DOWNWARD MOVE.mp4. This clip shows how the air bubble is created around the midleg during fast downward movements in the water. The movements are slowed down (0.03125 normal speed). *The movie (C0143) was captured in the field at 959.04 fps and saved in the format of 59.94 fps, which was additionally slowed down to 50%. Wikimedia: https://commons.wikimedia.org/wiki/File:Gigantometra_gigas (Gerridae)_DEAD_LEG_DOWNWARD_MOVE.webm* Youtube: https://youtube.com/shorts/-f6E80VricM?feature=share

JUMPING OF AQUARIUS PALUDUM:

Near-vertical jumps of water strider (*A. paludum*) **slow motion**. - The video clip shows a slow motion of a water strider jumping upwards. Note the "dimples" under the legs and how they increase in depth while the legs push down and "bend" the water surface without breaking it. The model in *Nat Comm* 7, 13698 (2016) <u>https://doi.org/10.1038/ncomms13698</u> focuses on this ability of insects to jump upward without breaking of the water surface. It shows that this ability results in maximization of the jump speed and minimization of the latency between the jump initiation and leaving the water surface in the response to attacking predators.

Wikimedia: Water-striders-adjust-leg-movement-speed-to-optimize-takeoff-velocity-for-their-morphology-ncomms13698-s2.ogv Youtube: <u>https://youtu.be/8sjSmX5pNw8</u>

Water strider A. paludum jump side view. - Slow motion video of the water strider Aquarius paludum jumping on the water surface. Side view reveals the backward leg movements. The speed is approximately 0.006 of normal speed. Wikimedia: <u>https://commons.wikimedia.org/wiki/File:Water_strider_A_paludum_jump_side_view.webm</u> Youtube: <u>https://youtu.be/cDwGRyFiNoM</u>

Waterstrider A. paludum jump frontal view. - Slow motion video of the water strider Aquarius paludum jumping on the water surface. Frontal view reveals the inward leg movements. The speed is approximately 0.006 of normal speed. Wikimedia: https://commons.wikimedia.org/wiki/File:Waterstrider A paludum jump frontal view.webm Youtube: https://youtu.be/GLy70bl6jLc

Water strider *A. paludum* **jump on solid substrate**. - This video of a water strider jumping on a solid substrate illustrates that the leg movements are composed of two phases. First phase comprises downward movement against the substrate surface (normally surface of water) dominates. The second phase comprises mostly horizontal movements: first backwards then inwards. When this happens on water, where each leg creates a dimple, the leg movements are associated with the dimple shifts backwards and then inwards. The speed is approximately 0.006 of normal speed. *Wikimedia: <u>https://commons.wikimedia.org/wiki/File:Water_strider_A_paludum_jump_on_solid substrate.webm</u>*

Youtube: https://youtu.be/4Sr0im-umSU
Supplementary Materials PART 3: Jumps of large-sized water striders.



Fig. 2-S2. Jumping in the natural habitat. An example of a record of one jump in the natural habitat. Three frames are put together here and photo-edited to represent the moment just before jump, the moment of reaching the highest point, and the moment right after landing on the water surface again. In the natural habitat, jumps of the giant water strider were triggered by imitating attacks from under the water surface using long bent sticks, or by creating a very fast visual stimulus by waving a sheet of paper in the visual field of the water strider. High speed movies were shot using Sony RX10-III. See details in Supplementary Movie 1.



Fig. 2-S3. Field methods. a) Study site in the natural habitat of the giant water strider in the Pumat National Park area. b) Experimental setup used in the field to obtain high-speed movies of jumping giant water striders. A water strider was put in a transparent water tank. Jumping was triggered by poking the insect gently from under water using a hook-shaped wire. Battery-operated high-speed camera (TS 1000) was used to film the jump, while standard camcorder filmed it from above to provide information about distance of the insect to the front wall of the tank (this distance was crucial to translate pixel coordinates into centimeters). A white sheet of fabric spread behind the water tank appropriately located relative to the direction of sun light was used as a background. For some jumps, Sony RX10-III was used instead of the TS 1000.

In the water container, the slow-motion movies used for the detailed analyses have been shot using Trouble Shooter camera (TS 1000) set to film at 500 fps. The set-up is shown in Fig. 2-S3. This setup requires two people for efficient work: one operates the high-speed camera, and the second person handles the water striders and triggers the jumps. Sometimes the water striders used their wings to escape from the water tank and these escapes were not analyzed.

Table	2-S6. O	bserved nur	nber of wate	r surface	breaking by	y midlegs	during ju	umping by	the large-si	zed water	striders: (G. gigas
and P.	tigrina.	Breaking wa	as defined a	s breaking	with at lea	ast one leg	j by assι	uming that	in asymme	tric jumps	the other	has
lighter	load.											

	G. gigas	P. tigrina	sum
Breaking occurred	57	21	78
No breaking	0	2	2
sum	57	23	80

Supplementary Materials PART 4: Duration of jump phases in Gigantometra gigas

The surface tension phase lasts for ~12-44 ms (27 ± 7 ms for a single leg, n = 15, mean±SD, Table 2-S7; ~ 26 ms in Fig. 2-3; ~22 ms in Fig. 2-S5; ~12 ms in Fig. 2-S6 where the legs are relatively non-synchronized and the second leg starts breaking the surface after ~22 ms). The transition phase lasts ~4-28 ms (12 ± 5 ms, for a single leg, n = 15, Table 2-S7; ~16 ms in Fig. 2-3; ~18 ms in Fig. 2-S5; ~28 ms in Fig. 2-S6, where it is relatively long because the two legs are not well synchronized). The drag phase lasts ~14-22 ms (15 ± 6 , for a single leg, n = 15, Table 2-S7; ~22 ms in Fig. 2-S6; ~22 ms in Fig. 2-S6). The duration from the breaking to the detachment of the air bubble lasts 10-32 ms (23 ± 7 , for a single leg, n = 15, Table 2-S7; marked with blue-shaded vertical bands in Fig. 2-S5, S6).

Table 2-S7. Duration of jump phases of *G. gigas* recorded in the water container at the field site. "Surface tension phase" lasts from the start of the jump until surface breaking starts. "Transition phase" lasts from the moment when the first point of surface breaking is detected along one of the two midlegs until the moment when both midlegs fully break the surface. "Drag phase" lasts from the end of the "transition phase" until both midlegs have reached or passed through the deepest point (i.e., none of the two midlegs moved downward anymore). Bubble attached phase is from the start of surface breaking until the air bubble is completely detached from the leg and floats upwards in the form of bubbles. Bubble attached phase is usually included in the "After breaking phase". "After breaking phase" is from the start of surface breaking until the midleg reaches the deepest point (hence it is a sum of "Transition phase + Drag phase"). See also

Video (leg)	Surface tension phase (ms)	Transition phase (ms)	Drag phase (ms)	Bubble attached phase (ms)	After breaking (ms)
EVT05 (2) left	22	12	16	32	28
EVT05 (2) right	30	10	12	10	22
EVT16 left	28	14	14	24	28
EVT16 right	26	8	14	36	22
EVT41 left	22	18	16	22	34
EVT41 right	12	14	32	28	46
EVT12	26	20	20	28	40
EVT14	26	10	16	22	26
EVT28	30	8	6	16	14
EVT33	24	14	12	20	26
EVT35	32	6	12	18	18
EVT45	28	6	18	28	24
EVT47	28	16	12	16	28
EVT65	44	4	12	18	16
EVT75	28	14	18	24	32
Mean (±s.d.)	27.1 (±6.7)	11.6 (±4.7)	15.3 (±5.7)	22.8 (±6.8)	26.9 (±8.6)



Fig. 2-S4. Box-whisker plots of phase durations (ms). Duration of each phase of the jumps of G. gigas in the water container. Surface tension phase is from start of the jump until surface breaking starts. Transition phase is from the start of surface breaking until end of surface breaking. Drag phase is from end of surface breaking until leg reaches the deepest point (i.e., leg stops moving downward). Bubble attached phase is from the start of surface breaking until the air bubble is completely detached from the lea and floats upwards in the form of bubbles. Bubble attached phase is usually included in After breaking phase. After breaking phase is from the start of surface breaking until leg reaches the deepest point (hence it is a sum of "Transition phase + Drag phase"). The data are in Table 2-S7.



Supplementary Materials PART 5: Additional Results from the detailed analyses of jumps

Fig. 2-S5. Kinematics and dynamics of the jumping on water by the giant water strider, *Gigantometra gigas*. Results from analysis of a jump "EVT05 (2)". a) variables obtained from the body movement: changes of body height above the water surface (a1), body velocity (a2) and body acceleration (a3) during the jump. Right side axes in a2 and a3 indicated the changes of body momentum (a2) and net force (a3) during the jump calculated from the body movement and body mass. (a4) shows the comparison between the values of momentum gained during the three phases of jump: the surface tension phase (green), the transition phase (yellow) and the drag phase (purple). b) and c) contain variables concerning movements of the left (b) and right (c) midlegs (blue circles) and hindlegs (red circles in b1, b2, c1, c2): angular downward velocity (b1, c1), depth (b2, c2), downward velocity (b3, c3) and downward velocity relative to the body position (b4, c4). Yellow background indicates the transition phase when surface is breaking. Blue background indicates the bubble detaching phase duration of each leg. Red triangle in a2 indicates the moment of maximal body velocity.



Fig. 2-S6. Kinematics and dynamics of the jumping on water by the giant water strider, *Gigantometra gigas*. Results from analysis of a jump "EVT41". a) variables obtained from the body movement: changes of body height above the water surface (a1), body velocity (a2) and body acceleration (a3) during the jump. Right side axes in a2 and a3 indicated the changes of body momentum (a2) and net force (a3) during the jump calculated from the body movement and body mass. (a4) shows the comparison between the values of momentum gained during the three phases of jump: the surface tension phase (green), the transition phase (yellow) and the drag phase (purple). b) and c) contain variables concerning movements of the left (b) and right (c) midlegs (blue circles) and hindlegs (red circles in b1, b2, c1, c2): angular downward velocity (b1, c1), depth (b2, c2), downward velocity (b3, c3) and downward velocity relative to the body position (b4, c4). Yellow background indicates the transition phase when surface is breaking. Blue background indicates the bubble detaching phase duration of each leg. Red triangle in a2 indicates the moment of maximal body velocity.

Fig. 2-3 of the main text, and Fig. 2-S5, S6 present the details extracted from the three best movies. In total we observed 65 jumps (51 in males and 14 in females) by 17 individuals (12 males and 5 females). We also recorded 43 jumps by 5 individuals of *Ptilomera tigrina*. In all observed jumps the water striders broke the water surface and the jump was produced by a mixture of two types of forces: surface tension followed by drag. In all 65 jumps the legs moving in the water were surrounded by the layer of air captured within the long hairs of tibia and tarsi. Also, in all jumps the midlegs moving upward eventually left some of the air in the form of bubbles. In all jumps, we observed the three main phases: surface tension, transition, and drag phase. See Table 2-S7 and Fig. 2-S4 for timing of each phase based on timing recorded in 15 leg movement events from 12 videos.

Supplementary Materials PART 6: Assumptions and methods of digitizing



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yellow lines in c, d).



Fig. 2-S8. Calculations of midleg-to-vertical angles for each frame. Points on the water surface (A, C), the deepest point (blue circle) of the middle leg, and the body center (B) were tracked in every frame using MaxTRAQ program. The vertical angle of a midleg (ABD) was defined as angle between section AB (along the leg) and vertical line (section BD). However, the camera (3D arrow along the lens's axis indicates the direction to where the camera was pointing) provided direct measure of distances and angles within the plane marked by the triangle CBD (i.e., plane perpendicular to the lens axis). Using this information together with the inferred distance AB (from insect empirical measurements of leg length and proportion of leg above water measured from the video) we estimated (by trigonometry) the angle ABD in each frame in movies with insects facing the camera while jumping. The formulas are explained below:

The angle between the midleg (section AB) and the vertical line (red shaded angle in Fig. 2-S8a; angle ABD in b) was calculated in each frame using the trigonometric functions and coordinates of the body center and the water surface contact point of the middle leg. The middle leg angle is expressed as below.

$$\angle \text{Middle leg} = \angle \text{ABD} = \tan^{-1} \frac{\overline{AD}}{\overline{BD}} = \tan^{-1} \frac{(\overline{AC}^2 + \overline{CD}^2)^{1/2}}{\overline{BD}}$$
$$= \tan^{-1} \frac{\sqrt{(Body_z - Surface_z)^2 + (Body_x - Surface_x)^2}}{Body_y - Surface_y}$$
$$= \tan^{-1} \frac{\sqrt{(B_z - A_z)^2 + (B_x - A_x)^2}}{B_y - A_y}$$

The angle between the hind leg's femur and the vertical line was calculated in each frame by the three-dimensional approximation using the second law of the cosines and using the known length of femur (Fig. 2-S9). The angle was calculated as below.

$$\angle \text{Hind leg} = \angle \text{ABD} = \cos^{-1}\left(\frac{\overline{AB^2} + \overline{BD}^2 - \overline{AD}^2}{2 \cdot \overline{AB} \cdot \overline{BD}}\right)$$
$$= \cos^{-1}\left(\frac{\overline{AB^2} + \overline{BD}^2 - (\overline{AC}^2 + \overline{CD}^2)}{2 \cdot \overline{AB} \cdot \overline{BD}}\right)$$
$$= \cos^{-1}\left(\frac{\overline{AB^2} + \overline{BD}^2 - [(\overline{AB^2} - \overline{BC}^2) + \overline{CD}^2]}{2 \cdot \overline{AB} \cdot \overline{BD}}\right)$$
$$= \cos^{-1}\left(\frac{\overline{BD}^2 + \overline{BC}^2 - \overline{CD}^2}{2 \cdot \overline{AB} \cdot \overline{BD}}\right)$$
$$= \cos^{-1}\left(\frac{(B_y - D_y)^2 + [(B_y - C_y)^2 + (B_x - C_x)^2] - (D_x - C_x)^2}{2 \cdot \overline{AB} \cdot (B_y - D_y)}\right)$$

$$= \cos^{-1}\left(\frac{(B_y - C_y)^2 + \left[\left(B_y - C_y\right)^2 + (B_x - C_x)^2\right] - (B_x - C_x)^2}{2 \cdot \overline{AB} \cdot (B_y - C_y)}\right)$$
$$= \cos^{-1}\left(\frac{B_y - C_y}{\overline{AB}}\right)$$

The angle was calculated since the length of the femur (\overline{AB}) was already known from empirical measurements.



Fig. 2-S9. Calculations of hindleg-to-vertical angles for each frame. Hind femur angle was calculated for each frame from the empirically measured femur length and information extracted from the video. As we knew the real femur length of the hind leg (\overline{AB}) , the angle $\angle ABD = \cos^{-1}(\frac{B_y - C_y}{AB})$. Femur tip (yellow) and the deepest point (blue) of the hind leg were also digitized.

Supplementary Materials PART 7: Extraction of information from empirical measurements for the model's assumptions about the functional/effective radius of the wetted leg.



Fig. 2-S10. Air sheath and air bubble exist in both dynamic and static situations. In the dynamic situation (a), the wetted leg captures both air sheath and air bubble. The high-speed video frames show that the leg slips out from the air bubble but still keeps the air sheath (a2). In the static situation, when the insect accidentally breaks the water surface, the leg only captures and keeps the air sheath (b2).



Fig. 2-S11. Tibia and tarsus of the real water strider leg (a) considered for calculations. At the first approximation, tibia and tarsus were assumed to form an ideal cone (b) of the length corresponding to the total length of tibia and tarsus, and the diameter (including hairs) ranging from the thickness of the proximal tibia at the femur/tibia joint to zero (at the tip). At the next step of approximation, we assumed the leg is a cylinder/rod with the diameter that results in the cylinder's volume in (c) equal to the volume of the cone in (b). Airbubble-including radius was calculated by assuming that the volume of the air bubble covers the ideal cylinder evenly (d): i.e., knowing the physical length and basal diameter of a wetted leg (a, b), we estimated the thickness of the leg assuming that air surrounds the leg in a uniform symmetrical manner (d).

The model dramatically simplifies some of the aspects of reality. One of the simplifications is an assumption about midleg shape that is used to estimate the drag force in the drag phase of a jump. The model assumes that the two midlegs are cylinders (or rods) of a diameter based on the empirical measurements of legs of water striders and on the empirical estimates of the air volume captured around the leg during a jump. The functional (effectively working for drag force) leg

diameter during the initial moments of the drag phase, when air bubble surrounds the leg, is calculated assuming that the air bubble surrounds the cylinder as a uniform layer (Fig. 2-S11d) of a thickness calculated from the empirically derived measurements of the volume of air caught around the leg (Table 2-S8). We measured the basal diameter of the legs, including their hair layer, as shown in Fig. 2-S11b, based on empirical data. This is because the air sheath captured by the hairs always remains attached to the legs in both dynamic and static situations, as depicted in Fig. 2-S10. As a result, we were able to estimate the volume of the air bubble (excluding the air sheath) by measuring the detached air bubbles (Fig. 2-S10a).

Table 2-S8. Calculated ideal radius of a cylinder imitating the midleg (Fig. 2-11c), and the ideal radius of a cylinder imitating the midleg surrounded by air bubble (Fig. 2-11d), as well as the ratio between these two radii (radius with bubble to radius without bubble). The observed minimum value is colored blue, and the maximum value is colored red in the table. Data derived from slow motion movies of *G. gigas*.

		Observed bubble volume for a	Ideal bubble included radius	
Videos	Ideal radius (mm)	leg (mm^3)	(mm)	Radius ratio
EVT16	0.131	43.4	0.520	3.96
EVT05 (2)	0.113	9.9	0.285	2.54
EVT41	0.137	36.3	0.482	3.52
EVT75	0.149	31.3	0.461	3.10
EVT45	0.137	26.9	0.421	3.07
EVT47	0.137	78.2	0.692	5.05
EVT63	0.117	7.8	0.261	2.24
EVT65	0.117	16.4	0.358	3.06
EVT67	0.117	23.1	0.419	3.58
EVT00	0.113	23.1	0.416	3.70
EVT03 (2)	0.113	18.2	0.373	3.31
EVT12	0.131	56.6	0.589	4.49
EVT14	0.131	61.1	0.611	4.65
EVT28	0.117	11.3	0.323	2.76
Mean (S.D.)	0.126 (0.012)	31.7 (21.2)	0.444 (0.126)	3.50 (0.81)

The model simulated several situations of different radius of leg cylinder (or rod) to imitate the leg with the air bubble around it assuming the "radius ratio" (Table 2-S8) of 2.24, **3.50**, and **5.05** to simulate the situation of the minimum, average, and maximal air volume of the air bubble trapped around the moving leg. We also imitated that ratio of 1 to simulate the leg without any additional air bubble trapped around the moving leg with air sheath in the hair layer.

Supplementary Materials PART 8: Observations of midleg dimple breaking and the role of the breaking process in the simulation model.



Fig. 2-S12. Examples of dimple breaking by the midleg of the giant water strider during jumps. The dimple starts to break when the leg reaches the maximum depth of the dimple (h_M , red arrows). However, the breaking of the dimple does not occur at the deepest point (i.e., not at the maximum depth, h_M), but rather at a certain depth (yellow arrows) that is shallower than the maximum depth: the depth of breaking initiation, h_B . The breaking initiation point vary and the breaking can start either at the middle of the dimple (a, b, d) or at the end of the dimple in the longitudinal direction (c). The breaking of the dimple expands along the leg in both longitudinal directions (a, b, d) or in a single direction (c), which can potentially affect the duration of the breaking process. The right panels of the figure depict this phenomenon 2 ms after the start of breaking.

The model is a simplified representation that does not directly simulate a leg breaking the surface at a different point along the wetted leg than the point of maximum dimple depth (Fig. 2-S12). In the previous model by Yang et al.¹, a leg was modeled as a horizontal cylinder (or rod) based on a theoretical model of a cylinder by Vella³, and it was assumed that water surface breaks at a critical dimple depth in a single moment. However, our model attempts to imitate an additional transition phase during which a combination of surface tension and drag contribute to the jump. We assume that the transition phase begins at the critical time, t_c , when the theoretically modeled dimple depth of the two horizontal cylinders (representing the two symmetrically moving midlegs in the model) reaches the critical depth, h_c . This critical depth is calculated in the model from the regression formula of h_c , (defined as the average between the breaking point depth, h_B , and the maximum dimple depth at the breaking moment, h_M) which was measured in videos of water striders of different sizes (Fig. 2-S12), on the wetted midleg length (Fig. 2-S13a). In addition, the model uses an empirical relationship shown in Fig. 2-S13b to calculate an index of maximum dimple depth, $i = h_M/l_c$, for water striders of different sizes. This index modifies Yang's formula¹, where the original $2l_c$ part (denominator) of the surface tension formula is replaced by " il_c " (Formula 6). This modification allows the deeper dimple depth for theoretical simulation of surface tension than the original Vella's model of a rigid cylinder³ used by Yang et al.¹. Once the transition phase starts at t_c , it lasts for a duration of D_b , which is calculated in the model from the empirically derived regression in Fig. 2-S14. During this time, the surface tension from the two midlegs is calculated based on the assumed dimple depth of h_c and the wetted length of the horizontal rod/cylinder (representing the midleg) that gradually decreases from 100% to 0% of the midleg tibia+tarsus length over the period D_b . Simultaneously, the drag force gradually increases over the same period, as the length of the horizontal cylinder (rod) moving downward in the water increases from 0% to 100% of the midleg tibia+tarsus length. The horizontal rod's downward velocity, which also contributes to the drag, is calculated in the model, considering the angular velocity of midleg rotation, ω , the height of the insect body above the surface, and the body's upward velocity, in accordance with Yang et al.¹.





The breaking point depth, h_B , and the maximum dimple depth at breaking moment, h_M , defined in Fig. 2-S12, were found to be linearly related to the wetted length of the middle leg among the studied species who use surface breaking jumps. The average of h_B and h_M was used to determine h_c for water striders with various sizes using linear regression of depth on middle leg constant wetted length (a). The h_M was used to determine index of dimple depth, *i*, in the model for water striders with various sizes using linear regression of depth on middle leg constant wetted length (b).



Fig. 2-S14. Relationship between midleg tibia + tarsus length (constant wetted midleg length) and the duration of dimple breaking phase of a midleg, D_b , which is also the duration of the transition phase if both midlegs act in an entirely symmetrical manner (assumed in the model). Several factors can affect the duration of dimple breaking, including the wetted length, leg downward velocity, and breaking point (whether at the center or end of the dimple in the leg's longitudinal direction). For simplicity, the regression of the empirically observed duration of breaking, D_b , on the midleg tibia + tarsus length (constant wetted midleg length) was used in the model to predict the duration of dimple breaking, D_b , for water striders of different sizes (midleg tibia + tarsus length). However, we were unable to include data from *A. paludum* in the regression because their complete breaking of the dimple was not observed under natural conditions.

Supplementary Materials PART 9: Observations of hindlegs in the jumps of Gigantometra gigas.



Fig. 2-S15. Hindlegs in the jump of the giant water strider. Changes of body pitch during the jump analyzed in Fig. 2-3 are the most pronounced in the final phase (40-60 ms in a), when a sudden short-lasting increase in the downward angular velocity of hindlegs occurs (in Fig. 2-3, S5, S6). Hindleg's dimples depth remain relatively stable at the initial phases (0-20 ms of surface tension phase in a) and subsequently during the transition and drag phases (30-40 ms in a), and they are generally similar to the dimples in resting position (b). During the jump, the angle between femur and tibia at the femur/tibia joint appears to remain roughly similar for extensive portion of the jump. The resulting dimples during jump may deepen slightly in the final stages of jump when the body pitch changes, and when the femur/tibia angle (marked red) becomes wider and approaches 180° in the final stages of the hindlegs' leaving the water surface. However, this typically this does not lead to breaking the water surface (a, c) because the hindlegs bend quite extensively (c) and because the water strider's legs at this stage are already moving upwards and do not push the surface (green shaded frames in a). Red arrows in (c) mark the femur-tibia joints for each hindlegs.

Supplementary Materials PART 10: Hindleg's constant depth, h_{hm} .

To calculate for each movie and species the theoretically assumed constant depth of a hindleg (h_{hm} ; see page 36 in Supplementary Materials PART 19), we used empirical maximum depth from high-speed videos. The constant depth used in the model (h_{hm}) was calculated from the empirical maximum depth, h_{hE} , and wetted leg length, l_h , assuming the wetted length as a half of an arc. The average depth of the arc was used as the constant depth.



Fig. 2-S16. The constant depth of hind leg. The constant depth, h_{hm} , was calculated as an average depth of a half of an arc that has same maximum depth, h_{hE} and wetted length, l_h , of empirical measurements of species.

Supplementary Materials PART 11: Maximum jumping height calculation.

If an object takes off vertically at the moment of takeoff, t_f , with the takeoff velocity, v_f , from initial height, H_0 , the object will be at the maximum height, H_m , when the total kinetic energy transferred to potential energy. Hence, the maximum jumping height, H_m , can be calculated as follows:

$$E_{potential} = mgh = mg(H_m - H_0) = E_{kinetic} = \frac{1}{2}mv_f^2$$
$$H_m = H_0 + \frac{v_f^2}{2a}$$



Supplementary Materials PART 12: Additional empirical results for G. gigas females and P. tigrina

Fig. 2-S17. Kinematics of the jumping on water by the giant water strider (*Gigantometra gigas***) females**. Extra results from analyses of jumps "EVT28", "EVT33", "EVT35". a1, b1, c1 show body height; a2, b2, c2 show body velocity; a3, b3, c3 show body acceleration during the jump. a4, b4, c4 show the comparison between the values of momentum gained during the three phases of jump: the surface tension phase, the transition phase (yellow), and the drag phase. The vertical yellow band across the panels indicates the transition phase when surface is in the process of breaking.



Fig. 2-S18. Kinematics of the jumping on water by *Ptilomera tigrina.* Results of analysis of jumps in movies "C0046", "C0049", "C0066". a1, b1, c1 show body height; a2, b2, c2 show body velocity; a3, b3, c3 show body acceleration during the jump. a4, b4, c4 show the comparison between the values of momentum gained during the two phases of jump: the surface tension phase, and the drag-breaking phase (corresponding to the transitional phase in *G. gigas*). Pure drag phase is not observed because *P. tigrina* legs go up before full breaking of the middle leg dimple. Yellow vertical bands across the panels indicates the "drag-breaking" phase (i.e., the transition phase) when surface is in the process of breaking under the midlegs (see also Supplementary Movie 3).

Supplementary Materials PART 13: Model validation - comparison with empirical observations

The supplementary results of model validation are similar to those shown in Fig. 2-3 in the main text. The results illustrate a reasonable match between empirical (Data from Supplementary Materials PART 12) and theoretical trajectories of body center for specific jumps of three females of *G. gigas* (Fig. 2-S19 a-c) and three individuals of *P. tigrina* (Fig. 2-S19 d-f).



Fig. 2-S19. The model results (simulations of separate jumps based on empirically measured input into the model) compared to the empirically observed trajectories for individual jumps of *G. gigas* females and *P. tigrina*. (a, b, c) - The theoretical model results and empirical results for the jumps of *G. gigas* females in movies EVT28, EVT33, and EVT35. (d, e, f) - The theoretical model results and empirical results for the jump videos of *P. tigrina*: C0046, C0049, and C0066. Horizontal axis represents time (ms); Vertical axis represents height of body center above water surface (mm). Blue line indicates model results and orange circles represent empirically measured values.

Supplementary Materials PART 14: Validation of the use of empirical constant angular velocity of the middle leg rotation, ω_e .

One of the model's simplifying assumptions is that the angular downward midleg movement occurs at a constant angular velocity that can be determined from empirical data on leg and body coordinates from the videos under certain assumptions described in Yang et al.¹, where it was shown to be acceptably close to the empirical average of angular velocity and resulted in valid conclusions regarding the surface tension jumps in small and medium size water striders. This angular velocity of middle leg rotation, ω_e , is empirically derived from basic observations of jump kinematics under the assumption that the empirically measured linear downward velocity of wetted midleg relative to water surface, v_l , can be approximated by the simple formula: $v_l = \omega(l_l - y_i)sin(2\omega t) - \dot{y}$, while the vertical distance from the tip of the legs to the body center, l_s , can be approximated by another formula: $l_s = \Delta l[1 - \frac{1}{2}\cos(2\omega t) + y_i]$ (see Yang et al.¹ and Supplementary Materials PART 19 for more details). To validate our use of the same procedure for determining the simple value of constant angular velocity of middle leg rotation, ω_e , we compared the empirically observed leg movements calculated using the constant value of ω_e extracted from each video separately. We did this for 3 jumps from each size class: *G. gigas* male, *G. gigas* female, and *P. tigrina* (data from Fig. 2-3, S13, S14, S15). The results suggest an approximate reasonable agreement between observed and theoretically predicted profiles of leg tip distance to body and leg tip relative velocity during jumps performed by relatively synchronized symmetrical movements by the left and right midleg.

In order to determine the theoretical performance for "virtual" water strider jumps that do not occur in nature we calculated the three hypothetical angular midleg velocities, ω_t , that are theoretically expected in the hypothetical situations of the three large classes using surface tension jumps and in the hypothetical situation of *A. paludum* using drag-involving jump. For the former, we first extracted ω_e values from the clips of jumps of *A. paludum*, which resulted in the range of ω_e between 23-41 rad/s (n = 7, Table 2-S9). This corresponds to 56-99% [calculated as $(\omega_e/\omega_c)\cdot100\%$] of the theoretical critical midleg angular velocity, ω_c , for *A. paludum* (marked as ω_c in Fig. 2-5) at which the water surface breaks. Then, we decided that the best feasible estimates of the hypothetical surface tension jumps' performance by the three large water strider classes are represented by the performance for the range the midleg angular velocity corresponding to 56-99% of ω_c for each of the three classes of large water striders (i.e., the range of ω_t is from $0.56\omega_c$ to $0.99\omega_c$). Similarly, we calculated theoretical performance in the hypothetical midleg angular velocity (ω_t) range from $1.13\omega_c$ to $1.69\omega_c$ (1.13 is the average of the three lowest ω_e/ω_c ratio, and 1.69 is the average of the three highest ω_e/ω_c ratio for the three classes of large water striders classes ω_e and from the theoretically calculated ω_c for each of the three classes separately; n = 6 for each class, Table 2-S9).



Fig. 2-S20. Three examples of the comparison of theoretical predictions based on constant midleg angular velocity, ω_e , extracted from the video analysis according to ¹ with empirically derived variables directly measured from the video of water striders (*G. gigas* male), who naturally use midleg angular velocity that varies during the course of a jump. (a1, b1, c1) – The profile of the vertical distance from body center to the leg tip in jumps by *G. gigas* males; (a2, b2, c2) - The profile of the velocity of the leg relative to the body center in jumps of *G. gigas* males. Data from videos EVT16 (a1, a2), EVT05 (2) (b1, b2), and EVT41 (c1, c2). Red dots indicate left leg and blue dots indicate right leg.



Fig. 2-S21. Three examples of the comparison of theoretical predictions based on constant midleg angular velocity, ω_e , extracted from the video analysis according to ¹ with empirically derived variables directly measured from the video of water striders (*G. gigas* female), who naturally use midleg angular velocity that varies during the course of a jump. (a1, b1, c1) – The profile of the vertical distance from body center to the leg tip in jumps by *G. gigas* females; (a2, b2, c2) - The profile of the velocity of the leg relative to the body center in jumps of *G. gigas* females. Data from videos EVT28 (a1, a2), EVT33 (b1, b2), and EVT35 (c1, c2). Red dots indicate left leg and blue dots indicate right leg.



Fig. 2-S22. Three examples of the comparison of theoretical predictions based on constant midleg angular velocity, ω_e , extracted from the video analysis according to ¹ with empirically derived variables directly measured from the video of water striders (*P. tigrina*), who naturally use midleg angular velocity that varies during the course of a jump. (a1, b1, c1) – The profile of the vertical distance from body center to the leg tip in jumps by *G. gigas* males; (a2, b2, c2) - The profile of the velocity of the leg relative to the body center in jumps of *G. gigas* males. Data from videos C0046 (a1, a2), C0049 (b1, b2), and C0066 (c1, c2). Red dots indicate left leg and blue dots indicate right leg.



Fig. 2-S23. Three examples of the comparison of theoretical predictions based on constant midleg angular velocity, ω_e , extracted from the video analysis according to ¹ with empirically derived variables directly measured from the video of water striders (*A. paludum* female), who naturally use midleg angular velocity that varies during the course of a jump. (a1, b1, c1) – The profile of the vertical distance from body center to the leg tip in jumps by *G. gigas* males; (a2, b2, c2) - The profile of the velocity of the leg relative to the body center in jumps of *G. gigas* males. Data from videos P_Female_evt25 (a1, a2), P_Female_evt32 (b1, b2), and P_Female_evt33 (c1, c2). Red dots indicate left leg and blue dots indicate right leg. We also extracted the values of constant empirical angular velocity of leg rotation, ω_e^{-1} , in these jumps in order to use the values in Fig. 2-5, 6 as the indicators of the range of values of midleg angular velocity by this species. Only one leg was digitized in P_Female_evt25 since the other was not visible.

Table 2-S9. Calculated empirical angular velocity of leg rotation, ω_e , for 6-7 jumps analyzed in details for each size class.

Species/sex	Individual	Mass (mg)	Video	Empirical angular velocity
				of leg rotation, ω_e
G. gigas male	19	374.76	EVT05 (2)	20
G. gigas male	20	483.23	EVT16	15
G. gigas male	12	315.64	EVT39	19
G. gigas male	13	325.41	EVT41	16
G. gigas male	3	424.01	EVT70 (2)	15
G. gigas male	4	404.27	EVT75	16
G. gigas female	8	315.64	EVT03	19
G. gigas female	8	315.64	EVT05	27
G. gigas female	21	305.67	EVT28	16
G. gigas female	11	226.81	EVT31	25
G. gigas female	11	226.81	EVT33	19
G. gigas female	11	226.81	EVT35	17
P. tigrina	1	134	C0044	31
P. tigrina	1	134	C0045	29
P. tigrina	1	134	C0046	41
P. tigrina	1	134	C0049	33
P. tigrina	2	106	C0061	27
P. tigrina	3	123	C0066	29
A. paludum female	1113	45.2	P_Female_evt1,2	33
A. paludum female	1113	45.2	P_Female_evt3,4	27
A. paludum female	1114	48.5	P_Female_evt7,8	27
A. paludum female	1114	48.5	P_Female_evt25,26	39
A. paludum female	2111	42.6	P_Female_evt31,32	40
A. paludum female	2111	42.6	P_Female_evt33,34	41
A. paludum female	2113	54.2	P_Female_evt35,36	23

Supplementary Materials PART 15: Additional simulation results for different Young's modulus of insect cuticle.



Fig. 2-S24. Theoretically predicted jump performance as a function of midleg angular velocity for four classes of water striders' body size based on *A. paludum* females, *P. tigrina, G. gigas* females, and *G. gigas* males when E = 15 GPa. Jump performance measured by three variables calculated by the model: takeoff velocity (a-d), maximum jump height (e-h), takeoff delay (i-l). Average empirical values (mass, leg length for each leg section, leg radius, initial height of the body; average values are shown in Table 2-S1, S3) for each body size class were used to simulate the jumps for each body size class across a wide range of angular velocity of leg rotation (*x*-axis). Orange dots represent surface tension jumps, and the other colors of dots represent drag-involving jumps. The performances of drag-involving jumps were calculated for various size of air bubble surrounding the leg. The radius ratio of 5.05, 3.5, 2.24, and 1 (i.e., no bubble situation) are represented as light blue, blue, dark blue, and black dots, respectively. The red vertical shades represent the ranges of the observed leg angular velocity (ω_e). The gray vertical shades represent the range of the hypothetical leg angular velocity (ω_t) for *A. paludum* using drag in their jumps, and for the other large species using surface tension jumps. The angular velocity (ω_t) for *A. paludum* using drag in their jumps, and for the other large species using surface tension jumps. The angular velocity (ω_t) for *A. paludum* using drag in their jumps, and for the other large species using surface tension jumps. The angular velocity (ω_t) for *A. paludum* using drag in their jumps, and for the other large species using surface tension jumps. The angular velocity (ω_t) and the values are listed in Table 2-S9.



Fig. 2-S25. Theoretically predicted jump performance as a function of midleg angular velocity for four classes of water striders' body size based on *A. paludum* females, *P. tigrina, G. gigas* females, and *G. gigas* males when E = 5 GPa. Jump performance measured by three variables calculated by the model: takeoff velocity (a-d), maximum jump height (e-h), takeoff delay (i-l). Average empirical values (mass, leg length for each leg section, leg radius, initial height of the body; average values are shown in Table 2-S1, S3) for each body size class were used to simulate the jumps for each body size class across a wide range of angular velocity of leg rotation (*x*-axis). Orange dots represent surface tension jumps, and the other colors of dots represent drag-involving jumps. The performances of drag-involving jumps were calculated for various size of air bubble surrounding the leg. The radius ratio of 5.05, 3.5, 2.24, and 1 (i.e., no bubble situation) are represented as light blue, blue, dark blue, and black dots, respectively. The red vertical shades represent the ranges of the observed leg angular velocity (ω_e). The gray vertical shades represent the ranges of the observed leg angular using drag in their jumps, and for the other large species using surface tension jumps. The angular velocity of leg rotation, ω_e , values were determined from slow motion jumping videos as explained in the Supplementary Materials PART 14 and the values are listed in Table 2-S9.

Supplementary Materials PART 16: Examples from the model simulations of the repeated "cycle" of dimple depth and body velocity fluctuations.

The simulation results show that the performance of surface tension jumps (takeoff velocity, maximum height, and takeoff delay) for the lower range of the angular velocity of leg movement has discontinuities as the angular velocity of leg rotation changes (Fig. 2-5). We propose the following explanation of this phenomenon (see details in Fig. 2-S26). During a jump, the dimple depth under insect's leg is initially getting deeper, leading to larger upward force which causes faster upward movement of the body. As the body ascends, the dimple depth becomes shallower because the leg is pulled upward from the water surface at a faster speed than the downward leg rotation. This leads to the weaker upward force causing decrease of body upwards acceleration to the point when gravitation slows the upward speed of the body, allowing the legs to "catch up" and to start pushing against the water surface increasing the dimple and the force. This repeated "cycle" of dimple depth and body velocity fluctuations can happen several times depending on the angular velocity of leg rotation. The takeoff velocity, maximum height, and takeoff delay change in an abrupt manner between jumps with different numbers of those cycles. These discontinuities do not happen if the downward leg rotation is sufficiently fast to always counteract the upward body velocity until the near end of the jump.



Fig. 2-S26. Examples of model simulation illustrating a repeated "cycle" of dimple depth and body velocity fluctuations during relatively short angular midleg velocities (b, d) compared to the changes of dimple depth and body velocity for larger angular leg velocity for water striders' body size based on *G. gigas* males. The figure illustrates body velocity (a, b) and dimple depth (c, d) of jumps when the angular velocities of leg rotation are 9 (a, c) and 7 (b, d). When the angular velocity of leg rotation is not high enough (b, d), one "cycle" cannot generate sufficient force for takeoff, causing the body to decelerate before takeoff (b) and resulting in the deepening of the dimple again (d). This phenomenon causes performance discontinuities of surface tension jump in Fig. 2-5, S24, and S25. These discontinuities do not happen if the downward leg rotation is sufficiently fast to always counteract the upward body velocity until the near end of the jump (a, c) The final performance of these jumps in (a, c/b, d) is illustrated in Fig. 2-5d.

4.50 1000 300 b С а 900 4.00 o Maximum height of fish jump (mm) 250 800 o 3.50 Maximum velocity of fish (m/s) 0 o 700 200 3.00 8 0 0 600 Maximum 2.50 0 height 500 150 0 2.00 8 Maximum 400 o height + 1/3 of 1.50 Х 100 8 300 body length 1.00 200 50 0.50 100 0.00 0 0

Supplementary Materials PART 17: Maximum jumping performance of fish.



Supplementary Materials PART 18: Weber number of the study species: *Gigantometra gigas*, *Ptilomera tigrina*, and *Aquarius paludum*.

Table 2-S10. Weber number of jumps respective to each size classes (same analyzed jumps from Fig. 2-6a). Calculation method for the Weber number was implemented from⁵.

Size class	Video	ho (kg/m ³)	σ (N/m)	w, Basal tibia thickness (m)	U (m/s)	$We = \rho U^2 w / \sigma^5$
G. gigas male	EVT05 (2)	998	0.072	0.00039	0.60	1.95
G. gigas male	EVT16	998	0.072	0.000455	0.49	1.51
G. gigas male	EVT41	998	0.072	0.000475	0.52	1.78
G. gigas female	EVT28	998	0.072	0.000405	0.39	0.85
G. gigas female	EVT33	998	0.072	0.00036	1.17	6.83
G. gigas female	EVT35	998	0.072	0.00036	0.46	1.06
P. tigrina	C0046	998	0.072	0.000261	0.73	1.93
P. tigrina	C0049	998	0.072	0.000261	0.65	1.53
P. tigrina	C0066	998	0.072	0.000327	0.51	1.18
A. paludum female	P_Female_evt25	998	0.072	0.000176	0.09	0.02
A. paludum female	P_Female_evt32	998	0.072	0.000194	0.28	0.21
A. paludum female	P_Female_evt33	998	0.072	0.000194	0.32	0.28

Supplementary Materials PART 19: Detailed description of the mathematical model of jumping.

I. Introduction

The mechanics of jumping of mid-sized Palearctic water striders, such as *Gerris latiabdominis*, *G. gracilicornis*, *Aquarius remigis* and *A. paludum* (Fig. 2-S28a) on water has been previously studied⁶. It has been shown that their leg stroke speeds are optimized to maximize their jumping speed and minimize time to take off given their mass and leg length¹, and that individual water strider are able to adjust their angular velocity of midlegs based on previous jumping experience⁷. By pressing the water surface until just before it breaks under water strider legs, these typically studied water striders make a full use of capillary forces that the water surface provides.

Water strider legs may be approximated as long thin cylinders (see also Supplementary Materials Part 7: Fig. 2-S10, 11 for link to empirically measured leg diameter and length). The surface is pierced when a very thin cylinder of a radius $r \ll l_c$ is pressed downward against the water surface in a quasi-static manner to a distance of the order of the capillary length $l_c = [\sigma/(\rho g)]^{1/2}$ with σ and ρ respectively being the surface tension coefficient and density of water, and g being the gravitational acceleration. When the legs sink into the water surface, the drag forces act on the legs, which are significantly smaller than the capillary forces for the mid-sized striders.

While the mid-sized water striders do not break water surfaces for efficient jumps, the larger species such as *Gigantometra gigas* (Fig. 2-S28b) do not follow the aforementioned rule of motion in jumping. *G. gigas* is up to ten times heavier than mid-sized water striders in leg length. Typical mass and middle leg length of *G. gracilicornis* (Fig. 2-S28a) are respectively 30 mg and 20 mm, whereas the giant water striders are up to 500 mg and 100 mm for *Gigantometra gigas* (Fig. 2-S28b). Figure 2-S29 shows a sequence of the jump of a *G. gigas* on water taken by a high-speed camera in a field experiment. We see that the middle legs pierce the water surface to a significant degree, which is not observed for mid-sized striders. Here, we describe the kinematic models of the two pairs of legs separately, and combine the models to predict the jump dynamics of the *G. gigas* and other water striders with similar jumping behavior.



Fig. 2-S28. Two different-sized water striders and basic parameters. (a) A mid-sized water strider, *Gerris gracilicornis*. (b) A gigantic water strider, *Gigantometra gigas*. (c) A side view of a *G. gigas* during its jump with parameters used in the theoretical model. Symbols and variables used in the model are explained in Table 2-S11 and Fig. 2-S32.



Fig. 2-S29. A sequence of the jump of *G. gigas* **on water.** (a) The initial posture of the *G. gigas* before jumping. (b) Surface tension phase. The middle and hind legs create dimples on the water surface. (c) The early stage in the drag phase. The middle legs break the water surface with air bubbles covering the legs (magnified image) until they reach the deepest location. (d) The late stage in the drag phase. The air bubbles surrounding the middle legs are absent. (e) Gravity driven phase. All the legs are completely disengaged from the water surface.

II. Kinematics of middle and hind legs

First, we consider the kinematics of middle legs. We assume that their movements comprise three phases: the surface tension phase, the transition phase, and the drag phase. In the surface tension phase, the middle legs push the water surface down with a constant wetted length, l_m (the length of tibia plus tarsus of the middle leg). Symbols and variables used in the model are explained in Table 2-S11 and Fig. 2-S32 in part IV. The dimple depth generated by the middle leg, h, grows, leading to the increase of the body center height, y, with time, t (Fig. 2-S28c). As the angular velocity of midleg's downward rotation can be approximated to be a constant, ω^1 , the downward linear velocity of the middle leg relative to the body center, v_m , can be written as:

$$v_m = \dot{l}_s = \omega(l_l - y_i)\sin(2\omega t), \qquad (1)$$

where $l_s = y + h$ is the vertical distance from the body center to the tip of the leg. l_l is the entire length of the leg consisting of femur, tibia, and tarsus, and y_i is the initial height of the body centre from the undisturbed free surface. Integrating v_m over time, t, gives:

$$l_s = \frac{1}{2}(l_l - y_i)[1 - \cos(2\omega t)] + y_i \text{ for } l_s = y_i \text{ at } t = 0.$$
(2)

Based on empirical leg measurements, we model the wetted middle legs as cylinders of diameter, d, and length, l_m , according to the details described in the Supplementary Materials PART 7. The water surface cannot withstand the depression of cylindrical legs when the dimple reaches a critical depth, h_c , which was determined by empirical measurement for different wetted leg length (Fig. 2-S13a, Supplementary Materials PART 8). When the dimple depth, $h = l_s - y$, exceeds h_c at time t_c , in the model, then the wetted part of the middle leg starts to pierce the water surface, entering the transition phase. In this phase, there are both sunk and unsunk part of middle leg. The unsunk part is supported by surface tension, while sunk part experiences drag. We assumed that the proportion of

wetted leg for surface tension, p_s , and for drag, p_d , gradually changes from only surface tension to only drag, i.e., from the start of sinking to completely sunk leg. The duration of this changing proportion was determined by empirical measurement (Fig. 2-S14, Supplementary Materials PART 8). After the transition phase, the drag phase begins at time t_d . During this third phase, the middle legs can only provide drag.

To calculate drag in both transition and drag phase, the middle legs are considered almost straight with the wetted length decreasing according to formula:

$$l_1 = l_l - \frac{y}{\cos\left(\frac{\pi}{2} - \omega t\right)},\tag{3}$$

that takes into account the ascent of the insect body. The downward linear velocity of a middle leg relative to the water surface is then given by:

$$v_l = \dot{l}_s - \dot{y} = v_m - \dot{y} = \omega(l_l - y_i)\sin(2\omega t) - \dot{y}.$$
 (4)

Because the legs penetrate the water with a high velocity, an air bubble forms around the leg, as shown in Fig. 2-S29c. We assume in the model that the air bubble detaches after the moment when the middle legs reach the deepest point in the water. Thus, the effective frontal area, the projected area of the leg with its diameter, d, along its moving velocity, is $A_f = d_b l_1$, thanks to the presence of an air bubble that increases the cylindrical leg diameter, to d_b ("b" stands for bubble of air) by the factor of 2.24-5.05 times (d_b =3.5*d in average value) as determined in empirical measurements (see Supplementary Materials PART 7: Table 2-S7).

We turn to the kinematics of hind legs which do not pierce the water surface during the jump. The stroke can be decomposed into two phases. In the first (pushing) phase, the hindlegs push the water surface down with a fully contacted constant wetted length, l_h (the length of tibia plus tarsus of the hind leg), with a growing dimple depth. We assume in the model that the depth of dimple created by a hind leg from the undisturbed free surface, h_h , grows at the same rate as dimple of the midleg until it reaches constant depth, h_{hm} . Constant depth of hindlegs is calculated using observed empirical maximum depth of hindlegs, h_{hE} , and wetted length of hindlegs assuming leg as half of an arc (see details in Supplementary Materials PART 9).

In the second phase, which starts when the dimple depth reaches its constant, h_{hm} , the legs slide on the water surface towards the body while detaching themselves from the surface. Thus, the wetted length eventually decreases by ascending the body while the dimple depth is constant. We calculate the wetted length of a hind leg, l_2 , based on body heights, y, constant wetted length of a hind leg, l_h , and femur length of a hind leg, l_{Hfemur} . We use a simplifying assumption that the hindlegs that are out of the water align with femur along the direction of jump and are being dragged out from the water surface vertically (Fig. 2-S30, Supplementary Movie 1), while the hindleg section on the water surface is bent creating a dimple without surface breaking. Therefore, the wetted length of a hind leg approximately follows:

$$l_2 = l_h - (y - l_{Hfemur}) \text{ when } y > l_{Hfemur} \text{ and } l_h > (y(t) - l_{Hfemur}).$$
(5)



Fig. 2-S30. Field jump of *G. gigas*. Field jump of *G. gigas* shows its hindlegs are almost vertical when the wetted length is pulled out from the water surface. Red arrows mark the femur-tibia joint.

Summarizing the simplified kinematics of both middle and hind legs in the model, we schematically plot the timeline of different phases of the jump for the four legs as shown in Fig. 2-S32. In the stationary phase, t = 0, both middle and hindlegs are in stationary situation with initial dimple depth, h_o . In the surface tension phase, $0 < t < t_c$, both the middle and hind legs moving with angular velocity, ω , are pushing the water surface with growing of dimple, and only dimple depth of hind leg, h_h , stops growing when it reaches specific depth, h_{hm} . In the transition phase, $t_c < t < t_d$, where t_d is the moment when the breaking ends, the unsunk part of the middle leg is supported by surface tension, while the sunk part experiences drag. The proportion of the sunk and unsunk length of the middle legs changes gradually during this phase. In the drag phase, $t_d < t < t_f$, where t_f is the moment of take-off, the middle legs continue to move in water with an angular velocity, ω , and are surrounded by air bubbles. The hind legs are being closed on the water surface with their wetted length being decreased with constant dimple depth, h_{hm} .

Phase	Stationary	Surface tension	Transition	Drag Drag with air bubble with l_l and v_l	
Middle legs	Stationary situation	Surface tension with l_m constant and h increasing	Surface tension with decreasing p_s at h_c and Drag with increasing p_d		
Hind legs	$l_m, l_h,$ and h_0 (Surface tension l_{h} with l_{h} constant and h_{h} increasing until h_{hm}	t_c t_d Surface tension with h_{hm} constant and l_2 decreasing when $v > l_{uc}$		

Fig. 2-S31. Timeline of the simulated phases of the jump for the middle and hind legs. Middle and hind legs contribute force resulting from surface tension to the jump until the critical moment of time when water surface is broken, t_c . After this moment of time, middle legs of diameter, d_b , resulting from the presence of the air bubble, create drag force until the moment, t_f . Between the moment of t_c and t_d , middle legs utilize surface tension and drag with unsunk and sunk parts, respectively. After t_d , the dimple under the middle legs is completely broken, and the middle legs exploit drag only. Hindlegs create force resulting from surface tension during the whole period from t_c to t_f . This force gradually decreases as the wetted leg length, l_2 , decreases, while dimple depth, h_{hm} , is assumed constant.

III. Jump dynamics of Gigantometra gigas

SURFACE TENSION PHASE

Symbols and variables used in the model, including geometric schematics for some of the variables, are explained in Table 2-S11 and Fig. 2-S32 in part IV. The water strider ascends from the water surface because the interaction of its legs and water produces upward thrust. Newton's second law of motion dictates $F = m\ddot{y}$, where F is the total force acting on the water strider legs and m is the water strider mass. We find the temporal evolution of the body center height and the take-off velocity by analyzing the forces produced by the movement of legs of angular velocity, ω .

During the "Surface tension phase" (Fig. 2-S32) of the simplified jump, various forces are exerted on the legs including the capillary force $F_c \sim \sigma l_w$, pressure force $F_p \sim \rho U^2 dl_w$, buoyancy $F_b \sim \rho g dh l_w$, added inertia $F_a \sim \rho d^2 l_w U^2/h$, viscous force $F_v \sim \mu r l_w U/l_c$, and the weight of the water strider (for the large *G. gigas* males it is ~ 5 mN). Here, l_w is the wetted leg length, and *U* is the rate of the vertical growth of dimple, which is a direct consequence of downward linear velocity of the middle leg, v_l that according to formula⁴ depends on, among others, on the leg angular velocity, ω .

Using the typical values for middle legs $d = 260 \ \mu m$, $l_w = 53.5 \ mm$, $h = 5 \ mm$, and $U = 0.4 \ ms^{-1}$, we found that the capillary force dominates the other forces, and we decided to ignore the other forces in the simplified model.

The capillary force acting on a pair of floating flexible cylinders is given by formula 6 below, which is a modified formula from Yang et al.¹ based on model for a cylinder by Vella et al.³.

$$F_c = 4C\rho g l_c l_w h \left[1 - \left(\frac{h}{i l_c}\right)^2 \right]^{1/2},\tag{6}$$

where *C* is the flexibility factor depending on the scaled leg length $L_f = l_w/l_e$. Here, $l_e = (Bl_c/\sigma)^{1/4}$ is the modified elastocapillary length of the leg with the bending rigidity $B = \pi E d^4/64$ and *E* being Young's modulus of insect cuticle. We approximate $C \approx (1 + 0.082L^{3.3})^{-1}$ for $L_f < 2$ and $C \approx (0.88L)^{-1}$ for $L_f > 2$. In comparison to the original model of Yang et al.¹, we modified the denominator in the formula 6 from $2l_c$ to il_c , by introducing the index of maximum dimple depth, $i = h_M/l_c$. The maximum dimple depth at surface breaking moment, h_M , was empirically derived for water striders of different sizes using the linear regression of h_M on the constant wetted length of midleg ($h_M = 0.1227l_m + 0.004$; Fig. 2-S13b, Supplementary Materials PART 8). This index allowed us to extend the range of the dimple depths beyond the mathematical limitation of $h \leq 2l_c$ from the original model¹.

We first model the stationary situation, "Stationary phase" (Fig. 2-S31). We assume the stationary dimple depth of each individual by calculating force balance between gravity and surface tension. When the water strider is on the water surface using their two middle legs and two hind legs, the stationary dimple depth, h_0 , satisfies the following formula by assuming the same dimple depth for middle and hind legs:

$$mg = 4\rho g l_c \left\{ C_{m0} l_m h_0 \left[1 - \left(\frac{h_0}{i l_c}\right)^2 \right]^{1/2} + C_{h0} l_h h_0 \left[1 - \left(\frac{h_0}{i l_c}\right)^2 \right]^{1/2} \right\}.$$
 (7)

In the surface tension phase, the dimple depth is given by $h = l_s - y$, leading us to write $\ddot{h} = \ddot{l_s} - (F - g)/m$. Here, *F* is the sum of the capillary forces acting on the middle and hind legs:

$$F = 4\rho g l_c \left\{ C_m l_1 h \left[1 - \left(\frac{h}{i l_c}\right)^2 \right]^{1/2} + C_h l_2 h_h \left[1 - \left(\frac{h_h}{i l_c}\right)^2 \right]^{1/2} \right\}.$$
 (8)

This gives a second-order nonlinear differential equation for h with the initial conditions of $h(t = 0) = h_0$ and $\dot{h}(t = 0) = 0$, which we solve using Matlab. Then we get the body centre height $y = l_s - h$ as a function of time for $0 < t < t_c$ (i.e., $h < h_c$).

TRANSITION PHASE

Once the middle legs start to pierce the water surface, $t > t_c$ (i.e., $h > h_c$; where h_c is empirically established for each water strider size; Fig. 2-S13a), the middle legs experience the drag force F_d of water in addition to the capillary force. The drag force acting on a pair of middle legs moving with the velocity $v_l = \omega(l_l - y_i) \sin(2\omega t) - \dot{y}$ as obtained above is given by

$$F_d = \rho C_D A_f v_l^2, \tag{9}$$

where $C_D = 0.8$ is the drag coefficient on the flexible cylinder⁸, taken to be about 30% lower than the value for a rigid cylinder at a Reynolds number, $Re = \frac{\rho v_l d}{\mu} \approx 100$. We simply assumed that the drag coefficient of the middle legs is the same as that of a solid cylinder. This is because calculating the exact drag coefficient of the middle legs would require detailed analysis of the movement of air inside the bubble around the leg and between hairs, which is beyond the scope of our study. The frontal area is $A_f = d_b l_1$ in transition and drag phase, $t_c < t < t_f$.

During the transition phase ($t_c < t < t_d$), the middle legs utilize both capillary and drag forces as the legs progressively sink. We assumed that the leg sinks continuously during a certain breaking duration, D_b , which was calculated from the wetted length using linear regression of empirical measurements (Fig. 2-S14, Supplementary Materials PART 8). Thus, the functional wetted leg length for each force is linearly changed by introducing the proportion of wetted leg length for utilizing surface tension, p_s , and for drag, p_d ($p_s + p_d = 1$). During the breaking duration, D_b , the proportion of wetted leg length for utilizing surface tension, p_s , linearly decreases from 1 to 0, while the proportion of wetted leg length for utilizing drag, p_d , linearly increases from 0 to 1. For a given moment, we write $p_s = (t_c + D_b - t)/D_b$ and $p_d = 1 - p_s$. In this phase, the dimple depth for capillary force is fixed at h_c since we observed that the breaking of the dimple expands laterally (as shown in Fig. 2-S12 in Supplementary Materials PART 8).

Then the total force acting on the middle and hind legs becomes

$$F = 4\rho g l_c C_m p_s l_1 h_c \left[1 - \left(\frac{h_c}{i l_c}\right)^2 \right]^{1/2} + \rho C_D d_b p_d l_1 v_l^2 + 4C\rho g l_c l_2 h_h \left[1 - \left(\frac{h_h}{i l_c}\right)^2 \right]^{1/2}.$$
 (10)

DRAG PHASE

In the drag phase, after the dimple is completely broken, $t > t_d$, the proportion of wetted leg for utilizing surface tension, p_s , becomes 0 and middle leg utilize drag only.

Then the total force acting on the middle and hind legs naturally becomes

$$F = \rho C_D A_f v_l^2 + 4C \rho g l_c l_2 h_h \left[1 - \left(\frac{h_h}{i l_c}\right)^2 \right]^{1/2}.$$
 (11)

In the transition and drag phase, solving $\ddot{y} = (F - g)/m$, a second-order differential equation with A_f , v_l , l_2 , h_h being functions of y and t, gives the body center height versus time. The initial conditions are provided from the results of the surface tension phase. From the relationship between the body center height versus time we predict time of take-off, t_f , and body speed at v_f . From the body speed and body mass, we predict that maximum jump height above the water surface as $H_m = y_i + \frac{v_f^2}{2g}$ (Supplementary Materials PART 11). These model predictions can be calculated for various vales of angular leg velocities, and for water striders of various body mass and leg lengths.

IV. Explanations of the symbols used in the paper

The symbols and variable names used in the model are listed here in Table 2-S11, and additionally some of them are shown in a graphical schematic in Fig. 2-S32. The font colors in the Table 2-S11 correspond to the colors used in the Fig. 2-S32.

Table 2-S11. Explanation	ons of the symbols in the model
r	Radius of legs as cylinder
σ	Surface tension coefficient of water
ρ	Density of water
g	Gravitational acceleration
$l_c = [\sigma/(\rho g)]^{1/2}$	Capillary length
	Entire length of the middle leg consisting of femure tible, and terrors
	Constant watted length of middle log (the length of tibia plus tarsus of the middle log)
	Constant wetted length of hind leg (the length of tibia plus tarsus of the hind leg)
	Eemur length of hind leg
•Hfemur	Dynamic wetted length of a hind leg
l = y + h	Vertical distance from the body center to the tip of the lea
$l_s = y + n$ $l_s = h - y/\cos(\pi/2 - \omega t)$	Decreased wetted length of middle leg for ascent of the body
$l_{1} = (Bl_{1}/\sigma)^{1/4}$	Modified elastocapillary length of the leg
h	Dynamic dimple depth generated by the middle leg
h_c	Critical dimple depth
h_o	Constant dimple depth at stationary situation
h_M	Maximum dimple depth at surface breaking moment
h_B	The breaking point depth
h_h	Dynamic dimple depth created by a hind leg
h_{hE}	Maximum dimple depth of hind leg by empirical observations
h _{hm}	Constant dimple depth of hind leg derived from h_{hE}
	Index of maximum dimple depth; $u_c = h_M$
	Critical moment of the start of water surface breaking
ι_c	Last moment of complete water surface breaking
t_d	Moment of take-off
0; D.	Duration of dimple breaking
n_{p}	The proportion of wetted leg for utilizing surface tension (i.e., proportion of the length of
F3	unsunk part from total wetted leg length); $p_s = (t_c + D_h - t)/D_h$
p_d	The proportion of wetted leg for utilizing drag (i.e., proportion of the length of sunk part
	from total wetted leg length); $p_d = 1 - p_s$
У	Body center location on vertical coordinate axis
ý	Time derivative of y in Newtonian calculus notation; vertical speed of body center
y_i	Initial height of the body center from the undisturbed free surface
m	Mass of the water strider
r	Radius of the wetted middle leg as a cylinder
	Reduce of the leg
	Angular velocity of middle leg rotation of a jump
w w	Derived angular velocity of middle leg rotation in a jump under the assumption that
ω _e	empirically measured linear downward velocity of wetted midled relative to water surface.
	and the vertical distance from the body center can be approximated using a constant
	value of ω , by two formulae: $v_l = \omega(l_l - y_i)sin(2\omega t) - \dot{y}$, $l_s = \Delta l[1 - \frac{1}{2}cos(2\omega t) + y_i]$.
ω_t	Hypothetical velocity of midleg rotation of the hypothetical jumps (i.e., surface tension
L	jumps of <i>G. gigas</i> and <i>P. tigrina</i> ; drag-involving jump of <i>A. paludum</i>)
v_m	Downward linear velocity of the middle leg relative to the body center
v_f	Take-off velocity

$v_l = \omega(l_l - y_i)sin(2\omega t) - \dot{y}$	Downward linear velocity of a middle leg relative to the water surface
U	Rate of the vertical growth of dimple
μ	Dynamic viscosity
F _c	Capillary force
$\vec{F_n}$	Pressure force
F_{h}	Buoyancy
F_{a}	Added inertia
F.	Viscous force
$L_f = l_w / l_e$	Scaled leg length
$B = \pi E d^4/64$	Bending rigidity
E	Young's modulus of insect cuticle
С	Flexibility factor; function of wetted length of a leg, l_w , and its bending rigidity, B
C_{m0}	Middle leg flexibility factor; function of wetted length of a middle leg,
	l_m , and its bending rigidity, B
C_{h0}	Hind leg flexibility factor; function of wetted length of a hind leg,
	l_h , and its bending rigidity, B
C_m	Middle leg flexibility factor; function of wetted length of a middle leg,
	l_1 , and its bending rigidity, B
C_h	Hind leg flexibility factor; function of wetted length of a hind leg,
	l_2 , and its bending rigidity, B
C_D	Drag coefficient
Re	Reynolds number
H_m	Maximum height of the jump
$\Delta l_l = l_l - y_i$	Maximal downward reach of the middle leg
L	Downward stroke; dimensionless maximal reach of the average of four legs
$\Omega = \omega (l_c/g)^{1/2}$	Dimensionless angular velocity of the average four legs' rotation of a jump
$M = m/(\rho l_c^2 C l_w)$	Dimensionless index of insect body mass with respect to the leg; body mass with respect
	to maximal water mass can be displaced by the average of four legs
$L_m = \Delta l_l / l_c$	Midleg downward stroke; dimensionless maximal reach of the middle leg
$\Omega_{\rm m} = \omega_e (l_c/g)^{1/2}$	Dimensionless angular velocity of middle leg rotation of a jump
$M_m = m/(\rho l_c^2 C_{m0} l_m)$	Dimensionless index of insect body mass with respect to the middle leg; body mass with
	respect to maximal water mass can be displaced by the middle leg



Fig. 2-S32. Schematics of geometric parameters. Geometric parameters used in the mathematical model in stationary situation (a) and dynamic situations (b, c, d). (a) illustrates stationary phase with initial height, y_i , initial dimple depth, h_0 . (b) illustrates dynamic variables during jumping situations: surface tension, transition, and drag phase. (c) illustrates the functional leg length for drag calculation in formula 3. Please note that while the drawing (c) shows a bent leg for dimple depth, h, and downward velocity, v_l , the leg length for drag, l_1 , was assumed to be a solid cylinder. (d) illustrates hindleg's dynamic wetted length, l_2 , in formula 5.

V. Model diagram



Fig. 2-S33. Simplified diagram of model workflow. After input of the empirical data, in the stationary phase, initial dimple depth, h_o , is calculated by solving simple equation for providing initial condition for the surface tension phase. In the surface tension phase, h, y, and F are calculated in the range of $0 < t < t_c$, by solving second-order nonlinear differential equation for h. In the transition and drag phase, h, y, and F are calculated in the range of $t_c < t < t_f$, by solving second-order nonlinear differential equation for y. The initial condition of the transition phase is fixed by calculation of the surface tension phase. Model provides distribution of body height, y, dimple depth, h, force, F, by time and take-off time, t_f , take-off velocity, v_f , and maximum height, H_m . The model simulations were conducted in Matlab. The Matlab code is available at https://doi.org/10.5281/zenodo.7847879.

VI. Values of empirical parameters used in model simulations

Parameter/	G. gigas male		G	G. gigas female			P. tigrina		
variable (units)	EVT05 (2)	EVT16	EVT41	EVT28	EVT33	EVT35	C0046	C0049	C0066
σ (N/m)					0.072				
ho (kg/m³)					998				
g (m/s²)					9.8				
<i>E</i> (N/m ²)				1e10					
C_D					0.8				
<i>r</i> (m)	11.3e-5	13.1e-5	13.7e-5	11.7e-5	10.4e-5	10.4e-5	7.5e-5	7.5e-5	9.4e-5
ω_e (rad/s)	20	15	16	16	19	17	41	33	29
m (kg)	374.76e-6	483.23e-6	325.41e-6	305.67e-6	226.81e-6	226.81e-6	134e-6	134e-6	123e-6
y _i (m)	0.00017	0.00165	0.00088	0.00333	0.00435	0.00274	0.00271	0.00473	0.00806
l_l (m)	88.64e-3	102.69e-3	103.17e-3	72.59e-3	70.13e-3	70.13e-3	44.72e-3	44.72e-3	50.63e-3
l_m (m)	45.78e-3	54.60e-3	54.05e-3	39.80e-3	38.87e-3	38.87e-3	22.70e-3	22.70e-3	25.56e-3
l_h (m)	63.36e-3	79.48e-3	77.17e-3	44.21e-3	36.98e-3	36.98e-3	16.34e-3	16.34e-3	14.46e-3
l_{Hfemur} (m)	42.74e-3	47.42e-3	48.24e-3	32.25e-3	31.11e-3	31.11e-3	24.30e-3	24.30e-3	28.88e-3
h_{hE} (m)	0.0039	0.0032	0.0062		0.0062			0.0065	
$r_{b} = d_{b}/2$	28.5e-5	52.0e-5	48.2e-5			3.	5 <i>r</i>		

Table 2-S12. Empirical parameters used to model jump in each video that has been analyzed in detail.

Table 2-S13. Empirical parameters used in size-specific simulations.

Parameter/variable (units)	<i>G. gigas</i> male	G. gigas male G. gigas female		A. paludum female
σ (N/m)		0.0)72	
ρ (kg/m ³)		99	98	
g (m/s²)		9	.8	
E (N/m ²)				
C_D		0	.8	
<i>r</i> (m)	13.14e-5	11.21e-5	8.934e-5	5.128e-5
<i>m</i> (kg)	413.7e-6	265.2e-6	115.4e-6	47.6e-6
<i>yi</i> (m)	0.900e-3	0.900e-3	5.17e-3	3.00e-3
l_l (m)	101.9e-3	71.7e-3	47.9e-3	25e-3
l_m (m)	53.5e-3	38.5e-3	23.9e-3	13.4e-3
l_h (m)	73.5e-3	40.6e-3	19.1e-3	9.5e-3
l_{Hfemur} (m)	49.5e-3	32.1e-3	27.6e-3	12.1e-3
h_{hE} (m)	0.00	041	0.0065	0.0041
$r_{b} = d_{b}/2$		2.24 <i>r</i> , 3.	5r, 5.05r	

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Chapter 3. Physics of sliding on water predicts morphological and behavioral allometry across a wide range of body sizes in water striders (Gerridae)

Abstract

Laws of physics shape morphological and behavioral adaptations to locomotion at different body sizes. Water striders serve as a model taxon to study how simple physical constraints of water-surface habitats affect their behavior and morphology, and hydrodynamics of rowing by midlegs on the surface is well understood. However, the physics of the subsequent passive sliding has been less explored. We created a model of sliding on the water surface to simulate the effect of body mass, striding type, and wetted leg lengths on an insect's ability to float on the surface and on the sliding resistance. The model predicts that to support their weight on the surface during sliding, the heavy species should either develop long forelegs that support the body during symmetrical striding (when two midlegs thrust) or use asymmetrical striding (when one forward-extended midleg supports the body while the other midleg and contra-lateral hindleg thrust). These predictions are confirmed by the behavior and morphology of various Gerridae species. Hence, the results illustrate how simple physical processes specific to a certain habitat type have far reaching consequences for the evolution of morphological and behavioral diversification associated with body size among biological organisms in these habitats.

Keywords: water strider, striding, sliding, water surface, Gerridae, drag, surface tesion, biomechanics, hydrodynamics, allometry

3.1. Introduction

Understanding how laws of physics may constrain morphological and behavioral evolution of biological organisms of different body sizes is of great importance not only to biology^{1,128,129} but also to the modern bioinspired engineering^{130,131}. Allometry, the study of how physics and biology affect the relationships between body size and other characteristics of an organism, has a long history^{5,11,139–142,12,132–138}. Distinguishing between specific biological and physical mechanisms/constraints responsible for allometry may often be challenging^{11,128}. However, some organisms may provide the more clear-cut situations where allometry can be attributed to physical constraints. Animals that live on the water surface are exposed to a very clear and specific physical constraints from the nature of the water surface, and it has been suggested that body size may shape the morphological and behavioral adaptations to semiaquatic locomotion in animals^{14–16,30}. Water striders, Gerridae, are ideal subjects to study those issues. However, although many studies have taken theoretical approach to understand the physics of water striders' locomotion^{16,28,40–46,48,50,29–34,38,39}, the research effort is confined to several small- and medium-sized water striders in spite of a wide range of body mass that spans over two orders of magnitude from less than 5²⁰ to about 500²¹ mg.

The typical locomotion mode (gait) of Gerridae comprises the ancestral symmetrical striding/skating^{17,26,50}, in which midlegs symmetrically push backwards (thrust phase) to create forward movement of the water strider body (passive sliding on the surface or leaping above the surface) while body is supported on the water surface by two forelegs and two hindlegs for the duration of the push and the subsequent sliding until the midlegs return to their original positions on the water surface and braking occurs (short-lasting braking phase). As the anterior body section remains supported on the forelegs only, the heavier the body the stronger the surface-tension force from the forelegs, otherwise the surface will break under forelegs. Hence, floating on the surface during sliding is the first theoretical consideration in predicting locomotive adaptations in large-bodied water striders. An additional consideration is the effect of body mass, wetted leg lengths (following the convention in the literature, we use the term 'wetted length' as water-contact length even though the leg is not technically 'wet' by its hydrophobicity), and sliding velocity on the resistance that the legs experience on the water surface according to general physics for water striders to slide over long distance and duration.

Entomological literature suggests that heavy water striders evolved unique foreleg morphology and/or striding behavior in order to support the anterior part of the body on the water. Firstly, disproportionately elongated wetted forelegs in the large-bodied water striders of the genus *Ptilomera*^{17,53} may help to support the anterior body during the thrust and passive sliding phases. However, as this type of morphology is also observed in small species of Gerridae (e.g., in Halobatinae^{20,40}), it may not necessarily be a specific adaptation to heavy body, but rather to the midlegs not being used for support on the water surface. Secondly, asymmetric striding that involves one midleg extended forward to support the heavy anterior body part while the other midleg provides thrust may be the specific adaptation to heavy body. This locomotive behavior was only reported in the world's largest water strider species, the giant water strider, *Gigantometra gigas*²¹. In the asymmetric striding, forelegs are not crucial for the body support, and *G. gigas* has relatively short forelegs. Hence, based on the above reasoning, and based on the brief review of morphological measurements of Gerridae from the literature (Fig. 3-S1), we introduce the concept of the "wetted leg geometry". The term refers to the proportions of wetted

forelegs, wetted midlegs and wetted hindlegs in the total length of the wetted legs (sum of wetted lengths of forelegs, midlegs, and hindlegs). Literature suggests that we can classify species into at least three types of "wetted leg geometry": the "intermediate-foreleg (or "standard") geometry" observed in the frequently studied small and mid-size genera *Gerris* and *Aquarius*, the "long-foreleg geometry" (e.g., in Halobatinae, Ptilomerinae) and the "short-foreleg geometry" (extremely developed in *Gigantometra gigas*), depending the proportion of wetted forelegs in the total length of the wetted leg (Fig. 3-S1 shows ranges of values of different taxa).

The two aspects, the support for the anterior part of the body and the resistance on the legs during the sliding, should be considered in building a theoretical model to predict the feasible combinations of the "wetted leg geometry" and striding gait (symmetric or asymmetric striding mode) for a given body mass of a water strider in a specific habitat. Here, we develop a theoretical model of the hydrodynamics of a passive sliding phase in symmetric and asymmetric striding modes for the three types of the wetted legs geometry across a range of the water strider body size. We use the model to predict allometric changes in morphological and/or behavioral adaptations to locomotion on the water surface among the species of Gerridae. The predictions can be tested in the future comparative studies once accurate behavioral and morphological data are collected.

3.2. Results

3.2.1. Theoretical model of a sliding water strider

Detailed technical explanations of the mathematical model are in the Methods and the Supplementary Materials. The model assumed the leg as a cylinder with smooth surface with the length and diameter imitating legs of water striders. We consider that the water striders can stride in symmetric or asymmetric manner with the body velocity, U, relative to the water surface. Hence, water striders can produce thrust symmetrically, by using two midlegs, or asymmetrically, by using one midleg and one contralateral hindleg (Fig. 3-1A), and they can either slide symmetrically on two forelegs and two hindlegs or slide asymmetrically on a midleg and two hindlegs. When a water strider is sliding on the water surface (Fig. 3-1B, C), the normal force (the anterior, N_a , and the posterior, N_p , normal force) keep the water strider afloat, while the resistance on the legs interacting with water (the anterior, R_a , and the posterior, R_p , resistance) gradually slows down the passively sliding water strider.

We assume that three types of resistance force are applied to the water strider during the passive sliding phase: hydrodynamic drag F_h (Fig. 3-1D), wave drag F_w (Fig. 3-1E), and surface tension force F_s (Fig. 3-1F). We first consider the resistance force on one leg of the water strider. We assume that the leg is sliding on the water surface oriented parallel to the direction of movement (Fig. 3-1B, C) and regardless of the water strider mass, the half of the surface of the wetted leg interacts with the water surface. The hydrodynamic drag, F_h , is dominantly caused by the shear stress acting on the wetted area of the leg (yellow color in Fig. 3-1D). It is a function of water properties (density, ρ , kinematic viscosity, ν), leg morphology (diameter and length; the effect of the morphological structures on the surface of the leg was not considered in this study), and water strider behavior (water strider velocity, U, relative to the water surface). The hydrodynamic drag is greater when wetted area of the leg becomes larger and the velocity becomes faster. The capillary-gravity wave drag, F_w , is induced by the wave on the waterfront of the cylindrical leg/water interface as shown in Fig. 3-1E. It occurs at body velocities larger than the critical value c = 0.2313 m/s, when a moving water strider creates a visible wave on the water surface (also empirically proven in Fig. 3-S2). We assumed that this drag is a function of water properties (density, kinematic viscosity of water, and surface tension coefficient), morphology (body mass and leg length), and water strider behavior (body velocity).

To obtain the surface tension force that contribute to resistance (F_s), we assumed that the slope of the water interface in front of the leg is nearly vertical (vertical blue arrow in Fig. 3-1F) while the slope behind the leg maintains horizontal as shown by horizontal blue arrow in Fig. 3-1F. Therefore, only the horizontal surface tension force at the posterior edge of the leg (dashed yellow half circumference in Fig. 3-1F) contributes to the surface tension resistance, F_s , which is a function of water property (surface tension coefficient) and leg morphology (leg diameter). We determine the resistance force on the anterior (R_a) and posterior (R_p) legs of a water strider as the sum of the three types of resistance (F_h , F_w , and F_s).

We derived a simple gravity-normal force balance formula, and we also derived the torque-balance formula for the posterior, N_p , and anterior, N_a , normal forces on the legs, which depend on water strider body mass, leg morphology (distances a, b, and wetted leg lengths on forelegs and/or hindlegs; Fig. 3-1A; see also Methods section), and the resistance force on the anterior and posterior legs (R_a and R_p ; details in the Methods). Finally, from the calculations of the system of equations from these two balance formulae, the model predicts the normal forces, the total resistance as a sum of anterior and posterior resistance on water strider legs, and deceleration caused by the resistance.

3.2.2. The "wetted leg geometry" of the studied species used in theoretical calculations

Although the individuals from the six study species measured in our study followed a general allometric relationship between body mass and the total wetted leg length (Fig. 3-S4), they differed in the relative proportions of wetted foreleg (Fig. 3-2A), midleg (Fig. 3-2B) and hindleg (Fig. 3-2C) lengths, and represent the three types of "wetted leg geometries": the "intermediate-foreleg geometry", the "long-foreleg geometry" and the "short-foreleg geometry".

The four small/medium size water striders that we have measured (*G. latiabdominis*, *G. gracilicornis*, *A. remigis*, *A. paludum*) form one cluster of "intermediate-foreleg geometry" with wetted forelegs comprising from ~4 to ~8% of total wetted leg length (Fig. 3-2A-C). We decided to use the specific values of the "wetted leg geometry" of *A. paludum* (marked as green triangle in Fig. 3-2) as the representative "intermediate-foreleg geometry" for comparisons with the two other "wetted leg geometries": the "long-foreleg geometry" with wetted forelegs comprising 12-14% of the total wetted leg length (represented by the subtropical water striders *P. tigrina*; Fig. 3-2A-C), and the "short-foreleg geometry" with wetted forelegs comprising 1-3% (represented by *G. gigas*; Fig. 3-2A-C). The "leg geometries" of our study subjects are also visualized in an alternative manner in Fig. 3-2D-F as ratios of wetted to un-wetted leg lengths.

3.2.3. Model predictions for five different size classes and three leg geometries

3.2.3.1. - General

After confirming that the theoretical model reasonably well simulates the empirically observed trajectories (Fig. 3-S3), we used it to predict how the three "leg geometries" ("intermediate-foreleg", "long-foreleg", and "short foreleg geometry" based directly on empirical measurements of our study species; see below "Empirical observations of the study species") would perform in terms of floating on the water without breaking the surface during sliding, and in terms of resistance and deceleration during symmetrical and asymmetrical sliding on the water surface, in five body size classes corresponding to the recorded body mass ranges of our study species: *G. latiabdominis* (12-32 mg), *A. paludum* (35-72 mg), *P. tigrina* (83-144 mg), *G. gigas* females (217-318 mg) and *G. gigas* males (316-511 mg). This resulted in predictions for 30 situations (5 body mass classes * 2 modes of locomotion [symmetrical or asymmetrical] * 3 "leg geometries") including 6 actually observed in our study subjects and 24 "virtual" ones that have not been recorded in our study species (Fig. 3-3).

3.2.3.2. - Theoretical predictions of conditions for floating during sliding

The maximum (critical) surface tension force that water provides to the anterior supporting leg(s) is the product of surface tension coefficient, σ , and the entire wetted length consisting of the length, L_a , and diameter, D; $(2\sigma(L_a + D))$ [28, 29]. Therefore, the anterior supporting leg(s) would pierce through the water surface when the force needed to support the anterior part of body, N_{aT} , is larger than $2\sigma(L_a + D)$. This force depends on multiple factors including water strider morphology, behavior, and body velocity (see details in the Methods). Using the theoretical model, we produced two-dimensional phase diagrams in Fig. 3-3A-E, with the anterior normal force, N_a , on the vertical axis and the wetted leg perimeter $(2(L_a + D))$ on the horizontal axis. In these diagrams, the conditions when the sliding water strider's anterior supporting leg(s) do not pierce the water surface correspond to the unhatched area below the line of the critical $N_a = 2\sigma(L_a + D)$. The hatched area above this line comprise situations in which the anterior supporting leg(s) will pierce the water surface.

The model predicts that the water striders do not drown if they perform asymmetric sliding in any of the 15 conditions defined by 3 leg geometries and 5 body size classes (all polygons with dashed edges in Fig. 3-3A-E) because the wetted length of the forward-extended midleg is sufficiently long compared to the forelegs. Water striders with "long-foreleg geometry" (red solid line polygons) are not predicted to drown regardless of the body size if they perform symmetric sliding (polygons with solid line edges in Fig. 3-3A-E). However, the three larger size classes of water striders with "intermediate-foreleg geometry" (green solid line polygons in Fig. 3-3C-E) and water striders with "short-foreleg geometry" regardless of the body size (blue solid line polygons) are predicted to drown if they perform symmetric sliding except for a very narrow range of conditions that locate them under the critical lines ($N_a = 2\sigma(L_a + D)$).

3.2.3.3. - Theoretically calculated sliding resistance

We calculated the relationship between body velocity and the total sliding resistance force (Fig. 3-3F-J) for all floatable conditions. The total sliding resistance depends on the body mass, leg geometry, sliding mode, and body velocity. In general, the asymmetric sliding (broken lines in Fig. 3-3F-J) generates lower resistance than the symmetric sliding (solid lines in Fig. 3-3F-J). The sliding resistance dramatically increases when body velocity exceeds the minimum threshold velocity at which surface waves are produced by legs sliding on water surface (c = 0.2313 m/s). For symmetric sliding, the resistance greatly depends on the leg geometry and body mass: sliding resistance increase reaches a peak of 0.1-0.2 mN for body speeds of about 0.5 m/s for "intermediate-foreleg geometry" in water striders from the two smaller size classes (green solid line in Fig. 3-3F, G) and a peak of 0.3-0.6 mN for body speed of about 0.25 m/s for "long-foreleg geometry" in water striders with large body weight (red solid line in Fig. 3-3H-J), while for smaller water striders this peak for "long-foreleg geometry" is much less pronounced (red solid line in Fig. 3-3F, G). For asymmetric sliding (broken lines in Fig. 3-3F-J), a steep increase in resistance is predicted as body velocity passes through the threshold critical velocity, c, and afterwards its slope becomes much milder (broken lines in Fig. 3-3F-J), but these patterns were predicted regardless of the leg geometry.

During the thrust phase of each stride, a water strider must create a total thrust force comprising a counterresistance component (to overcome the resistance) and a net thrust force that contributes directly to the water strider body's momentum change (and produces the body velocity observed at the start of the sliding phase). We compared the empirically estimated net thrust forces in a set of strides by our study species with the theoretically calculated resistance in those strides (Fig. 3-S5, S6), and found out that on average 85-95% of total thrust is converted into the water strider's body momentum. The remaining thrust is used up to overcome the resistance (Fig. 3-S5, S6), especially in the symmetrical sliding of *P. tigrina* and those striders that perform fast sliding and produce surface waves (above the critical velocity threshold of 0.23 m/s) when the wave resistance starts affecting the moving water strider (Fig. 3-S5, S6).

3.2.3.4. - Theoretically calculated sliding deceleration

As the resistance mainly contributes to the deceleration, the patterns of theoretically calculated deceleration were similar to those of the resistance. The theoretically predicted decelerations were smaller for asymmetric than for symmetric sliding for all five size classes (Fig. 3-3K-O). The deceleration experienced by the two smaller studied species at their actual sliding velocities (shown as horizontal box-and-whiskers plots in Fig. 3-3F, G, and marked by vertical gray shaded bars across Fig. 3-3F, G, K, L) ranges from less than 2 to 5 m/s² for symmetrical sliding and between 1.5 and 2 m/s² for asymmetrical sliding (the ranges are based on lower and upper quartile values of sliding velocity recorded in the species). As the average sliding velocity of these species is less than 0.5 m/s (Fig. 3-3F, G), these values of decelerations have relatively strong slowing-down effect compared to the larger species (see below). Additionally, the theoretically predicted difference between asymmetrical sliding in the deceleration at the empirically measured median body velocity is roughly twice as large in the medium-size *A. paludum* as it is in the small-size *G. latiabdominis* (black double

arrows in Fig. 3-3K, L), indicating that by using asymmetrical rather than the symmetrical striding the mediumsize species with "intermediate-foreleg geometry" may importantly increase its sliding performance.

The predicted deceleration experienced by the three studied large species/sex classes at their actual sliding velocities (shown as horizontal box-and-whisker plots in Fig. 3-3H, I, J and marked by vertical gray shaded bars across Fig. 3-3H-J, M-O) vary ~0.8-1 m/s² in the *G. gigas* males and females to ~1-1.5 m/s² in *Ptilomera*. As the average sliding velocity of the two larger classes is more than 1 m/s (Fig. 3-3I, J), these relatively small values of decelerations have relatively weak slowing-down effect, compared to the effect of deceleration expected in the two smaller species.

3.2.4. Empirical observations of the study species

We observed three combinations of thrusting-sliding phases of locomotion: symmetric thrusting – symmetric sliding (Fig. 3-4A, B, C), symmetric thrusting – leaping (Fig. 3-4A, B), and asymmetric thrusting – asymmetric sliding (Fig. 3-4B, D, E). The smallest species with "intermediate-foreleg geometry", *G. latiabdominis*, thrusts symmetrically (except for changing direction of the body), and slides symmetrically or leaps forward after symmetric thrusting (Fig. 3-4A; examples of digitized strides are in Figure 3-S7-S10). The larger species with "intermediate-foreleg geometry", *A. paludum*, used all three phase combinations (Fig. 3-4B) depending on their initial body velocity (the velocity at the end of the thrust phase). The large species with "long-foreleg geometry", *P. tigrina*, used only the symmetric thrust followed by symmetric passive phase (Fig. 3-4C). Only when forelegs were handling the food⁴⁷ or grooming, *P. tigrina* used asymmetric striding mode. Both sexes of the large species with "short-foreleg geometry", *G. gigas*, used only the asymmetric locomotion mode (Fig. 3-4D, E): at least one middle leg always supported anterior part of the body even in changing direction of the body.

The two smaller species with "intermediate-foreleg geometry" moved at relatively slower velocities than the three larger size classes using either symmetric or asymmetric mode (horizontal plots at the top of each panel in Fig. 3F-J; See also Fig. 3-S11). The largest class (asymmetrically sliding *G. gigas* males with "short-foreleg geometry") moved with the highest speed (compare the horizontal box and whiskers plots inserted in Fig. 3-3H, I, J; see also Fig. 3-S11). The medium size species, *A. paludum*, was observed to slide at the widest range of body velocities from near zero to near 1.5 m/s (horizontal box-and-whisker plot in Fig. 3-3G and Fig. 3-S11).

3.2.5. Locomotion mode depends on body speed – observations in G. latiabdominis and A. paludum.

Observations of *G. latiabdominis* revealed that they used symmetric thrust followed by either sliding or leaping (Fig. 3-4A). Leaping velocity of *G. latiabdominis* was significantly faster than that of symmetric sliding (Wilcoxon Signed-Rank Test, p<0.05, n=8, Table 3-S2). Hence, they seemed to avoid sliding on the water surface by leaping in conditions of high resistance, i.e., when body velocity is high.

Observations of A. paludum revealed that symmetric thrust followed by leaping occurred at high body velocities (>0.5 m/s for A. paludum; Fig. 3-5A). A. paludum switched between symmetric and asymmetric modes of sliding locomotion (Fig. 3-4B). Asymmetric mode was used at significantly higher velocities than the symmetric mode (Fig. 3-5A; statistics in Table 3-S3). While the symmetric sliding was used over a relatively wide range of body velocities including slow sliding (Fig. 3-5A), most of the asymmetric sliding occurred at body velocities that are larger than the theoretical threshold velocity (c = 0.231 m/s; marked with red unfilled circle in Fig. 3-5A, D), above which capillary-gravity wave resistance starts to slow down the water striders, especially during the symmetric sliding. 75% of asymmetric sliding occurred at the initial velocities higher than 0.258 m/s (lower quartile in Fig. 5A; marked by red arrow in Fig. 3-5A, D), when symmetrical sliding already results in twice as strong deceleration due to resistance as the asymmetrical sliding does (Fig. 3-5D, red double arrow shows this difference). Higher body velocity leads to increasingly larger difference in resistance between symmetric and asymmetric striding (Fig. 3-5D). It is illustrated by the relatively smaller predicted deceleration difference between symmetric and asymmetric sliding for the velocity ~ 0.37 m/s, corresponding to the median initial velocity of symmetric sliding (marked with green filled triangle on the velocity axes in Fig. 3-5A, D), and the relatively larger deceleration difference for the higher body velocity of ~0.44 m/s corresponding to the median (marked with un-filled green triangle on the velocity axes in Fig. 3-5A, D) initial velocity of asymmetric sliding (these differences in deceleration are marked with green solid and green broken arrows in Fig. 3-5D). The lower deceleration in asymmetric sliding seems to lead to the sliding distance (Fig. 3-5B; Table 3-S4) and sliding duration (Fig. 3-5C; Table 3-3-S5) twice as long for asymmetric as for the symmetric sliding (detailed results of all the statistical analysis are in Tables 3-S3-S5).

3.3. Discussion

Our analysis predicts that all six combinations of the three leg geometries and the two locomotion modes can theoretically be observed among the relatively small-sized water striders (~10 to ~30 mg) (considering floating ability during sliding). Although the symmetrical locomotion by the water striders with "short-foreleg geometry" is physically possible (i.e., water striders can stay afloat), this can be performed only in a narrowly constrained area of light body mass or slow motion (i.e., when relatively weak normal force is applied on the anterior body part) in these small-sized water striders, because if heavier the short wetted forelegs cannot create sufficiently large force upward to support the anterior body section on the water surface. This also indicates that very small water striders (smaller than the body size modeled here, i.e., <10 mg) could theoretically perform symmetrical mode of locomotion with the "short-foreleg geometry".

However, as those theoretically feasible combinations differ in resistance, and the resulting deceleration, and as water striders seem to pay attention to the resistance (as indicated by our observations on *A. paludum*), we hypothesize that natural selection or adaptive behavioral plasticity towards decreasing resistance may in certain conditions cause evolutionary or behavioral shifts from the ancestral^{17,26,50} symmetric striding of water striders with "intermediate-foreleg geometry" towards either the asymmetric locomotion mode or "long-foreleg geometry". Asymmetric locomotion mode substantially decreases resistance and deceleration and increases sliding distance but involves weaker thrust from only one midleg aided by contralateral hindleg (in the habitats

where the relatively strong thrust is less important). The "long-foreleg geometry" reasonably decreases resistance (longer forelegs create lower resistance by smaller wave drag) while maintaining high thrust from two symmetrically pushing midlegs, which will be especially important in ecological situations where frequent rowing with high thrust is highly beneficial (e.g., in fast flowing water). The presence of "long-foreleg geometry" (and apparently also the symmetrical gait) even in the small taxa typical for fast current (e.g., *Metrocoris*⁵⁰) or for turbulent oceanic waters (*Halobates*^{20,40}) is consistent with the idea that "long-foreleg geometry" is advantageous in the turbulent habitat where frequent thrust from midlegs is needed even in the smaller water striders.

When body mass reaches the range represented by *P. tigrina* and *G. gigas* (range of about 80-500 mg), water striders with typical "intermediate-foreleg geometry" of legs would not be able to support their bodies on the surface during symmetric striding/sliding (when the anterior body mass is supported by two forelegs). The model predicts, and literature⁵² suggests (Fig. 3-6 and Fig. 3-S13), that there are two solutions: either a shift to "long-foreleg geometry" by elongation of forelegs (recent studies in the genetics of morphology in Gerridae identified some genes that may be involved in the leg elongation^{143,144} while maintaining the standard symmetric locomotion mode like in Ptilomerinae, or a use of asymmetric locomotion mode, like in G. gigas. The difference between G. gigas, who lives in slower flowing waters, and P. tigrina, who lives in faster moving water, is consistent with the idea that even though the asymmetric sliding always creates less resistance than the symmetric sliding and does not cause sinking regardless of body mass and leg geometry, P. tigrina does not use the asymmetric sliding because of the importance of strong thrust in the very frequent short strides against the fast flowing water in their habitat⁴⁷. Hence, we propose that the habitat type may affect the evolutionary trajectories shaping the wetted leg geometry in large water striders leading to the asymmetrical locomotion in slow-flowing waters and to the long-foreleg/symmetrical locomotion combination in species from fast currents, where the requirements for frequent and strong thrust may additionally trigger evolution of special microstructures for rowing^{20,53,145} and the associated loss of the midlegs' function of supporting the water strider on water surface^{40,53}. If this is correct, then Gerridae illustrate how the physical environment channels the morphological and behavioral evolution^{146,147} towards either of the two physically feasible adaptive solutions for locomotion by large-sized water striders.

Asymmetrical mode provides a similar performance regardless of the relative wetted foreleg length and therefore it is not surprising that, in accordance with the rules of competition among water strider body parts¹⁴⁸, it is associated with shortening of the wetted forelegs that are no longer needed for support of the anterior body mass like in *Gigantometra gigas* and most likely in other large Gerrinae with "short-foreleg geometry" (Fig. 3-6 and Fig. 3-S13). Finally, as already speculated²¹, the asymmetric locomotion is associated with asymmetry in thrust (stronger on the side of the pushing midleg than on the side of the midleg stretched forward), which leads to torque in the horizontal plane. Therefore, the especially elongated wetted hindlegs characterizing the "short-foreleg geometry" (Fig. 3-S13C) of the asymmetrically striding species may play a role as a rudder preventing rotation of body axis. If this is correct, the hindlegs in heavy asymmetrically striding species serve two functions: adding to the thrust and counteracting the torque.

As we have discovered asymmetric locomotion mode in one of the common and widespread species, A. paludum⁴³, which has been the subject of multiple studies^{18,31,34,46,149}, and was believed to solely use the symmetric locomotion mode, we advise caution in using the traditional knowledge (in the literature) about locomotion modes of water striders in natural situations. Additionally, the data on the species-specific body size usually includes information on body length but not fresh body mass, and the existing literature on body lengthbody mass relationships in insects does not concern fresh body mass^{150–158} or it does not provide accurate formulas for the full body mass range of Gerridae^{159,160}. Hence, the information from the literature allows us to present only a very preliminary view on the relationship between relative body length and "wetted leg geometry" (Fig. 3-6 and Fig. 3-S13), which nevertheless confirms the model predictions. Our preliminary observations of a relatively little studied genus of large water striders, Cylindrosthetus costalis with "shortforeleg geometry" (Fig. 3-6), confirms that, similar to G. gigas, they use asymmetric striding mode. Once solid morphological and behavioral data on locomotion modes in natural habitats across a variety of species of different sizes are collected, the predictions from our theoretical model can be properly tested in quantitative comparative phylogeny-based analyses of evolutionary correlations between body size, morphological adaptations (leg geometry) and behavioral plasticity (locomotion mode), in a variety of habitats across a wide range of body weights from less than 5 mg (e.g., Halobatinae) to above 500 mg in G. gigas. Hence, the model provides a solid theoretical basis for the next comparative step of research to understand the evolution of allometry of striding in water striders. It also provides insights into bio-inspired engineering of water walking robots of various sizes^{31,45,116,122,131}.

3.4. Materials and Methods

3.4.1. Mathematical Model

The total resistance of each leg was calculated based on three forces: hydrodynamic drag¹⁶¹ (Fig. 3-1D), wave drag (Fig. 3-1E), and surface tension (Fig. 3-1F). The normal forces on a leg (Fig. 3-1B) supporting the anterior side (N_a) and the posterior side (N_p) were calculated by the force balance in the vertical direction and the torque balance about the center of the mass of the water strider. We computationally determined N_a , N_p , R_a , and R_p (Fig. 3-1B, C) for an empirical situation of an water strider sliding on the surface comprising the following set of empirically derived values: body mass, m, wetted leg lengths, L, wetted leg diameters, D, distances a and b (as defined in Fig. 3-1B, C), vertical distance between surface and water strider body, h, (Fig. 3-1C) and body velocity, U, during sliding.

The larger the resistance force is, the larger the rate of deceleration is pronounced, and the heavier the water strider is, the smaller the rate of deceleration is. We have estimated the deceleration rates (Fig. 3-3K-O) corresponding the lines of average resistance in all five panels in Fig. 3-3F-J. We calculated the body deceleration for the average mass of each size class from the standard equation: $\ddot{x} = F/m$ with F being the total external force.

To predict the model results for non-existing combinations of leg geometry, body mass and striding behavior, we chose leg geometry of *G. gigas*, *P. tigrina*, and *A. paludum* as representatives of "long-foreleg", "short-

foreleg", and "intermediate-foreleg geometry", respectively. As the leg geometries of the three small-sized species showed small differences (Fig. 3-2), *A. paludum* was chosen as the sole representative of the "intermediate-foreleg geometry". We used five size classes corresponding to empirically observed ranges and empirical distributions of body mass in our study species: *G. latiabdominis* (12-32 mg), *A. paludum* (35-72 mg), *P. tigrina* (83-144 mg), *G. gigas* females (217-318 mg) and *G. gigas* males (316-511 mg). We created morphological data for 15 separate situations (5 body mass classes * 3 "leg geometries"), including 5 empirically collected data (for the five size/sex classes in our study) and 10 "virtual" situations that have not been recorded in our study species. Each situation was represented by a population of individuals (actually measured or virtually created) with their morphological traits: body mass, body length, distance from the head tip to the location of foreleg attachment to the body, distance from the head tip to the location of midleg attachment (those distances expressed as proportion of body length), and leg measurements: femur, tibia, tarsus. Wetted leg length was assumed as tarsus (forelegs) or tibia plus tarsus (midleg and hindleg). For each individual in each empirical data set we additionally expressed the femur, tibia and tarsus lengths as proportions in the total length of the legs of that individual.

Based on the mathematical model we built a computational model in MATLAB. At the core of the model was numerical integration of the equation of force and torque balance using morphological data of the studied species and "virtual" re-distribution. Figures showing model output were also prepared using MATLAB. See Supplementary Materials for additional mathematical details of the model and re-distribution of leg geometry for virtual data set.

3.4.2. Measurements, observations, and experiments

We determined body mass and various morphological variables explained in Fig. 3-S14 for six water strider species: *G. latiabdominis* (n=16; Seoul, Korea), *Aquarius remigis* (n=6; Huyck Preserve, USA), *G. gracilicornis* (n=16; Seoul, Korea), *A. paludum* (n=21; Seoul, Korea), *Ptilomera tigrina* (n=18; Me Linh Station for Biodiversity, Vietnam) and *Gigantometra gigas* (n=25; Pu Mat National Park, Vietnam). Photographs were used for measurements by ImageJ (https://imagej.nih.gov/ij/). The research was permitted in Pu Mat National Park administration, and the study in and near the Me Linh Station for Biodiversity was permitted by the Institute of Ecology and Biological Resources, VAST, Vietnam.

We filmed *G. gigas* and *P. tigrina* in their natural habitats (standard and high-speed movies at 250, 500, and 1000 fps), and *A. paludum* and *G. latiabdominis* in acrylic containers filled with water (standard and high-speed at 1000 fps) with Sony RXIII-10 camera. A total of 50 striding events of *G. gigas* and 12 striding events by *P. tigrina* were filmed and used to determine their striding behavior, and a total of 236 striding events from 6 individuals of *A. paludum* and 13 striding behaviors from 5 individuals of *G. latiabdominis* were analyzed. The high-speed videos that were shot directly from above the water strider with scale at the level of the water surface were digitized and analyzed using Tracker program to determine the body velocity and acceleration.

For statistical comparisons of body velocity between different locomotion modes by *G. latiabdominis* (*n*=8 striding events by 4 individuals), we used Wilcoxon Signed-Rank Test (https://astatsa.com/WilcoxonTest/, https://www.aatbio.com/tools/mann-whitney-wilcoxon-signed-rank-test-calculator). For statistical comparisons of initial body velocity of sliding among three locomotion modes of *A. paludum*, (n=236 striding events from 6 individuals) we used *lmerTest* and *gamlss* packages (R version 3.6.1). The distance traveled, and the duration of sliding among three locomotion modes were also analyzed in a similar manner, but with only sliding events that were naturally ended by the water strider itself (e.g., excluding sliding event that ended by hitting wall, n=228 striding events from 6 individuals).

Additionally, we chose 72 striding events of *A. paludum* that have passive phase duration long enough (50-80 ms) to empirically evaluate the deceleration and subsequently the resistance. These data were analyzed using the general additive model (*gamlss* package in R). Finally, for a small subset of striding events (8 for *G. latiabdominis*, 16 for *A. paludum*, 8 for *P. tigrina*, and 5 for *G. gigas*) we digitized the striding from high-speed movies in a frame by frame manner in order to extract information for evaluation of acceleration and force generated during thrust phase of each species (seen in Fig. 3-S6).



Fig. 3-1. Graphical explanation of the basic concepts in the model of sliding of water striders.

(A) – Striding locomotion has two phases: thrust phase (when legs pushing backward create a thrust force forward), and passive phase (when water strider is sliding on water or leaping above water). The thrust plus sliding can either be symmetric (typical for most Gerridae) or asymmetric. The leaping is preceded by symmetric thrust. Colored legs indicate thrusting legs (blue) and sliding legs (red). (B, C) – schematics of asymmetric (B) and symmetric (C) sliding, and variables used in the model: anterior and posterior normal forces (N_a, N_p) , the anterior and posterior resistance forces (R_a, R_p) , wetted leg lengths (L) and diameters (D), horizontal distance along line parallel to the moving direction from the center of the mass to the center of the anterior and posterior wetted legs (a, b), body height above water surface (h), body velocity (U); (D, E, F) – explanations of the three main forces contributing to the total resistance: hydrodynamic drag (D; F_h), wave drag (E; F_w), and surface tension (F; F_s).



Fig. 3-2. Leg proportions ("leg geometry") and body masses of the study species.

Leg morphology is expressed as proportions of wetted forelegs, midlegs, and hindlegs in the total length of wetted legs of an individual water strider ("wetted leg geometry"; A-C), and as absolute lengths of wetted *vs*. un-wetted leg for forelegs, midlegs and hindlegs of each study species (D-F). From the four species with "intermediate-foreleg wetted leg geometry" (*Aquarius paludum*, *A. remigis, Gerris gracilicornis and G. latiabdominis*), we chose the wetted leg geometry of *A. paludum* (green triangles) to serve as the representative distribution of leg morphology in species with "intermediate-foreleg geometry" in the model. *Ptilomera tigrina* served as the representative of "long-foreleg geometry", and *Gigantometra gigas* served as representative of the "short-foreleg geometry" in the model.



Fig. 3-3. Model predictions of floating conditions, resistance, and deceleration.

Model results: predictions of the ability to float on the surface without breaking it (A-E), and calculations of resistance (F-J) and deceleration (K-O) during sliding for 30 different combinations of "leg geometry" ("intermediate-foreleg", "long-foreleg", "short-foreleg"), body size class (5 classes between about 10 and about 500 mg) and locomotion mode (symmetric vs. asymmetric). The large gray arrow under the figures represents size classes based on empirical data from five species/sex classes of our study organisms: G. latiabdominis, A. paludum, P. tigrina, and G. gigas females and males. Wetted leg geometries are marked as colors: "shortforeleg" (blue), "long-foreleg" (red), and "intermediate-foreleg" (green). Sliding locomotion modes are marked with line patterns: symmetric sliding (solid line) and asymmetric sliding (dashed line). (A-E) - phase diagrams of the total normal force applied on anterior supporting legs (N_{aT} ; vertical axis) and the perimeter of the anterior legs' wetted perimeter of the foreleg $(2(L_a + D))$; the diagonal black solid line in each figure corresponds to $N_{aT} = 2\sigma(L_a + D)$, and the hatched area above the line indicates conditions leading to meniscus breaking under the anterior supporting leg(s) and sinking of the water strider's forelegs. (F-J) The total resistance as a function of body velocity relative to the water surface for those conditions among (A-E), in which floating is possible. Geometries are marked as colors: "short-foreleg" (blue), "long-foreleg" (red), and "intermediate-foreleg" (green). Sliding types are marked with lines: symmetric sliding (solid line) and asymmetric sliding (dashed line). Empirical initial velocities observed in the study species within each size class are shown as small horizontal box plots (water striders in panels F-J), and also by vertical gray shaded rectangles across panels F-O. The resistance and deceleration differences between symmetric/asymmetric striding at the observed median velocity are marked as black arrows in F, G, K, L.



Fig. 3-4. Summary of observations of locomotion of the study species. The large gray arrow under the figures represents relative size of species. Geometries are marked as colors: "short-foreleg" (blue), "long-foreleg" (red), and "intermediate-foreleg" (green). Water strider size classes are arranged in order from the smallest to largest size: (A) *G. latiabdominis*; (B) *A. paludum*; (C) *P. tigrina*; (D) *G. gigas* females and (E) *G. gigas* males.



Fig. 3-5. Striding behavior of A. paludum and theoretical predictions of deceleration for A. paludum.

The box plot of initial velocity (A), sliding/leaping distance (B), and sliding/leaping duration (C) in a passive phase from empirical data of *A. paludum*. Symmetric sliding, asymmetric sliding, and leaping are marked as green solid, green dashed, and gray solid lines, respectively. Theoretical predictions of deceleration in (D) come from Fig. 3-3L. Median initial velocities of symmetric and asymmetric sliding are marked with filled and unfilled triangles in (A, D), respectively. Lower quartile of asymmetric sliding velocity is marked with red arrow in (A, D). Critical body velocity of wave-making, c = 0.231, is marked with unfilled red circle in (A, D). The statistical analysis results for (A-C) are in Tables 3-S3-5, and additional results for *A. paludum* are in Fig. 3-S12 and Table 3-S6.



Fig. 3-6. The body length and the proportion of wetted foreleg length in total wetted legs' length.

The gray shaded area helps visualizing that with increasing body size the water striders adopt one of the two "wetted leg geometries", either "long-foreleg" or "short-foreleg geometry". The subfamilies (according to Matsuda 1960) are indicated by different large unfilled circles: Gerrinae (blue), Ptilomerinae (orange), Halobatinae (purple), Rhagadotarsinae (gray), and Trepobatinae (brown). The species with our measured data are indicated by small-filled circles: G. gigas (blue), P. tigrina (red), A. paludum (green), G. latiabdominis (light green), G. gracilicornis (orange), A. remigis (yellow), and C. costalis (purple). The subfamilies follow Matsuda 1960, which may be not entirely consistent with the modern assignments of genera into subfamilies. Additionally, this is phylogenetically un-corrected relationship, and therefore it does not directly represent evolutionary processes shaping the evolutionary changes of leg morphology as a function of evolutionary changes of body size (the goal of the future studies). The figure is based on Table 16 in Matsuda 1960. See also Fig. 3-S13 for more details and comments about Table 16 in Matsuda (1960). The equations fitted to the data points for each family separately have the power form following the convention for allometric equations. However, we used body length because of the absence of data for body mass, absence of body width, height or diameter data, and absence of body length - body mass formulas for water striders over such a large body size range. We expect that from among the possible linear measurements of body (width, height, length) the length is relatively more correlated with the body mass (albeit not necessarily in a linear fashion) than are body width or height as they are relatively small and differ among species relatively less than the body length. We decided not to use *body length*³ (a possible alternative used occasionally in allometry) because of the elongated shape of the water striders.

Supplementary meterials

Model description

Hydrodynamic drag (F_h ; Fig. 3-1D)

The hydrodynamic drag on a cylindrical leg is a function of water properties (density and kinematic viscosity of water), morphology (diameter and length of the wetted leg), and insect behavior (water strider velocity, U, relative to the water surface). The hydrodynamic drag, F_h , is dominantly caused by the shear stress on the leg surface in contact with water (yellow color in Fig. 3-1D), and is represented as

$$F_h = \frac{1}{2} C_D \rho U^2 A,$$

where ρ is the density of water, U is the relative velocity of the water strider to the water, and A is the wetted area of the leg (the yellow-shaded part of the cylindrical wetted leg in Fig. 3-1D). The wetted area is assumed as a half of the curved surface of a cylinder, $A = \pi DL/2$. D and L are the diameter and the length of the wetted leg, respectively. C_D is the drag given by $1.328\sqrt{\nu/(UL)}$, where ν is kinematic viscosity of water [*reference nr 70 in the main text*]. The resistance by hydrodynamic drag is higher when wetted area of the leg becomes larger and the velocity becomes faster.

Wave drag (F_w ; Fig. 3-1E)

The capillary-gravity wave drag on a cylindrical leg is a function of water properties (density, kinematic viscosity of water, and surface tension coefficient), morphology (body mass, leg length and shape of the wetted area), and water strider behavior (water strider velocity, direction of movement relative to the leg orientation). When a floating object moves on the surface of water at velocity greater than $c = (4g\sigma/\rho)^{1/4}$, where σ is the surface tension coefficient of water and g is the gravitational acceleration, it generates capillary-gravity waves⁴⁵. The theoretical minimum critical velocity, c, that produces those waves on water is 0.2313 m/s, and observations of water striders are generally consistent with this value of c (Fig. 3-S2).

The wave drag, F_w , is induced by the wave generated by the cylindrical leg as shown in Figure 3-1e. The waves push the leg of the water strider with a force of

$$F_{w} = \frac{N^{2}}{4\pi^{2}L^{2}} \Re\left\{\int_{-\infty}^{\infty}\int_{-\infty}^{\infty}\frac{i|\widehat{\Psi}|^{2}k_{x}k}{\xi} \mathrm{d}k_{x}\mathrm{d}k_{y}\right\},\$$

where N is the normal force on the leg from the water (Fig. 3-1B) and k, the wave number, is represented for each x and y axis in Fig. 3-1E as $(k_x^2 + k_y^2)^{1/2}$ (\Re stands for "real part of"). ξ is represented as below to simplify the formula.

$$\xi = \rho g k + \sigma k^3 + \rho (2\nu k^2 - ik_x U)^2 - 4\rho \nu^2 k^3 \sqrt{k^2 - \frac{ik_x U}{\nu}}.$$

The shape of wetted area, Ψ , depends on the shape of the leg and its moving direction ($\hat{\Psi}$ stands for the Fourier transform of Ψ). We assume the shape of the wetted leg as a line with length L and the longitudinal

movement, then $\hat{\Psi} = (1 - e^{-iLk_x})/ik_x$. The resistance by wave drag is higher when the normal force is higher and the leg length is shorter.

Surface tension resistance force (F_s; Fig. 3-1F)

The surface tension force contributing to the total resistance during sliding is a function of water properties (surface tension coefficient) and morphology (leg diameter). For simplicity, our model assumes that the resistance by surface tension is a stepwise function that has zero value below the minimum velocity at which surface waves are produced, c = 0.2313 m/s, and increases beyond this threshold. As the waves are generated around a leg, we assumed that the slope of the water interface in front of the leg approaches the vertical while the slope behind the leg maintains horizontal as shown by blue arrows in Fig. 3-1F. Therefore, only the horizontal surface tension force at the posterior end of the leg, F_s , contributes to the resistance force. We assumed that the wetted length for the horizontal F_s is half of the cylindrical leg's circumference (yellow dashed line in Fig. 3-1F). The resistance by surface tension is higher when leg diameter, D, is larger:

$$F_s \sim \frac{\sigma \pi D}{2}$$
,

Total resistance on a leg

The resistance force on the leg, R, is the sum of F_h , F_w , and F_s :

$$R(U) \sim \frac{1}{2} C_D \rho U^2 A + \frac{N^2}{4\pi^2 L^2} \Re \left\{ \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \frac{i|\hat{\Psi}|^2 k_x k}{\xi} dk_x dk_y \right\} + \frac{\sigma \pi D}{2} H(U-c),$$
(1)

where H(x) is the Heaviside function to represent the surface tension force as a stepwise function based on velocity criteria, c.

Modeling normal forces responsible for water strider's floating on the surface and resistance forces acting on water strider's legs during symmetric and asymmetric sliding

Figures 3-1B and C show the schematics of a water strider sliding on the water surface. The normal force on a leg supporting the anterior side is N_a and the posterior side is N_p (Fig. 3-1B). The force balance in the vertical direction is represented as

 $mg = nN_a + 2N_p$, (2)

where g is gravitational acceleration, m is the mass of the water strider, and n is the number of legs involved in the anterior part and it depends on the sliding posture. At asymmetric sliding one midleg creating supporting force, N_a , supports the anterior side so n = 1, while during symmetry sliding two fore legs, each creating supporting force, N_a , are supporting the anterior side so n = 2. Hence, the total normal anterior force can be represented as: $N_{aT} = nN_a$. The body is represented in the model as a uniform rod with a length corresponding to the body length of the water strider and oriented parallel to the direction of movement (this is a simplification as water striders do not keep their body axis ideally parallel to the movement direction during asymmetric sliding). The torque balance about the center of the mass of the water strider is represented as:

$$nN_aa = 2N_pb + h(nR_a + 2R_p),$$
(3)

where h is the height of the center of the mass above the undisturbed water surface, a and b are the horizontal distances along the axis parallel to the moving direction from the center of the mass to the center of the wetted anterior supporting leg(s) and of the wetted posterior supporting leg(s), respectively. The values of a and b are calculated based on the leg segments' lengths, the body length, the leg attachment positions on the body, and the empirically measured angles at joints between leg segments (Fig. 3-S14); R_a and R_p are the resistance forces on each of the anterior supporting legs and on each of the two posterior supporting legs, respectively, and they contribute to the total resistance ($R_T = nR_a + 2R_p$).

If predicted N_a is larger than the maximal surface tension force produced by anterior legs (either two forelegs in symmetrical sliding or one midleg in asymmetrical sliding), the water strider cannot float on the surface. We use the theoretical model to determine the conditions for floating on the surface (i.e., sliding without surface breaking) during symmetric and asymmetric sliding, and to calculate the resistance values for water strider's legs that interact with water (red marked wetted legs in Fig. 3-1A, B, C) for various sliding velocities, body mass, and leg geometries. The theoretical model reasonably well simulates the empirically observed trajectories (Fig. 3-S3).

To check if the model imitates the behavior of water striders in a reasonable manner we numerically calculated the displacement, x, of a sliding water strider (from the equation of motion, $m\ddot{x} = -nR_a - 2R_p$, which expresses the effect of resistance on the displacement) based on empirically derived variables from two video clips, one for the *G. gigas* and one for the *A. paludum*, (Fig. 3-S3). The comparison confirmed that the theoretical model imitates reasonably well the real striding by the larger (Fig. 3-S3A) as well as the smaller (Fig. 3-S3B) water striders. We also confirmed that the shapes of the theoretically calculated curves of resistance fit reasonably well, considering the unavoidable scattering of empirical data, to the shapes of splines obtained from the generalized additive model analysis of empirical evaluations of resistance based on observed decelerations during sliding by *A. paludum* extracted from video clips (Fig. 3-S12).

Additional mathematical explanations for wave drag

(based on a large extent on Raphaël & De Gennes, 1996)-reference nr 45 in the main text

- Velocity potential

The velocity potential of the liquid is determined by solving Laplace's equation:

 $\nabla^2 \varphi = 0$

with the boundary condition, $\partial \varphi / \partial z \to 0$ for $z \to -\infty$. The formula of the velocity potential is

$$\varphi = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \frac{1}{4\pi^2} B(k_x, k_y) e^{i(k_x(x+Ut)+k_yy)} e^{kz} dk_x dk_y$$

where $k = (k_x^2 + k_y^2)^{1/2}$ and $B(k_x, k_y)$ is the constant. The coordinates at t = 0 are represented in figure 3-1D.

The vertical displacement of liquid surface, ζ , is obtained by $\partial \zeta / \partial t = (\partial \varphi / \partial z)_{z=0}$. The Fourier transform of the vertical displacement of liquid surface, ζ , is $\hat{\zeta}$ and satisfies the following equation:

$$\zeta(x, y, t) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \frac{1}{4\pi^2} \hat{\zeta}(k_x, k_y) e^{i(k_x(x+Ut)+k_yy)} \mathrm{d}k_x \mathrm{d}k_y$$

Therefore the velocity potential is represented as

$$\varphi = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \frac{1}{4\pi^2} \frac{ik_x U}{k} \,\hat{\zeta}(k_x, k_y) e^{i(k_x(x+Ut)+k_y y)} e^{kz} \mathrm{d}k_x \mathrm{d}k_y \tag{S1}$$

The relationship of $\hat{\zeta}$ and \hat{P} is determined from the Navier-Stokes equation and equation (S1):

$$\left[\rho g k + \sigma k^3 + \rho (2\nu k^2 - ik_x U)^2 - 4\rho \nu^2 k^3 \sqrt{k^2 - \frac{ik_x U}{\nu}}\right] \hat{\zeta} = -k\hat{P}$$
(S2)

where \hat{P} is the Fourier transform of the external pressure, $P(x, y) = N(H(x) - H(x - L))\delta(y)/L$ so $\hat{P} = N(1 - e^{-iLk_x})/ik_xL$.

- Wave drag

The wave drag is the total pressure per unit area of the liquid surface in the x-direction:

$$F_{w} = -\int_{-\infty}^{\infty} \int_{-\infty}^{\infty} P(x, y) \left(\frac{d}{dx}\zeta(x, y)\right) dx dy = -\int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \frac{ik_{x}\hat{\zeta}\hat{P}}{4\pi^{2}} dk_{x} dk_{y}$$
(S3)

From equation (S2) and (S3) we obtain the wave drag:

$$F_{w} = \Re\left\{\int_{-\infty}^{\infty}\int_{-\infty}^{\infty}\frac{i\hat{P}^{2}k_{x}k}{4\pi^{2}\xi}\mathrm{d}k_{x}\mathrm{d}k_{y}\right\} = \frac{N^{2}}{4\pi^{2}L^{2}}\Re\left\{\int_{-\infty}^{\infty}\int_{-\infty}^{\infty}\frac{i\left|\hat{\Psi}\right|^{2}k_{x}k}{\xi}\mathrm{d}k_{x}\mathrm{d}k_{y}\right\}$$

Re-distribution of leg geometry to create virtual morphologies of theoretical water strider species

We created 10 virtual data sets on the basis of the 5 empirically measured populations according to the following procedure. To create a virtual population of water striders with the distribution of body mass, and the total leg lengths observed in the sample of species A with the range and distribution of the "leg geometries" measured in the sample of species B, we redistributed the empirically measured total leg length of each of n_A individuals of species A (keeping wetted leg diameter of species A) into the lengths of femur, tibia and tarsus of forelegs, midlegs and hindlegs according to the length proportions observed in each of n_B individuals of species B). Hence, the virtual population comprised a total of $n_A n_B$ individuals. This process was designed to result in a conservatively wide range of estimated virtual morphologies in order to take into account all possible virtual combinations and to focus on the major differences among virtual species (differences observed even though the

virtual species include extreme morphological combinations). However, for the real water striders, we only used the morphological combinations observed in nature, i.e., we did not redistribute leg lengths of one individual of species *A* into proportions observed in the remaining individuals of species *A*. In this way, we intended to consider morphological variability that actually represents the reality observed in the real water striders.

Numerical calculations

To create Fig. 3-3A-E that maps each of the 30 different sets of water striders (either real or virtual one) representing different combinations of body mass, leg geometry and locomotion onto the phase diagram determining conditions for floating, for each individual (real or virtual) characterized by specific fixed body mass and leg morphology (Fig. 3-S14), including the value of $2(L_a + D)$ that comprises the horizontal axis of Fig. 3-3A-E, we determined the minimal and the maximal value of the total anterior normal force required to support the frontal part of body during sliding on the surface (N_{aT} , on the vertical axis in Fig. 3-3A-E) based on the combination of minimal and maximal values of the following four variables that affect the normal forces (N_a , N_p) and resistance forces (R_a , R_p) in the model (in the system of equations presented in *Model description* section):

- a horizontal distance from the body center to the center of anterior wetted leg(s) (Fig. 3-1B, Fig. 3-S14)
- b horizontal distance from the body center to the center of posterior wetted legs (Fig. 3-1B, Fig. 3-S14)
- h body height above the water surface (Fig. 3-1C)
- U body velocity.

In a similar manner, we determined for each individual the minimal and maximal value of total resistance R_T during sliding (if sliding is feasible) as a function of body velocity, *U*. The minimal and maximal values of total resistance depended on the combinations of the maximal and minimal values (for each individual) of the three remaining variables; *a*, *b*, *h*. (and the set of fixed variables for each individual such as body mass and leg morphology).

The maximal and minimal values of the total normal force (N_{aT}) for each individual were marked in the phase diagram leading to an outline in Fig. 3-3A-E for each of the 30 different sets of water striders representing different combinations of body size class, leg geometry and locomotion. The outlines of predicted total resistance (R_T) in Fig. 3-3F-J as a function of U were obtained in a similar way. We also calculated R_T values by using average leg morphology for each set of water striders (of given body size class, leg geometry, and locomotion mode) combined with the mid-range values of a, b, and h, (solid or dashed lines in Fig. 3-3F-J).

For each individual (real or virtual), the ranges of a and b were calculated by using the body size and leg morphology (fixed for an individual), and the minimal and maximal values of the empirically observed values of two angles (Fig. 3-S14):

 θ_a – angle between femur and tibia; tibia and tarsus (both angles were assumed the same) of the foreleg (in symmetrical sliding it is anterior leg - hence subscript *a*). The angle was measured in the plane common for all three sections of a leg (femur, tibia, tarsus). It was determined from photographs and videos to range from ~90 to ~135 degrees for all 5 size classes of water striders. These two extreme values were used to calculate the minimal and maximal values of *a* for each individual.

 θ_p – angle between body axis and femur; femur and posterior wetted leg (both angles were assumed the same) comprising tibia and tarsus assumed to form one straight section of hindlegs. The angle was measured in the plane common for all three sections of a leg (femur, tibia, tarsus). It was determined from photographs and videos to range from ~30 to ~60 degrees for all 5 size classes of water striders. These two extreme values were used to calculate the minimal and maximal values of *b* for each individual.

The values of a and b were calculated by assuming that a midleg is extended forward (femur parallel to body axis/movement direction) with the wetted midleg parallel to body axis and movement direction, and that wetted hindlegs are also parallel to the body/movement axis during asymmetric sliding (left), and by assuming that tarsi and femora of forelegs, as well as hind legs' wetted parts are always parallel to the movement axis during symmetric sliding (Fig. 3-S14).

We used the following formulas to calculate a and b.

In asymmetric sliding:

$$a = L_{mw}/2 + L_{mf} + Bl/2 - D_{hm}$$
$$b = L_{hw}/2 + L_{hf}\cos(\theta_p) + Bl/2 - D_{hh}$$

In symmetric sliding:

$$a = L_{fw}/2 + L_{ft}\cos(\theta_a) + L_{ff} + Bl/2 - D_{hf}$$
$$b = L_{hw}/2 + L_{hf}\cos(\theta_p) + Bl/2 - D_{hh}$$

We estimated the maximal and minimal values of h and U by observations and measurements from video clips and photographs of each species. The body height ranges were determined as 3-10 mm, 3-7 mm, 1-3 mm, and 1-3 mm for *G. gigas*, *P. tigrina*, *A. paludum*, and *G. latiabdominis*, respectively. The body velocity ranges were determined as 0-1.5 m/s, 0-1.3 m/s, 0-1.2 m/s, and 0-1.0 m/s for *G. gigas*, *P. tigrina*, *A. paludum*, and *G. latiabdominis*, respectively. Wetted leg diameter, *D*, was empirically derived by using average of the five wetted leg diameters for each size category. Each wetted leg diameter of individuals was calculated by the average of the diameter of the center of wetted foreleg (tarsus), wetted midleg (tibia+tarsus), and wetted hindleg (tibia+tarsus). The wetted leg diameters, *D*, were determined as 0.266 (±0.032), 0.221 (±0.006), 0.163 (±0.017), 0.127 (±0.009), 0.077 (±0.009) mm for *G. gigas* male, *G. gigas* female, *P. tigrina*, *A. paludum*, and *G. latiabdominis*, respectively (average ±s.d.).



Fig. 3-S1. Distribution of wetted leg geometries among Gerridae

(A) - 3D scattergram of proportions of wetted forelegs, midlegs and hindlegs (3 axes in the plot) in the total wetted leg length with data points color coded to highlight the differences among species in the proportion of wetted forelegs; (B) - 3D scattergram similar to (A) with data points marking different subfamilies (according to Matsuda 1960) and family-typical body size of a water strider (families with the typically larger size species are marked with color-coded unfilled circles and families with the typically smaller size species are marked with color-coded dots. Figure is based on Table 16 in Matsuda 1960. The concept of the "wetted leg geometry" is crucial in our theoretical model. The term refers to the relative proportions of wetted forelegs, wetted midlegs and wetted hindlegs in the total length of the wetted legs. The figure suggests that we can classify species into at least three types of "wetted leg geometry": the "intermediate-foreleg" or "standard" observed in the frequently studied small and mid-size genera *Gerris* and *Aquarius*, (wetted forelegs comprise from ~4 to ~8% of the total wetted legs length), the "long-foreleg geometry" known in the marine small water striders, Halobatinae, and in the medium and large water striders from the subfamily Ptilomerinae (wetted forelegs comprise less than ~4% of the total wetted legs length), and the "short-foreleg geometry" (wetted forelegs comprise less than ~4% of the total wetted legs length) well documented in at least one large species, *G. gigas*, (Tseng and Rowe 1999), and present in some larger species of Gerrinae.



Fig. 3-S2. Empirical validation of the assumptions about velocity that leads to capillary gravity waves that increase resistance

The minimum critical theoretical velocity resulting in capillary gravity waves, c, is 0.231 m/s (marked with the red doted horizontal line). Comparison of water strider *A. paludum* initial sliding velocity among sliding without visible waves, sliding with incomplete/weak waves and sliding with clearly visible waves shows that waves start being noticeable around the velocities similar to the critical velocity.



Fig. 3-S3. Validation of the theoretical model accurateness in imitating sliding of larger and smaller species of Gerridae

(A) Empirical displacement of the larger species (*G. gigas*) sliding asymmetrically. (B) Empirical displacement of the smaller species (*A. paludum*) sliding symmetrically. The red solid line corresponds to theoretical predictions and the circles correspond to empirical data extracted from two movie clips of sliding.



Fig. 3-S4. Relationship between the total wetted length and body mass of the six studied species

Body mass and total wetted length are marked for *Gigantometra gigas* (blue circles), *P. tigrina* (red diamonds), *Aquarius paludum* (green triangles), *Gerris gracilicornis* (plus signs), *G. latiabdominis* (black squares), and *A. remigis* (cross-marks) in normal (A) and log scale (B).



Fig. 3-S5. Three examples of digitized thrusting phase of a stride

Strides to explain the method of extracting the thrusting phase acceleration as a slope of a regression line fitted to the data on body velocity versus time for each stride, and five examples of strides for which the net thrust force (based on the acceleration and body mass: m * a) and the theoretically predicted resistance force are depicted. (A) – a thrusting phase of a symmetric stride of *Aquarius paludum*; (B) – a thrusting phase of a symmetric stride of *Aquarius paludum*; (B) – a thrusting phase of a symmetric stride of *Ptilomera tigrina*; (C) – a thrusting phase of an asymmetric stride of *Gigantometra gigas* male. These estimated accelerations for each stride of an individual of known body mass were used to calculate the thrust net force (F = ma) in each stride. Thrusting phase is defined as the duration from the first frame with leg pushing backwards till the frame with maximum body velocity; it varies among species as the range of velocity (m/s) on x-axis in d-h show. (D-H) – examples of force profiles during thrust phase where the empirically evaluated thrust net force (blue dots) and the theoretically predicted resistance (red dots) force (based on empirically measured body velocity profile) are displayed in single strides of *Gerris latiabdominis* (d; symmetrical stride), *A. paludum* (symmetrical stride in e and asymmetrical stride in f), *P. tigrina* (G) and *G. gigas* (H).



Fig. 3-S6. Empirical net force and theoretical resistance for each species

Comparison of empirically derived (see Fig. **3**-S5) the net thrust force (A), which directly contributes to the momentum change of insect body during thrust phase of the striding, the theoretically predicted resistance force (B), which has to be countered by the insect using the thrust. (C) The proportion of the resistance in the total thrust produced by an insect (resistance / (net force +resistance)) in the chosen sets of strides in the four study species: *Gerris latiabdominis* striding symmetrically (n=8), *Aquarius paludum* striding symmetrically (n=6), *A. paludum* striding asymmetrically (n=10), *Ptilomera tigrina* striding symmetrically (n=8) and *Gigantometra gigas* striding asymmetrically (n=5). The average values for each species are marked as horizontal lines in (C). Red circles indicate striding at the body velocities that are higher than the theoretical body velocity threshold associated with surface waves (0.2313 m/s).



Fig. 3-S7. Symmetric sliding and leaping of G. latiabdominis

Each one of the examples of symmetric sliding (A) and leaping (B) of *G. latiabdominis* Individual 1 (19.1 mg). Orange, green, and gray shading represent leaping, symmetric sliding, and resting phase, respectively.



Fig. 3-S8. Symmetric/asymmetric sliding and leaping of A. paludum

Each one of the examples of leaping (A), asymmetric sliding (B), and symmetric sliding (C) of *A. paludum* Individual 4093 (18 mg). Orange, blue, and green shading represent leaping, asymmetric sliding, and symmetric sliding, respectively.



Fig. 3-S9. Series of striding of *P. tigrina* in the field

Velocity of body center of *P. tigrina* relative to the water surface by considering water flow speed in the series of striding locomotion.



Fig. 3-S10. Series of striding of G. gigas in the field

Velocity of body center of *G. gigas* relative to the water surface by considering water flow speed in the series of striding locomotion. Blue shading represents asymmetric sliding.



Fig. 3-S11. Box-and-whiskers plot of initial striding velocity five size classes corresponding to five species/sex classes of study subjects

G. latiabdominis (n=13), *A. paludum* (n=236), *P. tigrina* (n=12), *G. gigas* females (n=23), and *G. gigas* males (n=27). For *G. latiabdominis*, velocities of symmetrical sliding (n=9) and leaping (n=4) are pooled. For *A. paludum*, velocities of asymmetrical sliding (n=136), symmetrical sliding (n=68), and leaping (n=32) are pooled. Initial velocity was chosen as a peak velocity before sliding in *G. latiabdominis*, *P. tigrina*, and *G. gigas* (See methods section for *A. paludum*).



Fig. 3-S12. Resistance for *A. paludum*'s sliding from mathematical model and from empirical calculations. Results of the model calculation and empirical analysis for symmetric (A) and asymmetric (B) sliding of *A. paludum*. Theoretical calculations from mathematical model are marked as green line and shadings. Black squares represent empirical data points, the generalized additive models from those data are marked as gray solid line, and 97.5% centiles are also marked with dashed lines. Resistance was calculated from the estimated deceleration (Table 3-S6 on the next two pages) and body mass for each stride. The theoretical predictions in this figure were also used in Fig. 3-5D of the main text.


Fig. 3-S13. Relationship between the body length and the "wetted leg geometry" Relationship between the body length and the proportion of wetted forelegs (A), wetted midlegs (B) and wetted hindlegs (C) in total wetted legs length. The gray shaded area in (A) helps visualizing that with increasing body size the water striders adopt one of two "wetted leg geometries", either "long-foreleg" or "short-foreleg geometry". The species are represented by subfamilies: Gerrinae (blue circles), Ptilomerinae (orange circles), Halobatinae (purple circles), Rhagadotarsinae (gray circles), and Trepobatinae (brown circles). The data re from Table 16 in Matsuda 1969, and the subfamilies follow Matsuda 1960, which may be not entirely consistent with the more modern assignments of genera into subfamilies. Additionally, this is phylogenetically un-corrected relationship, and therefore it does not directly represent evolutionary processes shaping the evolutionary changes of leg morphology as a function of evolutionary changes of body size. The equations fitted to the data points for each family separately have the power form following the convention for allometric equations. However, we used body length because of the absence of data for body mass, absence of body width, height or diameter data, and absence of body length – body mass formulas for water striders over such a large body size range. We expect that from among the possible linear measurements of body (width, height, length) the length is relatively more correlated with the body mass (albeit not necessarily in a linear fashion) than are body width or height as they are relatively small and differ among species relatively less than the body length. We decided not to use body $length^3$ (a possible alternative used occasionally in allometry) because of the elongated shape of the water

striders. For the overview of leg geometries in Gerridae of various subfamilies and various body sizes we used data from Table 16 in Matsuda (1960; see also Fig. 3-6 and Fig. 3-S13 based on the same data). We converted the Matsuda's units to metric measurement units (mm) using the information provided in Matsuda (1960) after one correction that was necessary to circumvent Matsuda's apparent mistake. On page 32 Matsuda states the following rules: "In table 16, 82 units are equal to 10 mm. For those values with asterisks, 173.7 units are equal to 10 mm.". However, we concluded that the correct rule is "10 mm is equal to the 173.7 units of measurements presented in Table 16" for all species listed there (regardless of whether they are marked with asterisk or not) because only this rule gives results that agree with body lengths of several species known to us, and with the body length of G. gigas listed by Matsuda in a different part of his book. Matsuda mentions on page 12 that that body length of G. gigas is 3.19 cm ("Gigantometra gigas (China), male. Length of body: 31.9 mm."; Fig. 3-3), which is consistent with the calculation using the "173.7 units" rule but not the" 82 units rule" (predicted body length of G. gigas: 67.08 mm), which according to Matsuda's erroneous advice is supposed to be applied to G. gigas as it is listed without an asterisk in Table 16. When we tried to convert body length by using the "173.7 units rule" to species without asterisks then the body lengths matched reasonably well with our observed data (e.g., ~31 mm for G. gigas, ~12 mm for A. paludum, ~13-16 mm for Ptilomerinae). Therefore, we used the "173.7 units rule" for data presented in Figures 3-6 and 3-S13.



Fig. 3-S14. Graphical schematics of the morphological variables used in calculations in the model.

See Supplementary Methods for detailed explanations of how the variables were used to calculate *a* and *b*.

Table 3-S1. Explanations of the symbols in the model

The lists of symbols used in the mathematical model. The symbols below black solid line used only in supplementary materials. Values used in the model are shown for variables that do not change values among different simulations. The symbols are listed in the order in which they appear in the text.

Explanations of the sym	bols in the model
R	Resistance force on a leg
F_h	Hydrodynamic drag on a leg
F_{w}	Wave drag on a leg
F_s	Surface tension force on a leg
ρ	Density of water, 997 kg/m ³
U	Velocity of sliding object on the water surface
D	Diameter of a wetted leg
L	Length of a wetted leg
$A = \pi DL/2$	Wetted area assumed as a half of curved surface of a cylinder with a length, L, and diameter, D.
ν	Kinematic viscosity of water, 1.003 mm ² /s
$C_D = 1.328 \sqrt{\nu/(UL)}$	Drag coefficient on the flat plate
σ	Surface tension coefficient of water, 0.0728 N/m
g .	Gravitational acceleration, 9.8 m/s ²
$c = (4g\sigma/\rho)^{1/4}$	The theoretical minimum critical velocity of a floating object on the water surface to produces capillary-gravity
Ν	Normal force on a leg from the water
$k = \left(k_x^2 + k_y^2\right)^{1/2}$	Wave number
Ψ	Shape of wetted area; the shape of the wetted leg assumed as a line with
	length L with the longitudinal movement
$\widehat{\Psi} = (1 - e^{-iLk_x})/ik_x$	Fourier transform of Ψ
H(x)	The Heaviside function
m	Mass of a water strider
n	The number of the anterior supporting legs
N _a	Normal force (perpendicular to the water surface) on an anterior leg: a wetted foreleg for symmetrical sliding; a wetted midleg for asymmetrical
N	sliding
N_p	Normal force on a posterior leg, i.e., on the wetted hindleg
N _{aT}	Normal anterior force on all anterior legs (total normal anterior force)
R _a	Resistance force on an anterior leg: a wetted foreleg for symmetrical
ת	sliding; a wetted midleg for asymmetrical sliding.
R_p	Resistance force on a posterior leg, i.e., on the wetted hindleg
n	surface
а	Horizontal distance in the parallel axis to the moving direction from the
	center of the mass to the center of the wetted anterior supporting leg
b	Horizontal distance in the parallel axis to the moving direction from the center of the mass to the center of the wetted posterior supporting leg
R_T	Resistance on all legs (total resistance, $nR_a + 2R_p$)
x	Displacement of a water strider by sliding
ÿ	Acceleration/deceleration of a water strider
L_a	A wetted length of all anterior leg(s) (total anterior wetted length)
L_{fw}	Length of foreleg wetted length (tarsus)

L_{ft}	Length of foreleg tibia
L_{ff}	Length of foreleg femur
L_{mw}	Length of midleg wetted length (tibia+tarsus)
L_{mf}	Length of midleg femur
L_{hw}	Length of hindleg wetted length (tibia+tarsus)
L_{hf}	Length of hindleg femur
D_{hf}	Distance from the head tip to the foreleg attachment
D_{hm}	Distance from the head tip to the midleg attachment
D_{hh}	Distance from the end tip of the abdomen to the hindleg attachment
Bl	Body length
$ heta_a$	Angle between femur and tibia; tibia and tarsus (both angles were
	assumed the same) of the foreleg
$ heta_p$	Angle between body axis and femur; femur and posterior wetted leg (both
	angles were assumed the same) comprising tibia and tarsus assumed to
	form one straight section of hindleg
arphi	Velocity potential
$B(k_x, k_y)$	Amplitude of the waves of the disturbance
t	Time
ζ	Vertical displacement of liquid surface
Р	External pressure
δ	The Dirac δ function

Table 3-S2. Initial velocity for locomotion type of G. latiabdominis

Individual	Video	Locomotion	Initial velocity (m/s)	
1	C0030	Sliding	0.282334	
2	C0040	Sliding	0.373578	
3	C0053	Sliding	0.209729	
5	C0069	Sliding	0.191976	
1	C0035	Leaping	0.574271	
2	C0042	Leaping	0.596126	
3	C0057	Leaping	0.506352	
5	C0073	Leaping	0.390899	

Results The initial velocities were significantly different between sliding and leaping of *G. latiabdominis* (Wilcoxon Signed-Rank Test, p-value = 0.029).

Table 3-S3. Linear mixed model of initial velocity and sliding type of A. paludum

Results of the linear mixed model: Velocity ~ (Sliding type) + (1 | Individual) + (1 | Video: Individual), No. of observations: 236, No. of Individuals: 6, No. of random factor (Video: Individual) groups: 100. The reference passive phase type is Asymmetry. Results are shown in Fig. 3-5A.

	Estimate	df	t value	Pr(> t)
(Intercept)	0.43565	5.40296	10.495	8.51e-05
Leaping	0.24132	188.12922	10.857	< 2e-16
Symmetry	-0.07078	159.28365	-3.525	5.54e-04

Table 3-S4. Generalized mixed model of sliding distance and sliding type of A. paludum

Results of the generalized mixed model: Sliding distance ~ (Sliding type) + (1 | Individual) + (1 | Video:Individual), Family: ("Inverse Gamma"), No. of observations: 228, No. of Individuals: 6, No. of random factor (Video:Individual) groups: 100, Degrees of Freedom for the fit: 48.63265, Residual Deg. of Freedom: 179.3673. The reference passive phase type is Leaping. $E(distance) = sqrt(exp(\sigma^2)) * exp(\mu)$. Results are shown in Fig. 3-5B.

cot +			D (L)
μ coefficient	Estimate	t value	Pr(> t)
(Intercept)	2.72043	57.657	< 2e-16
· • • ·			
Asymmetry	0 23134	3 665	3 25e-04
<i>i</i> isymmetry	0.25154	5.005	5.250 04
G (0.20200	5 702	4 77 00
Symmetry	-0.38289	-5.703	4.//e-08
σ coefficient	Estimate	t value	Pr(> t)
(Intercept)	-0.91275	-20	< 2e-16
(· · · · I · /			

Table 3-S5. Generalized mixed model of sliding duration and sliding type of A. paludum Results of thegeneralized mixed model: Sliding duration ~ (Sliding type) + (1 | Individual) + (1 | Video:Individual), Family:("Box-Cox-Cole-Green"), No. of observations: 228, No. of Individuals: 6, No. of random factor(Video:Individual) groups: 100, Degrees of Freedom for the fit: 52.71993, Residual Deg. of Freedom: 175.2801.The reference passive phase type is Symmetry. Results are shown in Fig. 3-5C.

	Estimate	t value	Pr(> t)	
(Intercept)	43.907	26.799	< 2e-16	
Asymmetry	37.615	11.913	< 2e-16	
Leaping	-15.615	-9.218	< 2e-16	

Table 3-S6. Deceleration data of the sliding of A. paludum

	Mass		Striding	Initial	Sliding/leaping	Duration	Last
Individual	(mg)	Video	type	velocity (m/s)	distance (mm)	(ms)	velocity (m/s)
4091	53	C0082	Asy.	0.283	6.432	45.879	0.077
4091	53	C0076	Asy.	0.133	7.792	60.477	0.133
4091	53	C0082	Asy.	0.148	4.812	32.324	0.139
4193	47	C0176	Asy.	0.257	14.146	66.733	0.143
4193	47	C0162	Asy.	0.184	10.036	64.648	0.149
4193	47	C0158	Asy.	0.309	16.721	66.733	0.167
4193	47	C0173	Asy.	0.201	12.991	72.99	0.176
4192	32	C0144	Asy.	0.256	11.019	45.879	0.179
4191	40	C0114	Asy.	0.227	12.461	56.306	0.185
4191	40	C0113	Asy.	0.291	13.867	54.221	0.191
4193	47	C0158	Asy.	0.256	16.158	71.947	0.192
4192	32	C0144	Asy.	0.244	10.768	44.837	0.196
4191	40	C0114	Asy.	0.312	15.115	55.264	0.197
4193	47	C0153	Asy.	0.413	23.942	68.819	0.211
4091	53	C0080	Asy.	0.265	16.433	58.392	0.213
4192	32	C0144	Asy.	0.361	18.669	65.691	0.225
4193	47	C0149	Asy.	0.241	17.414	68.819	0.227
4091	53	C0082	Asy.	0.379	22.887	63.605	0.244
4191	40	C0107	Asy.	0.269	13.093	43.794	0.245
4191	40	C0107	Asy.	0.403	25.87	71.947	0.256
4191	40	C0108	Asy.	0.308	13.699	39.623	0.261
4091	53	C0081	Asy.	0.353	18.556	56.306	0.264
4191	40	C0105	Asy.	0.412	19.452	46.922	0.266
4091	53	C0078	Asy.	0.44	27.142	70.904	0.278
4192	32	C0142	Asy.	0.47	28.366	60.477	0.287
4191	40	C0106	Asy.	0.378	17.043	43.794	0.288
4193	47	C0155	Asy.	0.569	30.39	67.776	0.3
4192	32	C0134	Asy.	0.397	15.897	33.367	0.303
4191	40	C0111	Asy.	0.399	25.955	62.563	0.307
4091	53	C0076	Asy.	0.365	23.597	57.349	0.313
4093	18	C0099	Asy.	0.496	30.037	64.648	0.316
4193	47	C0153	Asy.	0.488	28.948	64.648	0.316
4192	32	C0123	Asy.	0.517	26.537	52.135	0.331
4191	40	C0121	Asy.	0.439	23.082	47.965	0.348
4091	53	C0078	Asy.	0.483	23.584	46.922	0.361
4092	47	C0092	Asy.	0.467	26.894	54.221	0.375

Sliding events that have enough passive phase duration (50-80 ms) to calculate deceleration were chosen.

4092	47	C0093	Asy.	0.433	25.654	59.434	0.387
4093	18	C0099	Asy.	0.625	34.342	59.434	0.418
4193	47	C0172	Sym.	0.196	8.823	69.862	0.061
4193	47	C0148	Sym.	0.138	5.533	55.264	0.082
4193	47	C0158	Sym.	0.151	7.782	51.093	0.123
4193	47	C0162	Sym.	0.177	9.598	47.965	0.129
4191	40	C0113	Sym.	0.207	9.132	49.007	0.134
4192	32	C0134	Sym.	0.318	13.373	57.349	0.136
4193	47	C0162	Sym.	0.126	8.748	52.135	0.136
4193	47	C0151	Sym.	0.164	9.241	54.221	0.15
4193	47	C0162	Sym.	0.197	8.095	38.58	0.155
4193	47	C0151	Sym.	0.152	8.343	45.879	0.157
4091	53	C0079	Sym.	0.145	8.076	39.623	0.157
4193	47	C0149	Sym.	0.317	14.846	58.392	0.158
4192	32	C0144	Sym.	0.188	10.622	52.135	0.16
4192	32	C0135	Sym.	0.253	16.521	66.733	0.161
4093	18	C0095	Sym.	0.249	14.698	69.862	0.163
4193	47	C0156	Sym.	0.266	14.017	54.221	0.171
4193	47	C0155	Sym.	0.208	13.218	64.648	0.174
4191	40	C0120	Sym.	0.211	8.902	34.409	0.175
4091	53	C0077	Sym.	0.162	7.8	36.495	0.192
4191	40	C0116	Sym.	0.184	8.439	32.324	0.207
4193	47	C0149	Sym.	0.294	16.8	64.648	0.215
4191	40	C0121	Sym.	0.485	13.711	31.281	0.227
4191	40	C0121	Sym.	0.403	13.998	31.281	0.246
4192	32	C0126	Sym.	0.38	18.202	42.751	0.251
4193	47	C0178	Sym.	0.336	16.381	47.965	0.27
4193	47	C0173	Sym.	0.519	29.176	66.733	0.274
4193	47	C0178	Sym.	0.477	24.928	56.306	0.294
4192	32	C0139	Sym.	0.424	19.841	42.751	0.309
4093	18	C0101	Sym.	0.488	25.563	47.965	0.348
4193	47	C0176	Sym.	0.505	26.002	52.135	0.359
4193	47	C0170	Sym.	0.632	35.399	59.434	0.422
4093	18	C0100	Sym.	0.521	24.456	37.538	0.456
4092	47	C0092	Sym.	0.589	30.869	45.879	0.483
4193	47	C0164	Sym.	0.706	35.184	45.879	0.536

Chapter 4. Functional micro-morphology of setae on legs of the heaviest semi-aquatic insect, the giant water strider (*Gigantometra gigas*)

Abstract

The cuticular protuberances (hairs/setae) of arthropods, including Gerridae, are generally classified into macrohairs (macrotrichia) and micro-hairs (microtrichia, minute cuticular outgrowths). Typically, these structures are regarded for two general anti-wetting functions: maintaining the air layer in submerged animals ('water protection') and preventing permanent wetting of surfaces that are not under water ('water repellency'). Based on the studies of small/medium water strider species, it was hypothesized that morphological characteristics of special hair types play a role in three functions: support on the water surface, rowing/jumping locomotion, and cleaning/grooming of legs' hairs. It has also been reported that hindleg setae differ from the setae on midlegs without further studies on the functional significance of the differences. Gigantometra gigas is the heaviest Gerridae species and therefore its adaptations to live on the water surface are expected to be pronounced. We investigated the giant water strider's leg morphology using XRM, SEM, and optical micro photography, and we hypothesized about the functions of various hair types and their distribution on legs based on observations of leg use by animals in the natural habitat and leg-water interactions observed in the laboratory conditions. We described 12 types of hair structures on the leg of G. gigas: a comb, stump setae, spade-shaped, spine-like, haylike, grass-blade-like setae, three types of macrosetae, microsetae, leaf-shaped, and thorn-shaped setae. We divided them into non-water interacting and water-interacting functions, and we proposed their hypothetical functions based on our own data and the literature. The comb, stump setae, and spade-shaped setae are used for cleaning, without interacting with water. The spine-like setae are also used for cleaning, but with the hydrophobic property. The hay-like, grass-blade-like, and spoon-like setae may be used to pull the water surface up, which occurs at the end of the leg. The long macrosetae would improve jumping and striding locomotion. The microsetae can play the role of extra water-proofing in addition to the macrosetae. In the hindleg, leafshaped setae flanked by rows of thick thorn-like setae seem to increase the asymmetric striding thrust, help in the rudder function of the hindlegs, and reduce sliding resistance on the surface. Our results provide a detailed description and morphological differences of G. gigas from a typical-sized water strider. The results show that special hair structures of G. gigas are an adaptation to their heavy body and different types of locomotion which is also related to their size. We also propose the new hypothesis of pulling-up the water surface, which can be widespread in the locomotive mechanisms of large-sized water striders, and maybe even across Gerridae of various sizes. We expect G. gigas can be used as a model species for bio-inspired water strider robots and hydrophobic surfaces since their large size matches the size of many water walking robots.

Keywords: *Gigantometra gigas*, Water strider, Setae, Hair, Anti-wetting, Nano-grooves, Locomotion, Functional morphology.

4.1. Introduction

The hydrophobic surfaces in nature have drawn attention of biologists and engineers. For example, micro/nanostructures of hydrophobic leaves of lotus^{56,57}, anti-wetting wings of dragonflies, mayflies⁵⁸, butterflies⁵⁹, and cicadas^{60,61} have been studied because they exemplify adaptive traits and provide bioinspiration for new technologies^{62–64}. About 4,500 species in five (out of eight) infraorders of the order Heteroptera live in aquatic/semiaquatic habitats¹⁶². This is arguably the suborder that has one of the best-studied anti-wetting surfaces. It has been long known that the structural properties of hairs provide insects with protection against water¹⁶³. Water striders, *Gerridae*, is a typical taxon of semiaquatic insects that serve as study subjects in an examination of various types of hairs in semi-aquatic insects that live on the water surface.

The cuticular protuberances (hairs/setae) present on the body cuticle of arthropods, including water striders, are classified into macro-hairs (or macrotrichia) and micro-hairs (or microtrichia: minute cuticular outgrowths) by Andersen⁶⁶. They correspond to the classes of "setae" and "microtrichia" of Richards and Richards⁶⁵, respectively. Historically, these structures were regarded to have two anti-wetting functions: 'water protection' and 'water repellency'.

"<u>Water protecting</u>" refers to the general function of maintaining the air layer around the body and preventing water penetration under equilibrial pressure in the body of a liquid (water) in insects immersed in water. For water protecting function, it is favorable if the inclination of hair (the angle between the hair's longitudinal axis and the cuticle surface or leg's longitudinal axis) is small so that the hair along its longitudinal axis is in contact with the water surface, and spaces between hairs are small⁶⁸.

"<u>Water repellency</u>", also called "waterproofing" or 'rainproofing', refers to a general function of shedding liquid droplets from the hair-covered surfaces that are not immersed in water, and it requires stiffness of hairs against lateral (with regard to the hair axis) forces⁶⁹, the small surface area of insect surface-water interface, and large air (air caught among the hairs)-water interface ratio⁷⁰. Hairs, therefore, are needed to be thick or large (to provide stiffness), to have low density (to capture air among them), and to have a perpendicular angle to the cuticle/leg surface (i.e., small inclination to provide small contact area between the hair and the water surface).

These two very general categories of functions can be applied to any part of a water strider's body in any context of life, including their role in leg/water interactions during body support and during locomotion on the water surface. The hairs on water striders' legs are likely to be differentiated to serve at least two specific functions. Firstly, legs support an insect body on the water surface by exploiting the surface tension force created when the legs make dimples (menisci) on the water surface (without breaking it). Secondly, legs produce thrust for locomotion. It includes rowing^{17,28,40–42,164} that exploits forces resulting from interactions between horizontally moving midlegs and the water surface^{16,30,38,46}, and near vertical jumping that in small and medium size water striders occurs without breaking of the water surface³⁴. The support function requires that the ventral segments of the supporting legs are super hydrophobic so that the surface tension is large enough to support the insect's weight without breaking the surface. The importance of using legs for jumping on the water surface without breaking it (in order to exploit surface tension force) is well understood³⁴, and it was imitated in robotic devices³¹.

Based on the aforementioned literature, a "typical" water strider (genus *Gerris* or *Aquarius*) in a stationary situation uses all six legs placed on the water surface to create dimples (menisci) that result in surface tension

force that supports the insect body on the surface. The parts that interact with the water surface in this situation are the hairs on the ventral sides of tibiae and tarsi on hindlegs, the ventral side of distal tibiae and tarsi on midlegs, and the ventral side of tarsi on forelegs. The horizontal backward movements of midlegs on the water surface (without breaking it) create the horizontal forward force (thrust) for the water strider's forward skating^{16,28,30,38,46}. In this case, the hairs on the ventral and posterior surfaces of midleg tibiae and tarsi interact with the water surface, and we considered the possibility that some special microstructural characteristics of those hairs may help in this function, especially when the insect's body is very heavy.

During jumps, the vertical fast downward movements of midlegs against the water surface push the insect body upward. This is well understood and theoretically modeled for jumps without surface breaking commonly observed in small and medium water striders species³⁴. The hairs on the ventral, ventral-posterior, and ventralanterior surfaces of midlegs' tibiae and tarsi are the parts that interact with the water surface creating deep dimples (menisci) that provide force for jumping. We considered the possibility that some special microstructural characteristics and/or distribution of those hairs on the leg surface may help in producing thrust during the upward jumps, and that these features may be especially prominent in the heavy species.

Detailed information on setae (hair) diversity is available in only several classical reports on the "typical" common (Palearctic or Nearctic, where most university centers involved in this type of research are located) water striders from genus Gerris and Aquarius of small and medium size^{40,66,67}, and on small oceanic water striders from genus Halobates^{17,20,165}. These studies revealed that the midleg tarsus and tibia (leg sections that interact with the water surface) of the "typical" water striders (genus Aquarius and Gerris) are covered with diverse hairs (setae). At least five different types of hair types varying in length, diameter, and cross-section shape are present on legs in densities from hundreds (the larger hairs) to several thousand (the smallest hairs) per mm² with asymmetrical distribution consisting of especially diversified hair layers on the ventral side of tibia and tarsi (surfaces that interact with water surface). Andersen⁴⁰ already noted, before fluid-dynamic studies highlighting the importance of nano-grooves for hydrophobicity^{71,75}, that the hair surface consists of small grooves running along each hair. It was also noticed that all these morphological characteristics are less variable on the insect body, and that the special hair types on midlegs may play a role in the two functions of the midleg: support on the water surface and rowing^{40,166–169}. Finally, it has also been noticed that setae on hindlegs which mostly perform the supporting function, differ from the setae on midlegs⁴⁰ which perform both functions: supporting and providing thrust. Apart from these classical descriptive reports on the "typical" common species from genus Aquarius and Gerris, the connections between a specific function and a specific type of hair on legs have not been further explored (to our knowledge).

We expect that the natural selection for unique micro-morphological adaptations to life on the water surface may be especially strong in large water strider species, where these adaptations may be especially prominent. Therefore, they may illustrate the outcomes of natural selection for micro-structural adaptations to locomotion on the water surface, and they may provide further guidance in future studies on the diversity of Gerridae species across a full range of body weights from less than 5 mg in small water strider species^{20,165} to about 500 mg in the largest extant species, the giant water strider (*Gigantometra gigas*²¹). The large species may also serve better as an inspiration for the design of water walking robots because most of those robots are larger than a typical or small water strider^{31,45,116,122,131}. Here, we provide (for the first time to our knowledge) the very detailed descriptions of leg-involving behaviors in the world's largest extant water strider species, *Gigantometra*

gigas, followed by descriptions of setae on the legs and simple experiments to test hypothetical functions of some of the microstructures on their legs. We focus mostly on the possible adaptations to support the heavy body on the water surface and to locomotion (skating and vertical jumps). To hypothesize about specific adaptations to the large body size, we observe interactions between the water surface and legs separated from the body and we compare *G. gigas* to the medium-sized species, *Aquarius paludum*, one of the frequently studied "typical" water striders. The special structures for cleaning and brushing are also described, which are known to be present on water striders' legs.

4.2. Materials and Methods

4.2.1. The terminology used in this chapter

In order to accommodate detailed descriptions, we provide here an overview and classification (terminology) of the types of hairs/bristles/setae found in G. gigas and anatomical terms for indicating axis/direction/side. Firstly, we decided to consistently use the term setae (Latin for "bristles") for 'macro hairs' or 'macrotrichia'66. We found that the water-interacting setae on G. gigas legs can be categorized into four types. We suggest these types of setae as following names; 'macrosetae' for long setae, 'microsetae' for short fine setae (note that it is different from 'micro hairs' or 'microtrichia': minute cuticular outgrowths), 'thorn-shaped setae' for the thickest setae, and 'leaf-shaped setae' on the hindleg. Both macro and microsetae are equivalent to the 'macro-hair layer of long, pointed hairs (macrotrichia)⁶⁶ or 'long, evenly tapered setae⁶⁷, but more differentiated from other Gerridae. Secondly, we consistently used proximal, intermediate, and distal to indicate the longitudinal location of a leg. Therefore, the nine leg segments in order from the body to the leg tip are as follows: proximal femur, intermediate femur, distal femur, proximal tibia, intermediate tibia, distal tibia, proximal tarsus, intermediate tarsus, and distal tarsus. We used ventral and dorsal to indicate the upper and lower surfaces of a leg (and body). The terms anterior and posterior were used consistently to represent anatomy rather than behavioral use of the legs (Fig. 4-1). Hence, the leg sides directed towards the front of the animal were not always the ones classified as anterior and those facing backward were not always the ones classified as posterior due to the legs' configurations during natural behaviors. Therefore, anterior side of the foreleg and posterior side of the hindleg are the sides that can also be classified as "inward" (towards the animal body axis), and posterior side of the foreleg and anterior side of the hindleg can be classified as "outward" (away from the animal's body axis). Similarly, the dorsal side of foreleg's tibia is actually facing forward, and the ventral side of foreleg's tibia is facing backward (Fig 4-1b). For clarity, we use italic fonts in the figures to indicate segments/sides of legs, and non-italic fonts (non-italic capital in Figures) to indicate the perspective from which SEM camera view the specimen (i.e., this indicates the segment/side of leg that is facing the camera).

4.2.2. Observations of behavior

Behaviors of *Gigantometra gigas* were observed in the natural habitats on a flowing stream in Pu Mat National Park (Vietnam), in 2017 and 2018. Striding, resting, cleaning, and grabbing prey of the individuals were photographed and video-recorded in standard and high-speed videos with 30, 250, 500, and 1000 fps by cameras

(Casio Exilim 1000, Sony RX10 III). The menisci were visualized on the water surface using sunlight, or occasionally using shadows on the natural flat rock bottom. A total of 447 photos and 166 videos were collected, and subsequently carefully analyzed to extract information on how legs are used to support the water strider body on the water surface, and to produce thrust for locomotion (skating on the surface and jumping off the surface). Fast striding and upward jumping were induced by creating a threatening stimulus either above the insect or from below the water surface. Additionally, the interactions of legs with the water were observed in the plexiglass tank $(15 \times 15 \text{ cm}^2)$ situated in the natural habitat next to the stream (Chapter 2 in this thesis)

4.2.3. SEM imaging

The adults of *Gigantometra gigas* were studied in Pu Mat National Park (Vietnam), in 2016 and 2018. Adults of *Aquarius paludum* were collected in Korea in 2016. Specimens for Scanning electron microscopy (SEM) were kept in cold storage (in EtOH, 70%). Legs of five individuals in each species were dried in a vacuum chamber and attached to the aluminum mounts by carbon tape. Samples were coated with gold by a sputter coater (Cressington 108auto, Cressington Scientific Instruments, UK) for 100-200 s. Using Scanning Electron Microscopy (SEM, JSM-6390LV, JEOL, Japan) we obtained images at various magnifications of all nine segments (proximal femur, intermediate femur, distal femur, proximal tibia, intermediate tibia, distal tibia, proximal tarsus, intermediate tarsus, and distal tarsus) for each leg focusing on acquiring mainly the images of the anterior and posterior sides, and additionally also the ventral and dorsal sides.

4.2.4. Measurements of setae on midlegs from SEM images

Macrosetae – Five macrosetae were randomly chosen in an image of each leg segment of five individuals from on each side: ventral, dorsal, and lateral. For dorsal and ventral setae, I used ImageJ version 1.51j8 to measure the angle between the leg central axis and the slope of setae on its inflection point for bent setae or midpoint if there is no inflection point (since inflection points were approximately on midpoints). The basal diameters of the lateral (without differentiation between anterior and posterior side) setae were also measured. The basal diameters of the ventral setae were too difficult to measure due to the high density of the setae. Therefore, no comparison between setae diameters on the ventral and dorsal sides of the legs was conducted. For each leg segment of a leg, we measured the vertical leg diameter at five sites of a segment. We used the segment's average leg diameter to express the absolute values of setae lengths and setae diameters in relative units that control for the body size: we divided each of the absolute values of setae length and setae diameter by the absolute value of the leg diameter of the leg segment at which the setae were located. This relative length and relative diameter were used in comparisons between G. gigas and A. paludum in order to determine if the giant water strider (G. gigas) is equipped with disproportionately longer/thicker setae than the typical medium-size water strider represented by A. paludum. To statistically compare the species and segments with respect to the characteristics of setae, I applied linear mixed models with the individual ID as a random effect using *lme4* package version 1.1-21 in R version 3.6.1. The dependent variables were natural-log-transformed to normalize the model residuals when the residuals' normality was clearly violated without transformation¹⁷⁰).

4.2.5. Sample embedding and three-dimensional imaging

Tibiae and tarsi of the legs of both species were fixed in glutaraldehyde (2.5%) for 48 h and washed 3 times, 10 min each time, in the sodium cacodylate buffer (0.05 M). The samples were dehydrated in the procedure comprising six 10-minute log stages with increasingly concentrated EtOH in the following order: 70%, 80%, 90%, 100%, 100%, and 100%. Then, the remnants of the EtOH were washed away form the tissue in a procedure comprising two 10-minute long baths in propylene oxide (100%). The specimens were kept for 24 hours in the 1 : 1 mixture of propylene oxide (100%) and the prepared Spurr low-viscosity embedding resin from the Sigma Aldrich kit (ERL 4221 4.10 g : diglycidyl ether of polypropylene glycol 1.43 g : nonenyl succinic anhydride 5.90 g : dimethylethanolamine 0.10 g; Sigma-Aldrich, USA), followed by 24 hours in the Spurr's resin, and 4 h in a vacuum to remove air bubbles. Finally, the samples were kept for 48 h at 65-69 degrees Celsius (°C) to facilitate the polymerization of the resin. The resin blocks were prepared for microscopy by trimming away the resin. Then, we used X-ray microscopy (XRM; Xradia 520 Versa, ZEISS, Germany, - National Center for Inter-university Research Facilities at Seoul National University) to scan and reconstruct 3D morphology of the sample. The sample comprised approximately 1 mm of distal tibia, tibia/tarsus joint, and 1 mm proximal tarsus.

The scanned three-dimensional images of distal tibiae and proximal tarsi of these legs were overlapped in longitudinal directions (100 µm length of the legs) to produce cross-sectional images showing the cross-section of the leg with the surrounding setae (since distribution of surrounding setae was not visible in a single tomographic image). From this image, we measured the *vertical leg diameter* defined by the leg cuticle in the major axis and *cross-sectional length of the ventral setae* defined as the distance from the ventral cuticle to the farthest points reached by ventral setae's tips in the cross-section. The measurements were conducted along the line that goes from the ventral side that interacts with the water surface to the dorsal side of the leg. Hence, they were measured vertically in images with the ventral side oriented downward. Finally, to obtain a variable that represents the contribution of ventral setae to expanding of the *ventral setae* by the *vertical leg diameter*. We called this variable the "*relative leg diameter increment by setae*" as it reflects how much the functional leg diameter that does not consider the layer of setae. The images were made using Dragonfly version 4.0.1.

4.2.6. Visualizing the interaction between an isolated leg and the water surface

We used midlegs and hindlegs from recently collected specimens of *G. gigas* that were stored temporarily for several days in a solution (13%) of hydrogen peroxide. We did not use ethanol because it may dilute waxes and organic substances that may provide hydrophobicity. Immersion for a short duration of several days in the hydrogen peroxide did not have a noticeable effect on the soft tissues. After taking a leg out of the solution we made sure that the surface is dried but the leg interior (muscles and other tissues) remains wet imitating the leg freshly removed from an insect. The leg was then attached to the micromanipulator (MM-3, Narishige, Japan)

using a folded wire without any adhesive (Fig. 4-S1a). This setup allowed us to slowly and gradually move the leg downward pushing against the water surface in a transparent aquarium. Images of the leg's interaction with the water surface and subsequently of the leg in the water body were obtained through the glass wall of the aquarium using the portable microscope (Dino-Lite, AnMo Electronics Corporation, Taiwan, Fig. 4-S1a, b) and with the camera (RX10 III, Sony Corporation, Japan) mounted with 4x, 10x objective lenses (Olympus Corporation, Japan).

4.3. Results

4.3.1. Behavioral observations of leg use by G. gigas [Fig. 4-2,3,4]

4.3.1.1. General

The leg length of *G. gigas* reaches up to about 13 cm (Fig. 4-2a). The body weight of *G. gigas* reaches up to 500 mg (Table 4-S1; range from 217 to 511 mg) and is 4 to 14 times heavier that the body mass of one of the typical Palearctic water striders such like *Aquarius paludum* (Table 4-S1; range from 37 to 52 mg). During stationary posture (Fig. 4-2b, c), the insect is supported on the tarsi of the forelegs (Fig. 4-2e), tarsi and distal segment of the tibia of the midlegs (Fig. 4-2f, g, h), and on the full length of tarsi and tibia of the hindlegs (Fig. 4-2i-m). The extensive menisci under both the midlegs and the hindlegs (Fig. 4-2c) indicate that the main surface tension force supporting the insect on the water comes from the tibiae and tarsi of midlegs and hindlegs. The relatively wider (relative to their length), shadows created by the supporting legs (Fig. 4-2d), compared to typical small and mid-size water striders such as *A. paludum* (inset in Fig. 4-2d), indicate a relatively larger supporting force per unit of leg length in *G. gigas* than in *A. paludum*. However, even with one hindleg missing the water strider can live on the water surface (Fig. 4-S2a), indicating that without one hindlegs the surface tension force is sufficient to support *G. gigas*'s body on the water. Midlegs and hindlegs play a crucial role in locomotion on the water surface as shown in Fig. 4-3 and described below.

4.3.1.2. Forelegs

Foreleg femora and tibiae do not interact with the water surface and are used for prey grasping (Fig. 4-3a) and for grooming during cleaning of legs (Fig. 4-3b, c) and body. Prey may be held between the proximal section of the femur and the distal section of the tibia of a leg (Fig. 4-3a2) or between the left and right leg (Fig. 4-3a1). Small prey items are grasped by forelegs at the moment of capture, and subsequently are impaled on the insect's proboscis. During grooming (cleaning) the distal section of the tibia/proximal section of the tarsus on one leg rubs against the various sections of the second leg (Fig. 4-3b, c) or against body. In a stationary pose and during slow skating the ventral surface of the forelegs' tarsi interacts with the water surface (Fig. 4-2e, Fig. 4-S2b). However, during fast skating, the front legs are slightly lifted above the surface, and they normally do not interact with the water.

4.3.1.3. Midlegs

Midlegs are used for support as documented by dimples on the water surface (Fig. 4-2f, g, h). Based on the width of the shadows (which correlates with the dimple depth), the deepest dimple is observed around the proximal segment of the tibia, and it decreases towards the distal tibia (Fig. 4-2c). The tibia/tarsal joint often does not press the water surface leading to the creation of a second very small dimple under the tarsus only (Fig. 4-2c, f). However, the very tip of the tarsus often pulls the water surface upward (Fig. 4-2f-h, Fig. 4-S2c). Midlegs are also used in cleaning the legs when the water strider rubs the midlegs and hindlegs against each other, mostly using the tibia and tarsus sections (observed in the field).

All skating ($n \ge 150$) by *G. gigas* adults as well as late-stage nymphs observed in the field involved asymmetrical mode of leg movements (asymmetrical gait) as shown in Fig. 4-4a, b: one midleg provides a forward push by "rowing" movement of the leg in the backward direction on the water surface (without breaking the surface), the other midleg is stretched forwards and does not move providing support for an insect through interaction between the water surface and midleg tarsi and almost full length of the tibia (Fig. 4-4b). During rowing, the ventral and ventrolateral (posterior) sides of midlegs' tibiae and tarsi interact with the water surface by pushing it backward and creating an asymmetric meniscus (due to horizontal movement of legs the dimple is asymmetric) along the wetted leg length, which pushes the water strider forward. The leg sections involved in interactions near the femur/tibia joint (Fig. 4-4b). Only very occasionally, we observed that a water strider performed a very short backward stride by slightly pushing with anterior side of their midlegs' tibia and tarsi forward against the water surface of the meniscus.

Water striders use upward jumps from the water surface to escape from predators, and midlegs provide the main thrust. A jump has three phases (Fig. 4-4c, d). In the first phase (Fig. 4-4c, d1), the ventral sides of bent midleg tarsi and tibiae interact with the water surface without breaking it and create the surface tension force for a jump. In the subsequent phase, the water surface is broken, and the midlegs move downward pushing through the water with the tibia and tarsus that are surrounded by air sheath/air bubble attached to the leg surface and to the hair layer on the leg (Fig. 4-4d2-4). The drag phase creates an upward drag force that propels the insect's upward contributing to the final jump speed and height. In the third stage of a jump, the legs slide quickly upward leaving the air bubble in the water slowly floating upward (Fig. 4-4d5).

4.3.1.4. Hindlegs

Hindlegs provide support through interactions of the ventral side of the tarsus and tibia with the water surface. The dimple is created along the full length of the tibia and proximal tarsus (the tibiotarsal joint is barely visible), while the distal tarsus pulls the water surface upward (Fig. 4-2i-m, Fig 4-S3). Hindlegs are also used in cleaning the legs when the water strider rubs the midlegs and hindlegs against each other, mostly using the tibiotarsal sections (observed in the field).

Except for the initial stages of each skating, the orientation of hindlegs tibiae and tarsi is approximately parallel to the direction of movement and to the orientation of the forward-stretched midleg's tarsi and tibia during asymmetrical skating (Fig. 4-4b). During striding, the contralateral hindleg moves towards the body axis, contributing to the push forward as indicated by waves in Fig. 4-4b. During the pushing, hindleg's ventral and posterior surfaces of the tibia and tarsus interact with the water surface without breaking it.

During upward jumps, the hindlegs typically do not pierce the water surface, but the depth of the meniscus under hindlegs (tibia and tarsus) increases as the legs bend and move downward. In this case, the ventral, ventral-posterior, and ventral-anterior surfaces of hindleg's tibia and tarsus interact with the water surface of the menisci. Sometimes the hindlegs may break the water surface near the end of a jump.

4.3.2. Morphology

4.3.2.1. General

The behavioral observations described above have established which sections (femur, proximal tibia, intermediate tibia, distal tibia, proximal tarsus, intermediate tarsus, distal tarsus), and which sides of these sections (dorsal *vs* ventral, anterior *vs* posterior) interact with the water during locomotion. Therefore, in the descriptions of hairs' characteristics and distribution on legs, we pay special attention to comparing the leg segments that routinely interact with water and those that do not routinely interact with water during support and locomotion. We also refer sometimes here to the behavioral observations (previous section or Results) when they may provide potential explanations for the differences in morphology. Those are later all put together in the Discussion section.

4.3.2.2. Forelegs [Fig. 4-5,6,7]

Leg sections that do not normally interact with water surface – Femur (Fig. 4-5a, b, c) is mostly covered by two types of setae: about 40-60 µm long microsetae (blue shading in Fig. 4-6a-c) and about 70-90 µm long macrosetae1 (green shading in Fig. 4-6a-c). The setae on the femur's ventral side are longer and denser than on the dorsal side (Fig. 4-5a-c). Similar general asymmetry is noticeable on the tibia (Fig. 4-5d-f), which is oriented vertically in normal situations and setae on the ventral side of tibia face the ventral side of femur when the prey is held (Fig. 4-3a) between the distal tibia (Fig. 4-5f, i) and proximal femur (Fig. 4-5a). Therefore, the anterior and ventral surface of the femur in some individuals may have small patches of apparently broken hair (Fig. 4-5a), especially in the proximal area that is often used for prey handling in the manner shown in Fig. 4-3a.

The distal tibia has specialized 'stump setae' that have stump end that appears slightly wider than the rest of the setae (pink highlight in Fig. 4-5f, I; Fig. 4-6d; appears to be equivalent to the 'grooming hairs'⁴⁰; 'teeth of wide-tooth comb' or 'stout spine-like hairs'¹⁷¹) and 'spade-shaped setae' with wide head (yellow highlight in Fig. 4-5f and Fig. 4-6f). The heads of these spade-shaped setae had 6-11 µm width and 11-24 µm length including the pointy apex. The stump setae and spade-shaped setae do not have nano-grooves on the surface. A comb structure comprising a single row of about 20 setae is observed on the ventral side of the anterior ('inward') distal tip of tibia (aqua blue highlight in Fig. 4-5f and Fig. 4-6e; equivalent to the 'grooming comb'⁴⁰; 'transverse comb'¹⁷¹). For the proximal and intermediate tibia, there appears to be no clear difference between the anterior and posterior surfaces (Fig. 4-5d, e, g, h). A small comb-like structure is present at the base of the two claws at the distal end of tarsi (claw plate, unguitractor; Fig. 4-6g). While it does interact with water, we

mention it here because it probably has also a cleaning function like the transverse comb, stump-like setae, and the spade-shaped setae.

Leg sections that interact with the water surface – The macro setae on the foreleg tarsus were dense and long on the ventral side that faces the water surface (150-200 µm length macrosetae 2; Fig. 4-5j-l and Fig. 4-7b) and less dense, shorter and thicker (macrosetae 1) on the side and dorsal surfaces of tarsus (about 5 : 2 ratio for ventral : dorsal length; Fig. 4-5j-l; Fig. 4-7a, c). Additionally, flattened 'hay-like setae' were present on the ventral side of the distal sections of tarsus where they form a network of crisscrossing setae bent multiple times (Fig. 4-7e, f, g). Finally, some of the setae on the ventral and ventral/lateral sides of distal tarsus are groove-less and flattened, and we propose the term "grass-blade-like" setae (Fig. 4-6g, lower left corner) to differentiate them from the larger groove-less flattened "spoon-like" macrosetae with widened tips on midlegs (Fig. 4-9c). In general, the setae on the dorsal side were relatively straight (Fig. 4-7a), while those on the ventral tarsus were bent (Fig. 4-7b, e), including the "bent-tip" microsetae on tarsus tip near the claws (Fig. 4-6g). Except for the hay-like setae and the grass-blade-like setae (both located near the tarsus end), the remaining setae are covered with nano-grooves of about 300-600 nm width (Fig. 4-6a, b, f, 7b, d).

4.3.2.3. Midlegs [Fig. 4-8,9,10,11,12]

Leg sections that do not normally interact with water surface – The three basic types of setae were present on the femur: thorn-shaped setae (equivalent to the 'thorn'⁶⁷ and the 'conical thorn'⁴⁷; note that it is totally different hair with 'thorn-like outgrowth'¹⁷²), macrosetae 1, and microsetae (Fig. 4-9b). The thorn-shaped setae appeared most abundant in the posterior intermediate/distal femur (Fig. 4-8a-c), and in general were more abundant in the femur in comparison to tibia and tarsus (Fig. 4-8d-j). Additionally, the femora have shorter setae than those on the tibia and tarsi, especially in terms of the relative length, i.e., after controlling for larger femur (leg) diameter (Fig. 4-8).

Leg sections that interact with water surface – We distinguished 7 types of setae present on the sections of midlegs that interact with water: 'thorn-shaped setae', 'macrosetae 1', 'macrosetae 2', 'macrosetae 3 – spoon-shaped', 'microsetae – straight', 'bent-tip microsetae', and 'spine-like setae' (Fig. 4-9a-c, 10c, d). The thorn-shaped setae (Fig. 4-9a, b), which have an extremely large basal diameter (10-15 µm, Fig. 4-9e1), have a lower density than the macro and microsetae (Fig. 4-8d-j, 9d). The macrosetae were the longest setae (Fig. 9a-c) and distributed abundantly (Fig. 84-d-j, 10a, d), and their basal diameters (3-7 µm) were intermediate between thorn-shaped and microsetae (Fig. 4-9e2). Due to their length and abundancy, the macrosetae mainly formed the exterior outline of the middle tibiae and tarsi (Fig. 4-8d-j). There were 3 types of macrosetae: "macrosetae 1", "macrosetae 2", "macrosetae 3 – spoon-shaped". Macrosetae 2 were longer than other macrosetae (Fig. 4-9a, c) and their length was up to 290 µm. The macrosetae 2 were more abundant in the proximal tibia to intermediate tarsus on the ventral side (Fig. 4-8d-i) and their end is bent towards ventral (Fig. 4-8d-f, 9a) or distal directions (Fig. 4-8g, i). The groups of these hairs on the ventral side seem like blades from the ventral view (Fig. 4-10a). Spoon-shaped macrosetae (seems to be equivalent to the 'apically bent setae'⁶⁷) were observed on the ventral side of distal tarsus (orange shades in Fig. 4-8j). Their end parts were flattened and bent as parallel to the

longitudinal direction of the leg (Fig. 4-9c, 10c). The microsetae were the shortest (blue shades in Fig. 4-9a-c), finest (Fig. 4-9e), and most numerous setae (Fig. 4-9d). Straight and bent types were observed, and the bent type was more abundant on the ventral side than the lateral side (Fig. 4-9b, c). The anterior and posterior sides of distal tibia had spine-like setae (purple shades in Fig. 4-8f, 10d). These setae are a little flattened and have pointy tips giving them a resemblance to pine needles, Fig. 4-10d2). Except for the spoon-shaped setae, all the remaining types of setae had nano-grooves with similar spacing (300-800 nm) (Fig. 4-9e). Additionally, we observed that the leg surface had cuticular granulated outgrowths, which are more developed (higher density) on the surface of distal (Fig. 4-10e2; tarsus) than proximal (Fig. 4-10e1; femur) leg, the latter not used in direct interactions with water during support and locomotion.

Comparison with the "typical" water strider, Aquarius paludum – The *relative leg diameter increment by ventral setae* (the variable is defined in the Methods, and it corresponds to the cross-sectional hair length divided by leg diameter in Fig. 4-11a) of the distal tibia and proximal tarsus of *G. gigas* were both 0.68 (Fig. 4-11a1, 3), while those of *A. paludum* were 0.34 and 0.39 (Fig. 4-11a2, 4).

In *G. gigas* (Fig. 4-11b, Table 4-S3), the macrosetae along the tibia and tarsus were generally longer on the ventral than on the dorsal side of the leg (p < 0.001; detailed statistics in Table 4-S3). Generally, towards the distal tarsus, the setae length decreased, and the macrosetae on the distal tarsus were the shortest (p < 0.001; statistics in Table 4-S1). The difference between the ventral and dorsal side was observed on the proximal and intermediate tibia as well as on intermediate and distal tarsus which create dimples on the water surface (Fig. 4-2c, f), but not so clear on the distal tibia (the trend was even reversed in distal tibia; p < 0.001) and proximal tarsus (Fig. 4-11b; detailed statistics in Table 4-S3), that often do not contribute much to the midleg dimple (Fig. 4-2c, f). In *A. paludum* (Fig. 4-11b; detailed statistics in Table 4-S4), the length of macrosetae was different between leg segments. The macrosetae length on the distal tarsus was the shortest (p < 0.001).

The relative length of macrosetae (length per leg diameter; Fig. 4-11c, Table 4-S5) was different, in general, between species (*G. gigas* > *A. paludum*, p < 0.01) and sides (ventral > dorsal, p < 0.01). The length difference between sides was larger in *G. gigas* than in *A. paludum* (p < 0.01). The relative length also differed by the leg segment (intermediate tarsus > distal tarsus > others, p < 0.001), with the relatively short relative length near the tibia/tarsus joint, on the two segments (distal tibia and proximal tarsus), that often do not contribute much to the water surface dimple (Fig. 4-2c, f). For both sides (dorsal and ventral), the relative length differences between species were the most pronounced near the tip of the leg than on the tibia resulting in even reversing the difference (*A. paludum* > *G. gigas*, p < 0.001) on the proximal tibia.

The macrosetae inclinations (Fig. 4-11d, Table 4-S6) in *G. gigas* were different between sides (ventral > dorsal, p < 0.05). Distal tarsus had the highest inclination among all other segments on both sides (p < 0.05). The inclinations in *A. paludum* were different between sides except for the distal tarsus (ventral > dorsal, p < 0.05). In general, the ventral side of both species had larger inclinations than the dorsal side (p < 0.01), the differences were larger in *G. gigas* than in *A. paludum* (p < 0.01), and distal tarsus had the largest inclinations among all other segments (p < 0.001). The difference between species, however, was not clear. In separate comparisons for each leg segment, inclination differences between sides were clear (significant after the

Bonferroni adjustments) in both species except for distal tarsus. Inclination differences between sides on distal tarsus were only clear in *G. gigas* (significant after the Bonferroni adjustments).

The macrosetae basal diameter was different between species (p < 0.001), and in both species, it changed depending on the leg segment, with the smallest diameter on the distal tarsus (p < 0.05; Fig. 4-S4; details in Table 4-S7).

Midleg-water interactions – The midleg held the unbroken water surface by its setae (Fig. 4-12a), and leg bending facilitated deep dimples without surface breaking (Fig. 4-S1c, d). The long macrosetae that are bent on their tips, which create a relatively smooth profile on the water surface (Fig. 4-12b1), are pushed against the water surface producing a dimple (Fig. 4-12a2, a3, b1) without breaking the surface. As the dimple grew deeper, the surface became more stretched down, while still maintaining the relatively smooth leg/surface profile (Fig. 4-12b2) owing to the setae's bent tips and air captured in the space among the setae (air visible in Fig. 4-12b2) . Eventually, during the process of gradual pushing downwards, the surface broke. After the water surface was pierced by the leg (Fig. 4-12a4), the leg maintained a sheath of air caught in the layer of the setae covering the leg (Fig. 4-12a5, b3). The crucial role of the long-bending macrosetae in this process was clearly visible in the meniscus and air sheath (Fig. 4-12b2, 3).

4.3.2.4. Hind legs [13-15]

Structures not related to interactions with water surface – The thorn-shaped setae were observed in every segment of the legs. The distal and intermediate femora, however, had a numerous number of thorn-shaped setae on both leg sides (Fig. 4-13a-c). Femora were also covered with microsetae and macrosetae similar to those on midlegs (Fig. 4-9b, 14a).

Structures on leg sections that interact with water surface – Unlike other legs, the hindlegs had leaf-shaped setae on their ventral side that interact with water (tarsus and tibia; sky blue shading in Fig. 4-13d-i, 14b-d and on the ventral side of the leg in Fig. 4-14g-h). They appear to be equivalent to the previously described 'spatulate hairs, sometimes with bifid apices as in the *Cylindrostethinae* and *Ptilomerinae* '¹⁷ or 'flattened setae'⁶⁷). The flat leaf-shaped setae bend towards the leg and overlap each other forming an apparent ski-like beam located approximately 50 μ m from the leg cuticle and composed of 3-5 rows of smoothly overlapping leaf-shaped setae (Fig. 4-13d-i, 14c-f2, g-h). The basal parts of leaf-shaped setae have diameters (approximately 3 μ m) similar to those of macrosetae (Fig. 4-13c). The setae flatten and widen towards their tips and split to two pointy tips making them similar to the tail of a swallow with various degrees of asymmetry (Fig. 4-14b, c, d). They are approximately 10-20 μ m wide at the widest point. The shape of leaf-shaped setae depends on their location within the ski-like beam structure (Fig. 4-14c, d, f2). The ones on the posterior side of the leg, the side that pushes the water surface during asymmetrical striding, are extremely asymmetrical with the enlarged and elongated tip on the side facing the ventral direction, i.e., facing the water surface during thrust phases of asymmetric striding. The ones near the middle are roughly symmetrical and the ones on the anterior side are asymmetrical with the longer tip on the external edge of the ski-like beam (anterior edge). The surface of the

leaf-shaped setae has nano-grooves (approximately 600 nm spacing) aligned with setae and legs' longitudinal axis (Fig. 4-14b1), but only on the outer surface i.e., the surface that forms the surface of the ski-like beam that faces the water surface (Fig. 4-14b2, 3). Two rows of thorn-shaped setae are present on both sides of the leaf-shaped setae beam on tibia (Fig. 4-13d-f, 14c, d, f1, f2, g) but are not observed on tarsus (Fig. 4-13g-i, 14e, h).

Comparison with the "typical" water strider, Aquarius paludum – *A. paludum* also has a similar structure of a "beam" made of flattened setae (equivalent to the 'flattened setae'⁶⁷) on the leg's ventral side, but it is less developed and the setae have a different shape. A row of flattened setae was found on the ventral side of the tibia. The setae have the same general characteristics as leaf-shaped setae in *G. gigas*: thin basal parts, flattened shape, nano-grooves present on the side normally facing the water, overlapping each other to form a beam with a gap (approximately 20 μ m) between the "beam" and the leg cuticle, and the thorn-shaped setae alongside the "beam" (Fig. 4-14f2, f3). However, the leaf-shaped setae in *A. paludum* have only one apex, the beam comprises only 1-2 rows of the leaf-shaped setae, and the thorn-shaped setae are present only on the posterior side of the "beam" (Fig. 4-14f3).

Hindleg-water interaction. During the experiments with separated single legs of dead water striders, the beam structure held the water surface smooth and unbroken while the leg was pushed down against the surface (Fig. 4-15a). After breaking the water surface, the hind leg was covered with the air sheath captured in the ventral layer of setae, including the leaf-shaped setae (Fig. 4-15b). The smoothness of the air sheath exterior was different between the ventral side (leaf-shaped setae) and dorsal side (macrosetae) (Fig. 4-15b). The leaf-shaped setae made the ventral exterior of the hind leg smoother than that of the midleg interacting with water (Fig. 4-12b). The water surface/leg interface had a smooth outline when the ventral side was pushed down imitating the natural direction of leg movement during locomotion (Fig. 4-15c). However, when the hindleg was pushed down against the water surface in the upside-down position, dorsal side downward, the leg-water surface interface was uneven and bumpy, and some macrosetae stuck out into the water body on the dorsal side (Fig. 4-15d1, 2). Furthermore, at deeper dimple depths, the dorsal side occasionally failed to maintain the water surface unpenetrated, and in those cases, the surface of a dimple become attached to the beam-like structure in the upper part of the leg leaving the remaining sides of the leg inside of the water body i.e., below the water surface (Fig. 4-15d3, 4).

4.4. Discussion

In the discussion, we hypothesize about the functions of the different hair types by combining our behavioral observations about the segments of legs used in various types of behaviors such as prey handling or interacting with water during locomotion with the information on the uneven distribution of different types of hairs and hair characteristics among the various legs and leg segments as well as with the experimental observations of how different segments of legs interact with water. The resulting hypothetical functions may have different degrees of support in the evidence collected here as well as in the literature, and we suggest further studies to clarify their functions. All the characteristics and hypothetical functions of the setae are summarized in Table 4-1.

Brushing tools were found on the anterior side of the foreleg distal tibia: a comb and stump setae. Additionally, *G. gigas* have spade-shaped setae, which appear to be a more specialized hair type than the stump setae of *G. gigas* as well as other species like *Gerris lacustris*⁴⁰ or *Velia gridellii*¹⁷¹. The spade-like tips of the setae are widened and flat with a conspicuously pointy apex. We hypothesize that in addition to their cleaning/brushing function (where the widened tip may increase the ability to comb particles away from the hairs) they may also play a role in holding the prey because *G. gigas* were observed to use these sections of forelegs to held prey and the giant water strider's prey may occasionally be heavier than that of other smaller species (in this situation, more resistance is needed to hold the prey against gravitational force). Additionally, the spine-like setae were also observed on the lateral side of the distal tibia of the foreleg and midleg, which matches well with the observations of cleaning using the midleg tibia in *G. gigas*. Unlike other brushing tools, spine-like setae have clear nano-grooves on their surface, suggesting that they also can play a role in water-interacting (though it is not clear whether water protecting or water repellency).

The function of the small groove-less flattened hay-like setae, which were observed on the tips of forelegs only, remains unclear. Based on their location and their delicate structure they are unlikely used for cleaning or grooming. The lack of nano-grooves may imply that they may be less hydrophobic than the typical micro and macro setae, and this coincides with the observed "mini-dimple" differentiated from the main dimple under foreleg tarsus in Fig. 4-2e. Owing to the small surface area of this foreleg/water interaction, we were unable to ascertain whether the water surface is slightly pulled-upward (as opposed to the typical dimple's downward bending of the surface under the legs). Hence, it is unclear if these setae have an adaptive function. Detailed observations of behavior are needed to clarify the function of this special hair structure of *G. gigas*. The grass-blade-like setae were also observed on the tips of the forelegs, but they are also observed on the tips of the hindlegs. The tarsus of hindlegs, however, pull the water surface up, suggesting that grass-blade-like setae may function to pull the surface up, which coincides with the absence of nano-grooves on them. By the similarities of the distribution and groove-less surfaces between grass-blade-like setae and hay-like setae, it may possible to hypothesize that hay-like setae also pull the water surface up, and the "mini-dimple" is a pulled-up dimple.

Nano-grooves appear on every water-interacting seta except for hay-like setae, grass-blade-like setae, and spoon-like setae, all of which are located on distal segments of the leg, some just near the tip of the leg. The nano-grooves are important for hydrophobicity and they cause high values of contact angle⁷¹ and prevent the water surface from being pierced⁷⁵. This surface roughness is known to improve un-wetting¹⁷³ and waterproofing⁷². In addition, they can make the expulsion of condensed water drop to prevent legs from wetting⁷⁴. Additionally, the parallel direction of nano-grooves to sliding may reduce the resistance more than orthogonal direction like micro-grooves¹⁷⁴. In comparison to the hydrophobic structures of lotus¹⁷⁵ and silicon microstructure¹⁷⁶ were reported to lose their un-wetting properties by condensation of the water.

Macro-hair (setae) on the wetted leg segments of *G. gigas* were more differentiated than those of other species. Every leg of *G. gigas* has two different types of macro-hairs: macrosetae and microsetae. Even macrosetae can be divided into two types of different lengths of setae on the ventral side of the foreleg tarsus and the wetted length (tibia and tarsus) of midlegs. Since the setae in *G. gigas* are generally longer than in *A. paludum*, even after correction for the species size (leg diameter), this implies that those setae may be morphological adaptations to heavy body mass and to the specific asymmetric gait locomotion in *G. gigas*, who use mainly one midleg to push its heavy body forward making efficient interactions with water surface

especially crucial in locomotion. Water striders propel themselves by rowing associated with the creation of vortices and waves^{17,38,40,48,177,178}. The larger vertical extension of a leg diameter is expected to increase propulsion produced during rowing that involves drag forces from asymmetric dimples^{30,49} and curvature forces from the deformed meniscus¹⁶. This leg diameter extension is achieved by *G. gigas* through a combination of setae length, inclination, and the shape of setae by bending. The heavy body mass, asymmetric rowing²¹ (i.e., using one midleg to thrust; Chapter 3 in this thesis), and the different mechanism of jumping (i.e., utilizing drag to thrust; Chapter 2 in this thesis) require *G. gigas* to have long functional setae for large diameter extensions. The long length and large inclination of macrosetae on the ventral side and the distal segments of *G. gigas* will contribute to deep dimples assuring high propulsion. Setae on the lateral side will produce large projected areas to get large drag forces for jumping.

The large inclination of the ventral side of the midlegs also contributes to the diameter extensions. However, there is a trade-off issue, since the parallel hair inclination has the advantage of water protecting, and even high contact forces for lateral rowing¹⁷⁹. Moreover, the contact forces are larger when the water slides in the opposite direction of the tilted setae than along the direction of the setae^{179,180}. The bending of setae can help to have large diameter extension by the high inclination of the basal part of the setae, and also have high contact force by the parallel inclination of the end part of them. As the end of macrosetae 2 seems quite flexible to be able to bend in either other direction (compared to the macrosetae of A. paludum⁴⁶), it may help in asymmetric sliding when the stretched forward midleg slides on the surface in the direction opposite the direction of the inclined setae on leg's ventral surface²¹ (Chapter 3 in the thesis). Short and little-inclined setae on the dorsal side match with the locomotion of G. gigas. As the dorsal side interacts with the water only during the surface breaking jumps, end even then it does not contribute to drag, and therefore it does not need functional diameter extension but it benefits from water protecting while in the water. The macrosetae's short relative length and small inclinations on the distal tibia and proximal tarsus coincide with the gap of the midleg dimple near the tibiotarsal joint. However, cause and effect are not clear nor the reason for having the split dimple. Additionally, the presence of spoon-shaped setae without nano-grooves (hence less hydrophobic than the other setae or may be even hydrophilic) at the end of the midleg distal tarsus matches the pulled-up surface under it. However, the function of pulling the surface upward remains unknown.

We could not observe the actual function of microsetae, because macrosetae maintain air bubbles without clear contact of microsetae with water. Still, the nano-grooves on microsetae indicate their hydrophobic functions. Many types of small hairs on the body are known to create plastron that captures a thin air layer covering the insect body immersed in water^{20,67,68,181}. Therefore, we hypothesize the sublayer of microsetae may also sustain the air bubble less compressible to keep the certain size of leg diameter extensions when the macrosetae are bent by high pressure in fast locomotion (e.g., fast striding or jumping) or they may play a role as an assurance in case of water penetrating the outer layer of macrosetae. They also expected to expulse the condensed water drop⁷⁴, since they are more densely distributed and smaller than macrosetae. This would help in keeping the hydrophobicity of the legs in general.

What could be the functions of the "beam-like" structure on the ventral side of the hindlegs? Considering the main functions of hind legs, especially in asymmetric rowing, we expect this structure may reduce viscous forces during jumping and sliding while increasing the force during asymmetric thrusting. The direction of nano-grooves on the leaf-shaped setae also matches parallel to the direction of sliding and orthogonal to the

direction of thrusting. The beam may also increase the efficiency of the hindleg acting as a rudder in currents^{17,44,164}.

The pull-up surface under the hindleg tarsus may increase the thrust of the hindleg during striding. *G. gigas* may need more thrust from hindlegs because they use the asymmetric mode of striding. This can be indicated by their relatively long hindlegs compared to other species (Chapter 3 in this thesis) even though they do not need all this length for support: e.g., only one hindleg is enough to support the insect body mass (proven by observation in this chapter). *G. gigas* may need deeper dimple depth to increase thrust in striding, however, the dimple depth is naturally determined by the weight of the insect by gravitational force. If the tarsus pulls the surface up, the vertical adhesion from the surface tension can be added with gravity to press the hindleg's tibia down thus creating the deeper dimple needed for locomotion. In this scenario, the groove-less surfaces of leaf-shaped setae may hypothetically play a role in providing hydrophobicity for pulling up the surface to the interaction with the water surface. As the "beam" on the tarsus is not flanked on each side by the row of rigid thorn-shaped setae (present in the tibia, see discussion below) this may facilitate direct access of water to the surfaces of the leaf-shaped setae. The absence of a row or thorn-shaped setae also implies that the beam on the tarsus does not need thorn-shaped setae to support it, since it does not provide thrust itself. This hypothesis can be further tested in detailed calculations and additional experiments.

Thorn-shaped setae can play a role in shedding water drops. Hairs should be rigid, sparse, and perpendicular to the surface for waterproofing function⁶⁹. They are located abundantly in the leg segments which do not directly interact with water surface: midleg femora, the dorsal side of tibiae and tarsi, and the dorsal side of hind legs. The thick basal diameter, sparse distribution, inflexibility, and nano-grooves of thorn-shaped setae are also favorable for shedding droplets. In addition, besides the beam structure formed by leaf-shaped setae, thornshaped setae grow in two rows on the tibia, which is the main leg segment involved in providing support and providing thrust during asymmetric locomotion. We hypothesize that these rows of strong setae that flank the "beam" of leaf-shaped setae at a very close distance create a functional structure together with the beam. They may help in capturing air by the whole functional structure by blocking the access of water to the gap between the beam and the leg cuticle. They may also mechanically reinforce the leaf-shaped setae to sustain the force of thrust during asymmetric striding locomotion. As only one row is observed in smaller species like A. paludum⁶⁷, which do not use hindlegs mainly to thrust during locomotion, the presence of two-rows may be an adaptation to the use of hindlegs to push heavy body during locomotion. This shows that thorn-shaped setae can be used for multiple tasks. Additionally, the absence of these rows on the tarsus, which is not used to create dimple for support and locomotion but is involved in puling the surface upwards, match the aforementioned role of leafshaped setae in the pulling-up hypothesis.

In summary, we identified 12 types of hair structures on the leg of *G. gigas*: a comb, stump setae, spine-like, spade-shaped, hay-like, grass-blade-like setae, three types of macrosetae, microsetae, leaf-shaped, and thorn-shaped setae. The comb and stump setae are important for cleaning the legs, and spade-shaped setae may important to hold a prey item. The spine-like setae are used for cleaning, but may also have hydrophobic properties. The function of hay-like setae remains unclear. The macro and microsetae seem to be the crucial components for the locomotion of the midleg. The macrosetae were generally becoming longer close to the ventral and distal, and *G. gigas* has longer setae than *A. paludum*. Inclinations of macrosetae were generally

larger on the ventral side of each species. The long macrosetae seem to improve the jumping and striding locomotion of *G. gigas*. In the hindleg, leaf-shaped setae form a beam structure drawing a smooth outline of contact surface with water. This beam seems to increase the thrust of striding and may reduce the resistance of sliding. They may function to pull the water surface up under the hindleg tarsus to make deeper dimple under the tibia, which helps in thrust. Thorn-shaped setae seem to mainly play a role in shedding water droplets, and they reinforce the leaf-shaped setae on hindlegs. Nano-grooves were found in every type of water-interacting setae except for hay-like setae, grass-blade-likes setae, and spoon-like setae, all of which are present near the end of the leg, where water surface pulling is observed.

4.5. Conclusions

Our results provide evidence that the morphological characteristics and distribution of setae on the legs of *G. gigas* may be adaptations to the specie'' heavy body and to the different types of locomotion on the surface (asymmetric striding and surface-breaking drag-utilized jumping) that evolved due to the species' heavy body. Long macrosetae of *G. gigas* can have positive effects on water protecting, getting high thrust in rowing, and high drag in jumping. Leaf-shaped setae may affect sliding resistance, striding thrust, and orientating. Thorn-shaped could function for shedding water droplets and support the leaf-shaped setae structure on hindlegs. Nano-grooves are observed on the surface of functional hairs. The hydrophobic structures of *G. gigas* are potential models in the area of bio-inspired surfaces. Due to its larger size, it has the advantage of easier production than previous attempts with other smaller ones^{182,183}. The leaf-shaped setae of the hind leg, also, may be applied as a drag-reducing surface such as riblets^{184,185}. The hypothetical pulling-up function has to be tested in the future, since the pulling-up phenomenon may be widespread in large-sized water striders, and maybe even in the smaller species, and it was never reported in the literature (to the best of our knowledge).



Fig. 4-1. Terminology of direction. a View from above. b Lateral side view.



Fig. 4-2. The study species, *Gigantometra gigas*, and examples of how it uses its legs to support its heavy body on the water surface in its natural habitats. **a** Captured *G. gigas*. **b** G. gigas floating on the water surface. **c** Shadows of dimples for each leg of *G. gigas*. The gap between the tibia and tarsus dimple is visible. **d** Shadows of dimples for each leg of *A. paludum*. **e** Close-up photography of foreleg dimples. The inserted zoom-up image shows details of a dimple. **f-h** Close-up photography of midleg dimples. The surface pulled up at the end of the leg. The gap between the tibia and tarsus is visible in **f. i-m** Hindleg dimple and shadows. The pulled-up surface under the hindleg tarsus is visible.



Fig. 4-3. The role of forelegs in foraging and preening/cleaning of the giant strider, *Gigantometra gigas*. **a** For prey holding, the proximal femur and distal tarsus are often used and specific area depends on the prey size and shape. For cleaning, mostly the distal tarsus is used by rubbing it against another foreleg **b** or midleg **c**. Midleg's tibia also is used for rubbing the hindleg (not shown here).



Fig. 4-4. The role of legs in the locomotion of the giant water strider, *Gigantometra gigas*. **a** Static position of a floating insect. **b** A midleg and a contralateral hindleg stroked the water surface for thrust. The other midleg stretched forward and slid. **c** Jumping of G. gigas. **d1** A midleg started to break the meniscus. **d2-4** A midleg pushed the water with an air sheath around it. **d5** A midleg pulled out from the air sheath.



Fig. 4-5. Lateral views of the femur **a-c**, tibia **d-i**, and tarsus **j-o** of the foreleg of *Gigantometra gigas* (Scanning Electron Microscopy). The images are ordered from left to right by the relative distance from the body (proximal, intermediate, distal). Italic fonts were used to indicate the direction/side of the leg, and non-italic fonts were used to indicate the direction of the view of images. In the case of tarsus, the ventral side interacts with the water surface as marked by the label '*ventral (water surface)*' in **j-l**. In **f** and **i**, areas of a comb, stump setae, spine-like setae, and spade-shaped setae are indicated by the aqua blue, pink, violet, and yellow shadings, respectively. The spade-shaped setae appear on the anterior ("inward") side.



Fig. 4-6. Close-ups on setae on forelegs. **a-c** The two basic forms of setae, microsetae (blue shading) and macrosetae 1 (green shading) are present on the foreleg's femur sides **a**, as well as dorsally **b**, and ventrally **c**. **d** Brush of stump setae on the internal/inward side of the tibia. **e** A grooming comb. **f** A brush of spade-shaped setae with diamond-like apices on the internal/inward side of the distal tibia. **g** Claws with comb-like structures at the claw basis (claw plate, unguitractor). Grass-blade-like setae are also visible at the ventral site (lower left corner of the image), below the claw plate, and bent-tip microsetae are visible around the claw (upper-left corner of the image.



Fig. 4-7. Close-ups on setae on forelegs. **a-c** The three forms of setae: microsetae (blue shading), macrosetae 1 (green shading), and macrosetae 2 (red shading) are present. **a** Dorsal anterior proximal tarsus. **b** Ventral anterior distal tibia section; behind the layers of macrosetae 2, the layers of the spine-like setae (violet shading) are present on the posterior side of the distal tarsus as seen in **d**. **c** Side of the posterior intermediate tarsus. **d** Ventral anterior distal tibia, spine-like setae (violet shading) are present. The inserted zoom-up image shows nano-grooves. **e** Ventral posterior/dorsal tarsus, intermediate section. **f** Example of close-up on hay-like setae on distal tarsus. Hay-like setae are present mostly on the anterior distal part of the ventral tarsus. **g** Zoom-in on hay-like setae near the claw on the tip of the ventral tarsus.



Fig. 4-8. Lateral views of the femur **a-c**, tibia **d-f**, and tarsus **g-j** of the midleg of *Gigantometra gigas* (Scanning Electron Microscopy). The images are ordered from left to right by the relative distance from the body (proximal, intermediate, distal). Italic fonts were used to indicate the direction/side of the leg, and non-italic fonts were used to indicate the direction of the view of images. In case of tibia and tarsus, the ventral side interacts with water surface as marked by the label '*ventral (water surface)*' in **d-j**. In **f** and **j**, areas of spine-like setae and spoon-shaped setae are indicated by the violet and orange shadings, respectively.


Fig. 4-9. Close-up on setae on midleg. **a-c** The six forms of setae: thorn-shaped setae (yellow shading), straight and bent-tip microsetae (blue shading), macrosetae 1 (green shading), macrosetae 2 (red shading), and macrosetae 3 (spoon-like, orange shading) are present. **d** Example of distribution of thorn-shaped, macro, microsetae. Locations of thorn-shaped, macro, microsetae are marked as yellow, green, and blue shading, respectively in **d2**. **e** Zoom-in images of thorn-shaped, macro, and microsetae. The thickness of each seta and nano-grooves on the surface are visible.



Fig. 4-10. Close-up on setae on midleg. **a-d** The four forms of setae: macrosetae 2 (red), macrosetae 3 (spoon-like, orange), macrosetae 1 (green), spine-like seta (violet) are shaded. **a** The row of macrosetae 2 creates a ridge-like structure facing the view. **b** Sensory hair on the femur, nano-grooves straight along the hair's longitudinal axis. **c** The bent spoon-like setae do not have grooves. The inserted zoom-up image shows a comparison between spoon-like seta (orange) and microseta (blue) **d2** Spine-like setae located near the end of the distal tibia apparently on both the anterior and posterior side. **d1** Macrasetae 1 for comparison. **e** This illustrates the difference in the density of granules on the surface of the leg under the hairs on femur **e1** and tarsus **e2**.



Fig. 4-11. Macrosetae comparison between *G. gigas* and *A. paludum*; Box-whisker plot with average marked. **a** Cross-sectional images of the tibia and tarsus of two species from XRM imaging. The cross-sectional leg diameters with/without setae are visible for *G. gigas* tibia **a1**, *A. paludum* tibia **a2**, *G. gigas* tarsus **a3**, and *A. paludum* tarsus **a4**. **b** Macrosetae length for each leg segment. The statistical model is in Table 4-S3, 4. **c** Relative macrosetae length (setae length per leg diameter) for each leg segment. The statistical model is in Table 4-S5. **d** Macrosetae inclination for each leg segment. The statistical model is in Table 4-S6. Blue and red lines indicate *G. gigas* and *A. paludum*, respectively. Solid and broken lines indicate the ventral and dorsal sides, respectively. Averages are represented by overlapped line graphs over box plots.



Fig. 4-12. Midleg interaction with the water surface. **a** A midleg getting deeper into the water surface. **a1** A midleg, up in the air. **a2** A midleg pressing the water surface. **a3** A midleg stretching the water surface down. **a4-5** A midleg penetrated the water surface, air sheath around the leg is visible. **b1-3** Close-up photography for **a2**, **a3**, and **a5**.



Fig. 4-13. SEM images of the femur **a-c**, tibia **d-f**, and tarsus **g-i** of the hindleg of *Gigantometra gigas*. The images are ordered from left to right by the relative distance from the body (proximal, intermediate, distal). Italic fonts were used to indicate the direction/side of the leg, and non-italic fonts were used to indicate the direction of the view of images. In the case of the tibia and tarsus, the ventral side interacts with the water surface as marked by a label '*ventral (water surface)*' in **d-j**. In **d** and **i**, areas of leaf-shaped setae are indicated by sky-blue shadings.



Fig. 4-14. Close-up on setae on hindleg. **a-d** The four forms of setae: macrosetae 1 (green shading), microsetae (blue shading), thorn-shaped setae (yellow), and leaf-shaped setae (sky blue shading) are represented. **b** Zoomin on leaf-shaped setae with two apices. Leaf-shaped setae had nano-grooves on the outer surface **b2**, but the inner surface was smooth **b3**. **c** Ventral side of the cut-off tibia. Two rows of thorn-shaped setae are on both sides of the leaf-shaped setae structure. **d** Close-up image of the beam-like structure of leaf-shaped setae and rows of thorn-shaped setae. Leaf-shaped setae on the posterior side had a more dramatic asymmetry of two apices. **e** Comparison of the beam-like structure on ventral hindleg between *G. gigas* and *A. paludum*. **e** The tip of a hindleg. Grass-blade-like setae are on the lateral side of the leg. **f1** The beam is clearly visible by micrography. **f2-3** The structures were different between species. **f3** The structure of *A. paludum* has only one row of leaf-shaped and thorn-shaped setae. The leaf-shaped setae also had one apex. **g1-3** The tibia of the hindleg. A row of thorn-shaped setae is beside the beam structure. The row starts to disappear near the distal tarsus. **h1-3** The tarsus of the hindleg. A thorn-shaped seta is not visible. The thorn-shaped setae are marked with yellow stars in **g-h**.



Fig. 4-15. Hidleg interaction with the water surface. **a** Close-up of a hindleg pressing the water surface. **b1-3** Close-up of a hindleg with air sheath after penetrating the water surface. **c1-3** A ventral-side-down hindleg stretch the water surface, smooth air-water interface under the leg is visible. **d1-2** A dorsal-side-down hindleg (upside down) pressing the water surface, bumpy air-water interface under the leg, and some penetrating setae are visible. **d3** A hindleg lost the water surface under the leg. **a4** The leaf-shaped setae on the upper side of the leg holding the water surface.

Name of the setae type	Nano- grooves present	Characteristics	Location	Hypothetical functions	Comparison to previously described setae in smaller species
		SETAE PRESENT ON MA	NY SEGMEN	NTS AND SIDES OF LEC	ĴS
Thorn- shaped [Fig. 4-8, 9, 13, 14, 15]	Y	Short hairs of the largest diameter; sparsely distributed, relatively numerous on the femur; present in two rows beside a 'leaf-shaped setae beam' on the hindleg tibia	Midleg, hindleg	Shedding water droplets, supporting the beam of leaf- shaped setae on the anterior and posterior sides of a hindleg	Equivalent to the 'thorn' ⁶⁷ and the 'conical thorn' ⁴⁷ in <i>A. paludum</i> but not observed a row of thorn- shaped setae on the anterior side of hindleg beam (see details of leaf- shaped setae)
Micro- straight [Fig. 4-5, 6, 7, 8, 9, 10, 13, 14]	Y	Shortest, finest, most numerous	Every leg	Water protection: keep the air-water interface in higher pressure; the expulsion of condensed water droplets	Equivalent to the 'macro- hair layer of long, pointed hairs (macrotrichia)' ⁶⁶ or 'long, evenly tapered setae' ⁶⁷
Macro 1 [Fig. 4-5, 6, 7, 8, 9, 10, 12, 13, 14, 15]	Y	Medium density, pointed end, medium length	Every leg	Water protection: keep the air-water interface of a submerged leg	Equivalent to the 'macro- hair layer of long, pointed hairs (macrotrichia) ⁶⁶ or 'long, evenly tapered setae ⁶⁷ but differentiated (thicker and longer; less abundant)
SETAE	PRESENT	MAINLY ON SEGMENTS A	AND SIDES I	NTERACTING WITH TH	IE WATER SURFACE
Macro 2 [Fig. 4-5, 7b, 8, 9a, 10, 12]	Y	Longest, more abundant in proximal tibia to intermediate tarsus in ventral side of middle leg	Ventral foreleg tarsus, midleg tibia and tarsus	Water protection: keep the air-water interface of a submerged leg (e.g., during jumping); making deeper dimple and larger projected area of midleg to thrust for jumping and striding	Equivalent to the 'macro- hair layer of long, pointed hairs (macrotrichia)' ⁶⁶ or 'long, evenly tapered setae' ⁶⁷ but differentiated (thicker and much longer; less abundant)
Macro 3 (spoon- shaped) [Fig. 4-8j, 9c, 10c]	N	The end parts are flattened in a spoon-like shape and bent such that their surface is roughly parallel to the longitudinal direction of the leg	Ventral side of midleg distal tarsus	Pulling the surface up	Probably equivalent to the 'apically bent setae' found on the legs of nymphs of <i>A. paludum</i> ⁶⁷ , where the spoon-like wide end was less pronounced.
Hay-like setae [Fig. 4- 7e-g]	N	Flattened, ribbon-like, in- orderly crossing each other in the manner resembling hay, without nano-grooves	Ventral side of the foreleg distal tarsus	Unknown: as it is present only on the very tip of the wetted foreleg it may be related to the mini- dimple visible within the main dimple (Fig. 2e)	N/A
Grass- blade-like setae [<i>Fig. 4-5,</i> <i>6g, 13i,</i> <i>14e</i>]	N	Flattened setae, smoothly gradually bent distally; slightly wider towards the end	Tip of foreleg and hindleg tarsus near the claws	Pulling up: as it is present on hindleg tarsus. Also as it is on the very tip of the wetted foreleg, it may be related to the mini- dimple visible within the main dimple (Fig. 2e)	N/A

Bent-tip microsetae [Fig. 4- 6g, 9c]	Y	Microsetae with extensively bent tip that resembles a hook; the occasional very slight bending that occurs near the tip of microsetae 1 is clearly different from the extensive, sometimes abrupt (not arc-like) bending here.	Tarsus of foreleg and midleg	Unknown	Equivalent to the 'macro- hair layer of long, pointed hairs (macrotrichia) ⁶⁶ or 'long, evenly tapered setae ⁶⁷ but differentiated (the tip is bent)
Leaf- shaped [<i>Fig. 4-</i> <i>13, 14b-</i> <i>f2, g, h,</i> <i>15</i>]	Y (N) Only on the side facing water in a beam of leaf- like setae; absent on the side facing leg	Flattened, curved for the direction of covering leg (like a part of a curved surface of a cylinder) and split to two pointy tips asymmetrical/symmetrical, overlapped and form a beam-shape	Ventral side of hindleg tibia and tarsus	Water protection: stabilize and keep the air-water interface solid; making a smooth air-water interface to make less resistance of sliding; making higher resistance of hindleg thrust; function as a rudder	Equivalent to the previously described 'spatulate hairs, sometimes with bifid apices as in the <i>Cylindrostethinae</i> and <i>Ptilomerinae</i> ' ¹⁷ but in several rows; 'flattened setae' ⁶⁷ seem to also equivalent but the flattened setae are only in a row and less developed with only one apex, with one row of thorn-shaped setae on the posterior side of the beam.
SETAE	/STRUCTU	URES WITH THE MAIN HYP SUPPOR	POTHETICAI Γ AND LOCO	L FUNCTIONS NOT DIR DMOTION	ECTLY RELATED TO
Comb [Fig. 4-5f, 6e]	N	In a single row of about 20 setae	Ventral side of the anterior ('inward') distal tip of foreleg tibia	Cleaning legs	Equivalent to the 'grooming comb' ⁴⁰ ; 'transverse comb' ¹⁷¹
Stump setae [Fig. 4-5f, i, 6d]	N	Stumped end, occasionally slightly enlarged	Anterior side of the distal tibia on the forelegs, next to the area with spade- shaped setae	Cleaning legs	Equivalent to the 'grooming hairs' ⁴⁰ ; 'teeth of wide-tooth comb' or 'stout spine-like hairs' ¹⁷¹
Spade- shaped [Fig. 4-5f, 6f]	N	Have a flattened wide head with the pointy apex	Anterior side of distal foreleg tibia	Cleaning legs, holding prey	N/A
Spine-like [Fig. 4-5f, i, 7d, 8f, 10d]	Y	Resemble spines or pine needles i.e., the thinning at the tip is abrupt, the tip is pointy (not roundish)	Lateral side of distal foreleg and midleg	Cleaning legs with water-interacting functions as it has nano-grooves	N/A

Supplementary materials



Fig. 4-S1 Field experiment setting and macrography of midleg-water interaction of *G. gigas*. **a** Field micrography setting and leg grabbed by folded wire. **b** Close-up photo of field microscope and leg-water interaction. **c** Macrography from the diagonal view of midleg getting deeper into the water surface. **d** Macrography from the longitudinal view of midleg and menisci.



Fig. 4-S2 *Gigantometra gigas* on the water surface in their natural habitats. **a** A water strider missing one hindleg has no problems supporting itself on the surface. **b** Foreleg tarsus pushes the water surface down and the claw on the tip of the tarsus touches the surface. **c** Images of midleg dimple of G. gigas in their natural habitats.



Fig. 4-S3 Hindleg dimple of *G. gigas*. **a** The hindleg was moving during the thrust phase of an asymmetric striding and the upward bending water surface at the distal section of the tarsus was still present during the pushing against the water surface. **b-e** Hindleg and its dimple in a static position.



Fig. 4-S4 Box-whisker plot of macrosetae basal diameter of each leg segment of *G. gigas* and *A. paludum*. Blue and red boxes indicate *G. gigas* and *A. paludum*, respectively. Statistical models are in Table 4-S7.

Species	G. gigas	A. paludum
Minimum	217	37
Maximum	511	52
Average	360	42
s.d.	90	4
Sample size	25	11

 Table 4-S2 Body mass of G. gigas and A. paludum

ements
f

All lengths are in µm, ir	Gigantom	etra gigas	Aquarius p	Aquarius paludum		
Tarsus distal	Leg diameter	96.19	(10.72)	65.69	(10.72)	
	Seta basal diameter	3.41	(0.59)	1.74	(0.31)	
	Seta length (dorsal)	77.48	(14.74)	35.59	(6.02)	
	Seta length (ventral)	89.29	(11.27)	39.07	(6.85)	
	Seta inclination (dorsal)	34.80	(7.03)	36.27	(7.85)	
	Seta inclination (ventral)	47.20	(13.47)	36.92	(8.92)	
Tarsus intermediate	Leg diameter	102.64	(17.85)	73.63	(13.83)	
	Seta basal diameter	4.31	(0.64)	2.00	(0.56)	
	Seta length (dorsal)	90.16	(12.88)	41.62	(4.28)	
	Seta length (ventral)	110.98	(24.35)	48.56	(9.83)	
	Seta inclination (dorsal)	25.09	(5.13)	31.80	(5.97)	
	Seta inclination (ventral)	30.99	(3.78)	36.81	(4.94)	
Tarsus proximal	Leg diameter	175.05	(17.64)	97.66	(18.11)	
	Seta basal diameter	4.35	(0.47)	2.33	(0.69)	
	Seta length (dorsal)	109.43	(10.67)	48.60	(7.95)	
	Seta length (ventral)	118.19	(19.24)	54.29	(11.60)	
	Seta inclination (dorsal)	26.69	(3.10)	30.72	(2.91)	
	Seta inclination (ventral)	38.50	(7.40)	40.98	(5.18)	
Tibia distal	Leg diameter	230.67	(20.28)	123.76	(23.13)	
	Seta basal diameter	5.25	(1.10)	2.29	(0.73)	
	Seta length (dorsal)	125.26	(10.24)	48.61	(9.04)	
	Seta length (ventral)	118.36	(16.86)	48.57	(9.55)	
	Seta inclination (dorsal)	29.76	(2.31)	31.63	(3.76)	
	Seta inclination (ventral)	34.28	(4.13)	41.89	(6.53)	
Tibia intermediate	Leg diameter	269.71	(35.85)	116.01	(12.26)	
	Seta basal diameter	5.15	(0.29)	2.76	(0.18)	
	Seta length (dorsal)	125.91	(10.44)	49.05	(3.26)	
	Seta length (ventral)	176.44	(51.77)	50.36	(8.82)	
	Seta inclination (dorsal)	27.66	(1.97)	24.88	(4.10)	
	Seta inclination (ventral)	44.61	(11.72)	35.17	(4.60)	
Tibia proximal	Leg diameter	409.10	(52.69)	151.99	(11.69)	
	Seta basal diameter	4.90	(0.45)	2.86	(0.49)	
	Seta length (dorsal)	113.91	(16.65)	50.67	(6.18)	
	Seta length (ventral)	174.10	(34.31)	58.70	(14.10)	
	Seta inclination (dorsal)	28.70	(2.35)	25.08	(4.81)	
	Seta inclination (ventral)	43.39	(6.83)	34.17	(3.88)	

Table 4-S3 Linear mixed model of log macrosetae length of G. gigas

Results of the linear mixed model: $\ln(\text{macrosetae length}) \sim (\log \text{side}) + (\log \text{segment}) + (\log \text{side}):(\log \text{segment}) + (1 | \text{individual})$, No. of observations: 300, No. of Individuals: 5. The reference treatment is the dorsal side, distal tarsus. Data are shown in Fig. 4-11b.

	Estimate	df	<i>t</i> -value	<i>p</i> -value	
(Intercept)	4.32951	7.02666	75.420	1.75e-11	***
Ventral	0.15201	284.00000	3.614	0.000357	***
Tarsus intermediate	0.16164	284.00000	3.843	0.000150	***
Tarsus proximal	0.35971	284.00000	8.552	7.63e-16	***
Tibia distal	0.49654	284.00000	11.805	< 2e-16	***
Tibia intermediate	0.50057	284.00000	11.901	< 2e-16	***
Tibia proximal	0.39069	284.00000	9.288	< 2e-16	***
Ventral: Tarsus intermediate	0.04400	284.00000	0.740	0.460145	
Ventral:Tarsus proximal	-0.08339	284.00000	-1.402	0.162039	
Ventral: Tibia distal	-0.21815	284.00000	-3.667	0.000293	***
Ventral: Tibia intermediate	0.15166	284.00000	2.550	0.011311	*
Ventral:Tibia proximal	0.26446	284.00000	4.446	1.26e-05	***

Table 4-S4 Linear mixed model of log macrosetae length of A. paludum

Results of the linear mixed model: $\ln(\text{macrosetae length}) \sim (\log \text{side}) + (\log \text{segment}) + (\log \text{side}):(\log \text{segment}) + (1 | \text{individual})$, No. of observations: 300, No. of Individuals: 5. The reference treatment is the dorsal side, distal tarsus. Data are shown in Fig. 4-11b.

	Estimate	df	<i>t</i> -value	<i>p</i> -value	
(Intercept)	3.557405	8.325968	64.968	1.46e-12	***
Ventral	0.088387	284.000001	1.970	0.049853	*
Tarsus intermediate	0.161817	284.000001	3.606	0.000367	***
Tarsus proximal	0.314091	284.000001	6.999	1.86e-11	***
Tibia distal	0.311169	284.000001	6.934	2.76e-11	***
Tibia intermediate	0.331603	284.000001	7.389	1.65e-12	***
Tibia proximal	0.359482	284.000001	8.011	2.97e-14	***
Ventral: Tarsus intermediate	0.050627	284.000001	0.798	0.425691	
Ventral:Tarsus proximal	0.007922	284.000001	0.125	0.900746	
Ventral: Tibia distal	-0.091398	284.000001	-1.440	0.150917	
Ventral: Tibia intermediate	-0.072871	284.000001	-1.148	0.251836	
Ventral: Tibia proximal	0.039139	284.000001	0.617	0.537908	

Table 4-S5 Linear mixed model of log macrosetae length per leg diameter

The dependent variable is log macrosetae length per leg diameter. The reference treatment is *A. paludum*, dorsal side, and distal tarsus if they are used. TiPr, TiIn, TiDi, TaPr, and TaIn stand for tibia proximal, tibia intermediate, tibia distal, tarsus proximal, and tarsus intermediate, respectively. The individual was treated as a random variable. Every significant estimate is marked in bold. No. of observations: 600, No. of Individuals: 10. Data are shown in Fig. 4-11c.

Model explanation				Independent v	ariables							Leg segment	interaction			
Independent																
variables	Interaction	Subset	AIC	G. gigas	Ventral	G. gigas:Ventral	TiPr	TiIn	TiDi	TaPr	TaIn	:TiPr	:TiIn	:TiDi	:TaPr	:TaIn
Species, side, segment	s Species:side	None	-181.15	.2484**	.0773***	.1012**	6951***	3207***	3716***	1781***	.1016***					
Species, segments	Species:segments	Dorsal	-224.52	.3851***			4876***	2430***	3192***	0786*	.0515	5669***	1949***	.0499	1614**	.0529
Species, segments	Species:segments	Ventral	-99.65	.4486***			4485***	3159***	4106***	0706	.1021*	3415***	.0297	0768	2527***	.0463
Species, side	Species:side	TiPr	-53.91	1818	.1275†	.2889 †										
Species, side	Species:side	TiIn	-72.80	.1902	.0155	.2882 †										
Species, side	Species:side	TiDi	-79.42	.4350	0030	0631										
Species, side	Species:side	TaPr	-125.86	.2237†	.0963 †	0277										
Species, side	Species:side	TaIn	-73.37	.4380 †	.1390†	.0570										
Species, side	Species:side	TaDi	-71.29	.3851†	.0884	.0636										

'*' < 0.05, '**' < 0.01, '***' < 0.001, '†': significant after Bonferroni adjustments, n = 600

Table 4-S6 Linear mixed model of log macrosetae inclination

The dependent variable is log macrosetae inclination. The reference treatment is *A. paludum*, dorsal side, and distal tarsus if they are used. TiPr, TiIn, TiDi, TaPr, and TaIn stand for tibia proximal, tibia intermediate, tibia distal, tarsus proximal, and tarsus intermediate, respectively. The individual was treated as a random variable. Every significant estimate is marked in bold. No. of observations: 600, No. of Individuals: 10. Data are shown in Fig. 4-11d.

Model explanation				Independent v	ariables							Interactions				
Independent																
variables	Interaction	Subset	AIC	G. gigas	Ventral	G. gigas: Ventral	TiPr	TiIn	TiDi	TaPr	TaIn	:TiPr	:TiIn	:TiDi	:TaPr	:TaIn
Side, segments	Side:segments	G. gigas	-145.36		.2975***		1753***	2101***	1381**	2508***	3276***	.1071	.1512*	1611*	.0581	0733
Side, segments	Side:segments	A. paludum	-170.47		.0080		3552***	3599***	1141*	1458**	1198**	.3108***	.3434***	.2668***	.2810***	.1459*
Species, side, segment	s Species:side	None	-225.36	0338	.2327***	.0785**	1607***	1613***	0997***	1135***	2055***					
Species, side	Species:side	TiPr	-63.16	.1473	.3188 †	.0858										
Species, side	Species:side	TiIn	-68.76	.1172	.3514 †	.0974										
Species, side	Species:side	TiDi	-117.55	0567	.2749 †	1384†										
Species, side	Species:side	TaPr	-84.12	1376	.2890 †	.0666										
Species, side	Species:side	TaIn	-96.23	2404	.1539 †	.0704										
Species, side	Species:side	TaDi	-31.74	0326	.0080	.2895 †										

"*" < 0.05, "*" < 0.01, "**" < 0.001, " \dagger ": significant after Bonferroni adjustments, n = 600

Table 4-S7 Linear mixed model of log macrosetae basal diameter

Results of the linear mixed model: ln(macrosetae basal diameter) ~ (species) + (leg segment) +
(species):(leg segment) + (1 individual), No. of observations: 300, No. of Individuals: 10. The reference
treatment is A. <i>paludum</i> , distal tarsus. Data are shown in Fig. 4-S4.

	Estimate	df	<i>t</i> -value	<i>p</i> -value	
(Intercept)	0.53321	10.60392	7.620	1.28e-05	***
G. gigas	0.68056	10.60392	6.877	3.21e-05	***
Tarsus intermediate	0.12034	280.00000	3.059	0.00244	**
Tarsus proximal	0.27966	280.00000	7.109	9.77e-12	***
Tibia distal	0.25840	280.00000	6.568	2.47e-10	***
Tibia intermediate	0.47700	280.00000	12.125	< 2e-16	***
Tibia proximal	0.50390	280.00000	12.809	< 2e-16	***
G. gigas: Tarsus intermediate	0.11454	280.00000	2.059	0.04045	*
G. gigas: Tarsus proximal	-0.03322	280.00000	-0.597	0.55089	
G. gigas:Tibia distal	0.16402	280.00000	2.948	0.00347	**
G. gigas: Tibia intermediate	-0.05331	280.00000	-0.958	0.33880	
G. gigas:Tibia proximal	-0.13332	280.00000	-2.396	0.01722	*

Chapter 5.

The micro-morphology of ribbon-like setae on midlegs of a large water strider from lotic habitats, *Ptilomera tigrina*, and their role in locomotion on the water surface

Abstract

Water striders (Gerridae) are known for their adaptations to various water-surface habitats. Recently, the research has focused on hydrodynamic principles of locomotion and the role of the basic morphological micro-structural and nano-structural adaptations of their hairs to locomotion on the water surface. However, most of these studies consider only the basic hair types and use mostly the small-sized water strider species from one subfamily, Gerrinae, that live on slowly flowing or stagnant waters. This narrow scope precludes the diversity of water strider species that live in diverse water-surface habitats and use various locomotion modes. In this research, we chose to study locomotion and midleg-microstrutures used in strokes providing the thrust for sliding on the water surface, in a typical representative of the large-sized subtropic water striders, Ptilomerinae. Ptilomera tigrina lives on the fast-flowing water and is known to have a special hair brush on its midlegs. This hair brush is suggested as an adaption for rowing, but there is a lack of detailed information about its structure and its role in generating thrust. By using optical and scanning electron micrography as well as high-speed videography, we provide detailed descriptions of the complex structure of the midleg brush and how it is used during rowing that generates thrust. Through experimental removal of the hair brush in live animals, we determined that it considerably enhances the thrust force generated during a stroke. These results prove that *P. tigrina*'s hairbrush and the associated structures that form a functional "oar" are morphological adaptations for rowing, which is an important locomotion type for insects living on fast-flowing water. We propose new hypotheses about how the morphological characteristics of the hairbrush may improve rowing. Our study demonstrates the importance of considering detailed morphological/behavioral variety across a variety of Gerridae species for research on the hydrodynamics of locomotion on the water surface.

Keywords: water strider, *Ptilomera tigrina*, rowing, water surface, Gerridae, micro-morphology, hairbrush

5.1. Introduction

Water striders, Gerridae, have evolved a variety of adaptations to their semi-aquatic niches^{17,23,91,186} including morphological adaptations to locomotion^{28,34,41,42}, which have recently been explored from the developmental genetics point of view^{18,19,26,187}. Hairs (also referred to as setae in entomological literature; we use both terms here) are an important part of semi-aquatic insects' morphology that provides hydrophobicity important in their semiaquatic lifestyle. The basic morphological types of hairs present on the legs of the common Palearctic and Nearctic genus Gerris^{24,40,66} and their role in providing hydrophobicity¹⁸⁸ have been described 60-50 years ago. However, only recently the theoretical physics of their hydrophobic function has been explored^{71–76}. Unlike the old classical research^{40,66}, the recent studies are conducted within a certain research paradigm that seems to justify focusing on only basic aspects of leg morphology of only several small and midsize species from the subfamily Gerrinae, while most of the diversity of hairs on legs within Gerrinae as well as among-species diversity within the whole family Gerridae is little studied. We choose to explore the diversity that includes species that are less easily available and require expedition-type of research to explore their adaptations to locomotion on the water surface. One such species is *Ptilomera tigrina* from fast-flowing streams in South East Asia. It belongs to the subfamily Ptilomerinae that has been rarely studied, except for systematic reports^{37,77,78}.

Some of the less studied aspects of leg morphology are specialized structures on midlegs that are apparently used as oars providing thrust for the striding on the water surface while not being used for support at all. One taxon with those special leg microstructures described about 40 years ago^{17,40} is the small-sized oceanic water striders genus, *Halobates*^{17,20,40,165}. Another taxon is the subfamily *Ptilomerinae* of water striders from fast-flowing waters of mostly South East Asia^{35–37,53}, many of which have large body sizes. As the detailed physics of water strider striding on the water surface has only been based on the very basic aspects of leg microstructures in the few studied species from the subfamily *Gerrinae*^{16,30,38,43,44,48,49,67}, the exploration of the role of those specialized oars is crucial to fully understand adaptive evolution of morphology used in locomotion by water striders in a variety of their water surface habitats.

Species in the family Gerridae have evolved adaptations to a variety of habitats including fresh water, intertidal, marine, humid terrestrial, and marginal aquatic habitats^{17,18,23–25,40}. In general, it seems that various species of water striders developed different preferences regarding their position on the water (e.g., middle, margin, edge of the water^{79–81}, temperature⁸², or shade/vegetation/cover^{82–85}. Most of these studies concerned species from stagnant/slow-flowing habitats, and relatively less attention has been given to lotic habitats. The speed of water current is one of the important characteristics of lotic freshwater habitats of water striders¹⁷. Water striders from lotic habitats are required to use constant rowing in order to maintain their position in the stream. *Aquarius remigis* is one example of a water strider species that prefers a certain, albeit relatively low, flow speed range⁸⁹. Maintaining a certain position in a stream (at a location with a certain flow speed) is crucial for foraging: while increased flow speed requires higher energy expenditure from water striders to maintain their position, the food delivery rate is higher at higher flow speed⁸⁸. Therefore, water striders are able to choose and keep their position in their habitat according to the trade-off between the costs and benefits⁸⁸. They seem to use visual cues from the surroundings^{86,87}

to maintain their position. The study species, *Ptilomera tigrina*, is known to prefer relatively fast-flowing water current and to perform constant rowing with its midlegs that are not used for support of the realtively heavy body on the water surface⁵³. It belongs to the subfamily *Ptilomerinae* known to occur in fast flowing waters.

Ptilomerinae is a subtropical subfamily^{17,37}, with well-studied taxonomy^{37,77,78}. They have three behavioral and one morphological characteristic different from the commonly studied subfamily *Gerrinae*. First, unlike the commonly studied Gerrinae, they have a brush of ribbon-like hairs on the midleg's tibia and tarsus that are used in rowing. Second, they use only forelegs and hindlegs to support their body on the water surface, while midlegs are used only as oars⁵³. Third, they are known to prefer fast flow speed⁵³. Fourth, they use 'protean'¹⁸⁹ anti-predatory movements, which are extremely fast back-and-forth striding in various directions propelled by their midlegs⁵³. These behaviors strongly suggest that their special hair structure on midlegs is an adaptation to rowing ^{17,35–37}. Repeated frequent rowing using backward midleg's strike in order to propel the insect forward is indeed very important for them in order to maintain their position in a stream of fast-flowing and often turbulent water⁵³. However, except for one short report⁵³, this rowing structure has not been well described, the kinematics of striding behavior have not been quantitatively measured, and the effects of the hair structure on rowing performance have not been determined. Therefore, I decided to describe the details of their rowing micro-structures and determine their role in producing thrust during rowing.

5.2. Results

5.2.1. Morphology of the midleg's microstructures

Fig. 5-S1 shows the terms subsequently used to describe the distribution of hairs on the midleg. The midlegs of *Ptilomera tigrina* have five distinct types of hairs, which are absent from other Gerridae species that have been studied. One of the hair types is not used in locomotion: the mating brush of hairs appears only on the middle femora of males (Fig. 5-1A, 2A). These long hairs (487-688 μ m) are densely grown on the ventral side of a femur, and they get curly near their ends (Fig. 5-2A). The hairs appear to have a round cross-section (1.7-3.3 μ m in diameter), and they do not have nano-grooves on their surface (Fig. 5-2B1, 2). As a femur does not interact with water during locomotion the male-specific brush of hairs on the femur is not important in locomotion. During mating season, the brush of these hairs in a male often may have indentations made probably in the place where the midleg male's femur presses against the female's midleg during mating/mate guarding situation when a male stays on top of a female. Mating/guarding mostly happens at night, as suggested by observations of the population of *Ptilomera tigrina* at the Melinh Biological Station, Vietnam.

In contrast to the male-specific brushes of hairs on a femur, the ribbon-like hairs on a tibia and tarsus appear on the ventral side along the tibiotarsal segments (Fig. 5-1A-D, F) that are used by both sexes in rowing to provide thrust for sliding. The hairs were present on the wetted legs' (i.e., legs interacting with water surface during locomotion) sections of tibiae and tarsus (Fig. 5-2C) spanning from the intermediate-proximal tibiae to the proximal tarsi. These hairs were no longer observed on the intermediate and distal tarsus (Fig. 5-1E, 2E). The ribbon-like hairs are much longer (336-757 μ m in length) than the diameter of the wetted leg segment (approximately 200-300 μ m). They appear roughly tubular/rod-like at their bases (Fig. 5-2J, K), but they become flat-shaped and ribbon-like within approximately 50 μ m from the base. The flattened ribbon-like section of each hair appears solid inside near the basis (Fig. 5-2J, left-lower panel), and becomes partly hollow through most of its length (Fig. 5-2J, right-upper panel) when it is 0.27-1.23 μ m thick and 3.26-12.06 μ m wide (Fig. 5-2J). The surface of the ribbon-like hairs is flat without any nano-grooves (Fig. 5-2G, J, K) that are typically present on other types of hairs on legs and body (e.g., microsetae in Fig. 5-2B2). The hair's general angle to the leg axis is usually less than 45 degrees and they are directed towards the proximal leg end (Fig. 5-1A-C, F, 2J, K). Occasionally the ribbon-like hairs' arrangement is disturbed in SEM images when hairs bend extensively near the basis leading to images similar to Fig. 5-1D, 2H, I, which illustrate the length of the ribbons relative to the leg width well.

The ribbon-like hairs on a tibia are arranged in bundles. Each bundle consists of 3-7 of the hairs (typically 7 hairs; Fig. 5-2I, J, K) with distances between them 13.4-25.6 μ m. A row of basal hair tips of each bundle does not run parallel to the leg axis, but it forms a shallow angle with it (Fig. 5-2K). The edges of the ribbons' blades face posteriorly (Fig. 5-2J, K). As they are organized in bundles of ribbons touching each other with their flat surfaces, each bundle presents a surface of ribbons' edges (Fig. 5-2G). This posteriorly directed surface of each bundle is reminiscent of the nano-grooved surface of typical hairs in water striders (Fig. 5-2H, lower panel), but the distances between grooves are smaller: 200-1300 μ m for ribbon bundles compared to 200-400 μ m for typical grooves on hairs. In many images, we have observed that the bundles are joined together, especially in their distal sections, and form one larger surface of the ribbon's edges (Fig. 5-2H, lower panel). During backward strokes needed for the forward locomotion by individuals maintaining their position in flowing water in natural habitats, these posterior surfaces of edges of ribbon-like hairs press against the water surface (see also behavioral analysis below).

In the posterior view, another type of hair is visible behind the bundles of ribbons, especially through their basal sections: grass-leaf-like hairs (111-136 μ m in length, 7.7-10.3 μ m in width) that grow at an angle of more orthogonal angle to the ventral leg surface (typically more than 45 degrees) than the ribbon-like hairs (compare the angles in Fig. 5-2J, K). They form an almost continuous surface behind the ribbon bundles (Fig. 5-2I, J, K). Hence, the row of these grass-leaf-like hairs is located on the anterior side of the row of ribbon-like hair bundles (Fig. 5-2J, K). They are thin and have nano-grooves on their flat surfaces (Fig. 5-2K). The nano-groves occur at distances of 2.4-4.8 μ m each other.

These two types of hair form the main structure used as an oar during rowing. They are surrounded by rows of additional hair types, two on the posterior and one on the anterior side. A row of thin arc-shaped microsetae occurs posteriorly just next to the row of ribbon bundles (Fig. 5-2I, J), and one or two rows of thorn-shaped thick and short hairs appear on the posterior side along the row of the ribbon bundles about 70 µm from it (Fig. 5-2H). A row of long macro setae that are bent at their tips occurs on the anterior side of the row of the grass-leaf-like hairs (Fig. 5-2H). Proximal tarsus has only a single row of ribbon-like hairs on its ventral /posterior surface (Fig. 5-2D, F). Those

long ribbon-like hairs are absent on the intermediate and distal tarsus (Fig. 5-1E, 2E), but another type is present on the distal, intermediate tarsus's ventral side: short ($30-50 \mu m$) spatula-like ($5-10 \mu m$ wide) hairs (Fig. 5-2L, M). Considering the lengths of ribbon-like hairs to be about $300-800 \mu m$, and the length of the tibia-tarsus section on which they appear to be about 20-25 mm, and the hairs angle to the leg's ventral surface to be 30-50 degrees to the ventral leg surface, I roughly estimated the approximate surface area of the functional oar that presses against the water surface during strokes as $180-1000 mm^2$.

5.2.2. Locomotion powered by midlegs – observations

The striding behavior of *Ptilomera tigrina* happens as a series of backward strokes against the flow (i.e., insect moves forward) in their natural habitat with fast-flowing water. In contrast, they keep moving forward and backward if they are on a stagnant water surface. Tibia (especially the intermediate and distal segments) and proximal tarsus interact with the surface during the strokes (Fig. 5-3A, E). While the midleg tibiotarsal segments touch the water when the insect strides, the end of the tarsus is bent as an arc and only the leg tip touches the water (Fig. 5-2C, 3A, B, E) except for extremely fast strokes. The tarsal arc is trailing behind the leg during a stroke (Fig. 5-3C, D), and its ventral side points towards the posterior direction in the backward stroke (Fig. 5-3C, F) and towards the anterior site in the forward stroke (Fig. 5-3D, F). Both backward and forward strokes produce anteroposteriorly asymmetric dimples in the water surface (Fig. 5-3C, D, F), and the legs do not break the water surface. During a stroke, ribbon-like hairs interact with the water surface at approximately less than 60 degrees (Fig. 5-3E, right upper panel, approximately 60 degrees by the vertical distortion through the hill of water being pushed). The strokes generated vortices and surface waves traveling backward (at about 0.2 m/s for the wave and about 0.1m/s for the vortex; Fig. 5-3G).

Unlike in natural situations of striding in flowing water where *Ptilomera tigrina* uses symmetrical backward strokes repeatedly to maintain its position in the flowing creek, the insects in our laboratory containers used the two midlegs symmetrically to perform back-and-forth striding (Fig. 5-4A). During such a symmetrical striding they slide almost along the same line (Fig. 5-4B) and maximum speeds during these strides reached 0.42-1.13 m/s. The *wetted leg speed* (see details in the Methods section) of striding generally was faster than 0.23 m/s (wave making velocity, Chapter 3 in this thesis) while the midlegs thrust (Fig. 5-4A, shaded area). The peaks of *contributing leg velocity* (0.23-0.44 m/s, see details in the Methods section) synchronized with the peaks of total force during thrust (Fig. 5-4A, shaded area).

The insects switched to the protean striding movements when they get threatened (e.g., by a visual stimulus from above). In the protean striding, the water striders moved not only in the longitudinal but also in diagonal/lateral directions (Fig. 5-4C) with their body maximum speed of up to 2.22 m/s. These lateral strides were powered by asymmetrical action of the midlegs represented by different angular velocities of the two midlegs (velocity difference up to 1541 degrees/s, 0.27-29 ms in Fig. 5-4D). The *wetted leg speed* of striding was up to 2.6-3.1 m/s, but the peaks were not in thrust phase (i.e., midlegs are not touching water surface, Fig. 5-4D, unshaded area). The peaks of *contributing leg velocity* (0.38-0.99 m/s, see details in the Methods section) synchronized with the peaks of

total force during thrust (Fig. 5-4D, shaded area). The *contributing leg velocity* difference between the two legs was up to 0.72 m/s by asymmetrical striding.

5.2.3. The effect of midleg's ribbon-like hairs on striding performance – a field-based experiment.

We used symmetrical locomotion performed by insects in the plastic containers to compare rowing performance between control insects and insects with shaved brushes of ribbon-like hairs (Fig. 5-5A; see Methods). Firstly, for this purpose, we looked at the relationship between maximum midleg angular velocity during a stroke and a resulting maximum body speed (i.e., the highest value of velocity calculated from the scalar quantity of body moving vector, see details in the methods). The maximum body speed increased as the maximum angular velocity increased (linear mixed model, *p*-value = 6.38E-10; Fig. 5-5B, Table 5-S1), and this increment was larger in the unshaved group than in the shaved group (linear mixed model, *p*-value = 0.00808; Fig. 5-5B, Table 5-S1). Next, to compare the thrust phase between the two groups, we looked at the relationship between maximum *contributing leg velocity* and a resulting maximum body force calculated from the body mass and acceleration. The component (vector) of stroke velocity of the 2/3 location of the wetted length (tibia+tarsus) that directly contributes to the thrust forward or backward (*contributing leg velocity*) is represented by a projection of the stroke velocity vector measured in the absolute coordinate system (*wetted leg velocity*) onto the axis of body's movement (Fig. 5-4E, see also methods).

Maximum body force increased as the maximum *contributing leg velocity* increased (linear mixed model with natural log transformation, *p*-value = 0.00022; Fig. 5-5C, Table 5-S2), and the maximum body force of shaved group was proportionally 0.458 times smaller ($e^{-0.7814} = 0.458$, a linear mixed model with natural log transformation, *p*-value = 0.00324; Fig. 5-5C, Table 5-S2, also in logarithmic scale in Fig. 5-5F) than that of the unshaved group, suggesting that the slope this relationship at any *contributing leg velocity* of the shaved group was also proportionally 0.458 times smaller than non-shaved group ($e^{-0.7814} = 0.458$, by differentiating the linear mixed model with natural log transformation, *p*-value = 0.00324; Fig. 5-5C, Table 5-S2). The time difference between the moment reaching maximum force and the moment reaching maximum *contributing leg velocity* does not have statistical evidence that it is different from zero in both groups (*p*-value > 0.5; Fig. 5-5D, Table 5-S3). The *wetted leg speed* (scalar quantity of *wetted leg velocity*) at the moment of maximum *contributing leg velocity* was generally faster than wave making velocity (0.23 m/s), suggesting that thrusting midlegs exploited wave drag in the legs' maximum contributing moment for the thrust.

5.3. Discussion

The removal of the midleg ribbon-like hair structure led to the lower striding performance. Hence, the results prove that the hair structure is an adaptation that helps to produce thrust during the rowing of *P. tigrina*. As the *Ptilomera*'s brush is made of bundles of extremely long flat ribbon-like hairs and the associated grass-leaf-like, arc-

like and cone-shaped hairs seem to function like a functional oar for rowing (not for support) and are morphologically different from the typical rod-like macrosetae on ventral sides of legs of the typical *Gerrinae*, where they are used for both, the support and rowing (e.g., in *Aquarius paludum*, the ventral macrosetae on legs may reach up to ~100 μ m length hairs of *Aquarius* and *Gerris*^{40,47,71}), the flatness, the ribbon-like shape, the arrangements in bundles and the association with the surface of the grass-leaf-like setae are all unique morphological features in *Ptilomera* that appear to be adaptations to midleg's rowing in natural situations. This is additionally supported by the fact that the hairs of the male-specific brush on *Ptilomera's* femur that is not used for locomotion on the water surface, do not have the same morphological characteristics as the hairs on tibia and tarsus. Instead, they are long, curly, and without any nano-grooves that are known to provide hydrophobicity^{16,71,72,190}.

Some of the specific morphological details can be interpreted as adaptations to specific aspects important for rowing during a midleg stroke. As the bundled flat ribbons face, during a backward stroke, the water surface with their edges rather than the flat surface, and as they are hollow inside (except basal sections), they may be able to withstand stronger forces without considerable bending than in a hypothetical situation of ribbons' flat surfaces facing the water during a backward stroke (Fig. 5-3E). The organization of ribbons into bundles, rather than single hairs, can also serve the similar adaptive function of increasing their mechanical stiffness. We also suggest that the wall of grass-leaf-like hairs behind the ribbons (anterior from ribbons) may serve a function of further supporting the ribbon-like hairs during backward strokes, which is the most frequent natural direction of striding.

As midleg strokes are performed without breaking the water surface, it indicates that *Ptilomera's* oar structure on midlegs is hydrophobic. In the typical Gerrinae, the flexible midleg's hairs, their inclination (relative to the leg), and their longitudinal nano-grooves improve water repellency^{16,71,72,190} help to produce thrust through midleg strokes¹⁷⁹. However, there are no nano-grooves on the ribbon-like hairs in *Ptilomera*. We hypothesize that the multiple edges of the overlapping ribbons organized in bundles, and multiple bundles jointed together into a larger surface, may serve as equivalents of the hairs and nano-grooves in *Gerrinae* where they help in generating thrust in strokes on the water surface without breaking it^{16,71,179,190}. Alternatively, the absence of typical nano-grooves on the ribbon-like hairs may imply that a different mechanism is involved in providing hydrophobicity to the surfaces used by midlegs as water surface oars during strokes. For example, we hypothesize that when midlegs press backward during a stroke a layer of air may be trapped between the water surface pushed by the surface of edges of ribbon-like hairs and the surface of grass-leaf-like hairs behind them, and that it may facilitate the creation of the relatively deep asymmetrical dimple without surface breaking during the rowing strokes. Our observations indeed suggest that the dimples generated by *P. tigrina* show extreme anteroposterior asymmetry, and that *P. tigrina* can fully utilize the surface tension to gain thrust since the dimple is almost folded (as viewed in a plane perpendicular to the leg axis) in the direction of a stroke (Fig. 5-3F).

As the dimple created during a stroke is much deeper than the dorsoventral width of the ribbon brush + tibia's diameter, we suggest that the increase in the surface area of the brush due to the extreme length of ribbons is less crucial than the role of the brush length (and its structure) in causing the deeper asymmetrical dimples, which result in large anterior surface area of the dimples that functionally serve as oars during a backward stroke. The ribbon-like hairs could play a critical role in making this asymmetry since the insect seems to rotate their leg to use their hair

structure in a proper direction towards the stroke (Fig. 5-3F, red lines under ellipsoid leg cross sections). The segments with these hairs (from intermediate-proximal tibia to proximal tarsus) also match the midleg's wetted segments that produce thrust (the ribbon-like hairs were not observed in the distal tarsus).

Although the maximum body force and velocity were negatively affected by the absence of the midleg brushes, there was no difference in the maximal size of the *contributing leg velocity* (the velocity vector that contributes to forward thrust) and the *wetted leg speed* (the scalar quantity of the vector of a point at 2/3 of wetted length) at the moment of maximum *contributing leg velocity* between the shaved and non-shaved insects. This *wetted leg speed* over wave making velocity (0.23 m/s) was also observed in a series of slow back-and-forth movements (Fig. 5-4A) and fast protean movements (Fig. 5-4D) during thrusting phases. Consistency of the *wetted leg speed* on the water surface during thrusting may imply there is a preference for a certain *wetted leg speed* range by the insects. The insect may control their *wetted leg speed* to exploit wave drag by exceeding the wave making velocity, which would be beneficial to achieve high thrust in *P. tigrina*'s natural flowing habitats.

The *Ptilomera's* response to predation risk from above involves extremely rapid backward, forward, and diagonal strides on the water surface that differ from upward jumps, sometimes repeated jumps, observed in similar situations in *Gerrinae*^{80,107}. The reason for this difference may be the relatively heavy body of *P. tigrina*, which causes the slower velocity of upward jumps (Chapter 2 in this thesis) making jumps less efficient antipredatory escape and lower resistance during horizontal sliding (Chapter 3 in this thesis), making fast sliding more efficient locomotion that in smaller species. Another reason for using sliding rather than jumping movements in response to predation risk may be the relatively high efficiency of rowing (rather than jumping) due to the presence of the specialized "oars" (brushes of ribbon-like hairs and the associated structures). Additionally, *Ptilomera tigrina* lives on fast, turbulent flows that create a variety of surface waves, and the unpredictable "chaotic" movements that create an additional set of waves on the surface may lead to difficulty in visual detection by predators from above⁵³. If this type of predators exerted natural selection on *Ptilomera*'s behavior then their cryptic striding might have helped water striders avoid predation, and might have additionally contributed to natural selection for the development of the midleg brushes.

In summary, we provided the first (to our knowledge) detailed description of the micro-morphology of a set of specialized hair types that altogether serve as functional water-surface oars on the midlegs of the species representative of the rarely-studied water strider subfamily *Ptilomerinae*: a taxon known to prefer fast flowing waters where frequent rowing is important. We proved that *P. tigrina*'s special ribbon-like hair structures are a specific adaptation to rowing behavior and proposed hypotheses about thrusting mechanisms in the particular aspects of the new morphology may improve rowing performance, which is different from the hydrodynamic mechanism of well-studied hairs of *Aquarius* and *Gerris*^{16,71,72,179,190}. These new characteristics (e.g., smooth surface, gathered as a bundle, flat-shaped, directionally aligned) open new fields of research that remain unknown. We suggest the detailed variety across Gerridae species now should be considered in hydrodynamic research, since the solid understanding of the typical hair structure of water strider had been built.

5.4. Materials and Methods

5.4.1. Study sites, observations, and locomotion description

Ptilomera tigrina were captured and observed in their natural habitats in the creek near the Me Linh Station for Biodiversity, Vinh Phuc Province, Vietnam. Natural locomotion was filmed as high-speed and standard movies using Casio Exilim Z-1000 and Sony RX10-III cameras. Close-up high-speed movies were captured from above/side/under the water surface to observe detailed movements of midlegs and the water surface during stroke in 30x30cm² wide acrylic container. Striding locomotion was also filmed by a high-speed camera with visualization of the water surface by using microspheres (Glass spheres, 9-13 µm, Sigma-Aldrich). 43 strides from 4 individuals' 11 series of rowing were analyzed for locomotion description. The head, body center, and tibiofemoral joints of the midleg were digitized by MaxTRAQ program (Fig. 5-S1). The speed of a body was calculated from body center displacement divided by time differences between every frame. The force of a body was calculated from speed difference divided by time differences between every frame. The angle of the leg was calculated as the angle between the axis of a body (the theoretical axis that includes points of a head and body center) and the axis of a leg (the theoretical axis that includes points of a body center and tibiofemoral joint) (Fig. 5-4E, 5-S1). Wetted leg speed was calculated from the displacement of *wetted leg points* and the time difference between every frame. The coordinates of the *wetted leg point* were calculated from extrapolation of the leg axis by adding 2/3 of tibia+tarsus length to the femur (Fig. 5-S1). Contributing leg velocity was calculated as a projection of the wetted leg speed onto the axis of a body's movement from each frame (Fig. 5-4E). Contributing leg velocity has plus values when the wetted leg moves opposite direction of the body movement. We used a moving average of three for every variable to reduce the random noise in the data. The leg angle, angular velocity, wetted leg speed, and contributing leg velocity were calculated separately for each leg. Data sets are included in Supplementary materials (Table 5-S5).

5.4.2. Imaging and measurement

The leg morphology of the water striders was photographed by using Scanning Electron Microscopy (JSM-6390LV, JEOL, Japan). Before SEM imaging, legs were dried in a vacuum chamber, attached to the aluminum mounts by carbon/copper tape, and coated with gold by a sputter coater (Cressington 108auto, Cressington Scientific Instruments, UK) for ~ 200 seconds by 30 mA. Hairs were measured from SEM images by using the ImageJ program. 23 ribbon-like hairs, 12 short hairs beside ribbon-like hairs, and five mating hairs were measured for hair length. 75 ribbon-like hairs and five mating hairs were measured for hair thickness. 14 ribbon-like hairs' widths were measured. 28 ribbon-like hair bundles were counted for the number of hairs in a bundle. 24 gaps between ribbon-like hair bundles were measured for the distance between them. Length, width, and size of nano-grooves of five grass-leaf-like hairs were measured. Five spatula-like hairs were measured for their length and width.

5.4.3. Shaving experiments

<u>Experimental design</u> - Water striders were captured in their natural habitats and put in $30x30cm^2$ wide acrylic containers filled with water from the creek. The containers were located near the creek in an open area to assure

sufficient sunlight for high-speed filming. One group of water striders was shaved their midleg brushes by using a razor under a field microscope. Another group was shaved with a stick without a blade as a control group. Water striders were shaved with 'field lab equipment' to avoid touching their legs (Fig. 5-S2).

The weight of each water strider was measured by scale (GEM20 High Precision Digital Milligram Jewelry Scale, Smart Weigh, 0.001 g). The 25 strides of 10 individuals' high-speed movies were digitized by the Tracker program. The head, body center, and tibiofemoral joints of midlegs were digitized (Fig. 5-S1). The angle of the leg was calculated as the angle between the axis of a body (the theoretical axis that includes points of a head and body center) and the axis of a leg (the theoretical axis that includes points of a body center and tibiofemoral joint) (Fig. 5-4E, 5-S1). The quartic equations were fitted in excel to describe the x, y coordinates of head and body center, and leg angle as functions of time during a stroke (Fig. 5-S3) to remove the random noise in the data. The velocity vector of a body was calculated by differentiating the quartic equation of a body center, separately for x and y coordinates. The body speed was calculated as the scalar quantity of this vector. The acceleration vector of a body was calculated as second-order derivatives of the quartic equations of body center, separately for x and y coordinates. The body force was calculated as the multiplication of body mass by the scalar quantity of the acceleration vector. The angular velocity of a leg was calculated by differentiating the quartic equation of the angle, separately for left and right legs. Wetted leg speed was calculated from angular velocity and body velocity. Wetted leg velocity relative to the body was calculated from the orthogonal vector of angular velocity with the radius of the angular circle as a length of femur+ 2/3 of tibia+tarsus (Fig. 5-S1). This relative wetted leg velocity vector was added to the body velocity vector to calculate the *wetted leg velocity* vector in the absolute coordinate system. The scalar quantity of this vector was used as wetted leg speed. Contributing leg velocity was calculated as a projection of the wetted leg velocity vector onto the axis of body velocity vector (Fig. 5-4E). Contributing leg velocity has plus values when the wetted leg moves opposite direction of the body movement. Average values of two legs were used to find the maximum values of the angular velocity and the contributing leg velocity. The average wetted leg speed of two legs was used in Fig. 5-5E. All the quartic equations and variables were calculated for a time step of 1/960.04 s (determined to follow the time resolution of the original data from the video with a capture speed of 960.04 fps). Time differences between the moment of maximum force and moment of maximum *contributing leg velocity* were calculated by the frame differences between them.

<u>Statistical analysis</u> - The maximum body velocity depending on maximum leg angular velocity and the maximum body force depending on the contributing leg velocity were compared statistically between shaved/unshaved groups using the linear mixed models (*lmer* functions of *lmer4* and *lmerTest* package in R version 3.6.1). The time differences between the moment of maximum force and moment of maximum *contributing leg velocity* of the two groups were statistically tested whether they are different from zero. The residual normality of every model was tested by the Shapiro-Wilk normality test (*shapiro.test* function of stats package). The residual of the maximum force was natural log-transformed for the normality of model residuals. No outlier was detected in every model (the Bonferroni outlier test, *outlierTest* function of car package). Data sets are included in Supplementary materials (Table 5-S6).



Fig. 5-1. Optical microscopy of midleg hair structures of *Ptilomera tigrina*. (**A**) – Femora and tibia near the tibiofemoral joint of a male's midleg. Mating hairs are on the ventral side of the femur, and ribbon-like hairs are on the ventral side of the tibia, but not on the ventral side of the very proximal tibia. (**B**) – Tibia and tarsus near a tibiotarsal joint. Bundles of ribbon-like hairs are clearly visible on the ventral side of the tibia, but ribbon-like hairs on the tarsus do not appear in bundles. (**C**) – A midleg segment from a distal femur to the tip of the leg in the water. Ribbon-like hairs are visible on the ventral side of the tibia. (**D**) – A tibia submerged in the water. Bundles of ribbon-like hairs are attached to the tibia segment of a tarsus. Ribbon-like hairs are absent. (**F**) – A wet tibia in the air. Ribbon-like hairs are attached to the tibia and make an oar structure.



Fig. 5-2. SEM images of midleg hair structures of *Ptilomera tigrina*. (**A**) – Femur and tibiofemoral joint of a male's midleg. Mating hairs are on the ventral side of the femur. (**B1**) – Close-up SEM image of 'mating hairs' on a middle femur. (**B2**) – A basal part of 'mating hair' in comparison with a microsetae on a middle femur. Nanogrooves only exist on the surface of the microsetae. (**C**) – Start of thrusting. Tibiotarsal segments are used to push the water surface. The end of the tarsus is bent as an ark and only the tip of the leg touches the water surface. (**D**) – Scanning Electron Micrographs of shaved proximal tarsus. A row of the shaved 'ribbon-like hair' is visible. (**E**) – SEM image of intermediate tarsus. The ribbon-like hairs are absent. (**F**, **G**) – Longitudinal view of the mid tarsus and ribbon-like hair on the ventral side. (**H**, **I**) – Scanning Electron Micrographs of the intermediate tibia, with ribbon-like hair bundles and grass-like hairs on the anterior side. (**J**) – Closs-up SEM image of ribbon-like hair bundles. Basal parts of ribbon-like hairs are visible. The hairs are grown in diagonal rows of 6-7 hairs. A thin cross-section of the basal part without vacancy is shown in the lower panel. A vacancy inside the middle of the ribbon-like hair is shown in the upper panel. (**K**) – Closs-up SEM image of grass-like hairs. Overlapping of the hairs and nano-grooves on them are visible. (**L**) – Spatula-like hairs on the ventral side of a leg tip. (**M**) – Spatula-like hairs on the ventral side of proximal-like tarsus.



Fig. 5-3. Striding behavior of *Ptilomera tigrina*. (**A**) – Frontal view of striding. Menisci under wetted length and tip are visible. The insect uses tibiotarsal segments to thrust, the end of the tarsus bent as an arc, and only the tip of the leg touches the surface. (**B**) – Lateral view of striding under the water surface. Menisci of the wetted length and the tip are visible. (**C**) – Backstroke of the forward stride. The tarsus arc shows the ventral side of the leg is pointing posterior direction. (**D**) – Forward stroke of the backward stride. The tarsus arc shows the ventral side of the leg is pointing anterior direction. (**C**, **D**) – Menisci are anteroposteriorly asymmetric in strokes of both directions. (**E**) – A hill of water generated by stroke. Ribbon-like hairs are visible through the hill of water with distortion. (**F**) – Schematics of the cross-sectional view of backward and forward strokes. Surface tension can be exploited by anteroposteriorly asymmetric dimples. Trapped water makes a hill by stroke. (**G**) – Striding of the insect on the water visualized by microspheres, view from above. The surface wave and vortex were made, and the vortex was moving after the wave. Black and white arrows indicate the stroke direction. Yellow, blue, purple, and dark blue arrows indicate the leg tip, the arc of tarsus, surface wave, and vortex, respectively.



Fig. 5-4. Kinematics of normal and protean strides on the stagnant water. (**A**, **B**) – Series of normal back-andforth strides on the water. (**A**) – Femur-body angle, femur angular velocity, body speed, total force, wetted leg speed, and contributing leg velocity are shown with time. The left and right legs move symmetrically. (**B**) – Cartesian coordinates of moving body insects. The insect moves along the same line. (**C**, **D**) – Series of antipredatory protean strides on the water. (**C**) – Cartesian coordinates of moving body insects. The insect moves in a diagonal direction. (**D**) – Femur-body angle, femur angular velocity, body speed, total force, wetted leg speed, and contributing leg velocity are shown with time. The left and right legs move asymmetrically. The angular velocity difference between the two midlegs was up to 1541 degrees/s in 0.27-29 ms, and these made diagonal movements in the last stride of (**C**). Green and orange arrows indicate forward and backward body movements, respectively. Blue and red dots indicate left and right legs, respectively. Violet, yellow, and pink dots indicate braking (when midleg touches the water to decelerate body), start, and end of thrusting, respectively. Green and orange shades indicate forward and backward thrusting (i.e., touching the water surface), respectively. The time difference between dots is 4 ms. (**E**) – A schematic of striding variables.



Fig. 5-5. Midleg and striding of shaved and unshaved water striders. (A) – Midleg tibiae of shaved and unshaved insects. Ribbon-like hairs were absent in the tibia of the shaved insect. (B) – Maximum body speed and maximum leg angular velocity. The statistical model prediction is in Table 5-S1. (C) – Maximum body force and maximum *contributing leg velocity*. The statistical model prediction is in Table 5-S2. Natural-log transformed values are represented in (F), since the force was natural-log transformed in the statistical model. (D) – Time difference between the moment reaching maximum force and the moment reaching *contributing leg velocity*. There was no statistical evidence that the time difference is different from zero (Table 5-S3). (E) – Wetted leg speed at the moment of maximum *contributing leg velocity*. Wave-making velocity (0.23 m/s) is indicated by the green dotted line. Unshaved and shaved groups are marked by blue and red colors, respectively.

Supplementary Materials



Fig. 5-S1 A schematic of a water strider. Red dots indicate digitized points in the analysis. The terminology of leg segments used in the paper marked with blue arrows. The black dashed line shows extrapolation of the line between the body center and tibiofemoral joints, which was used to determine the coordinates of 2/3 of wetted leg.


Fig. 5-S2 Field lab equipment to shave and shaving process. (A) – A aluminum foil roll was put inside a paper roll to make a holder for live insects. Paper rolls provide mechanical stiffness while aluminum rolls provide a smooth inner surface of the holder. Shaving tools were made with broken cotton sticks and shaving razors. (B) – The process of putting a water strider inside a holder. (B1) – A water strider is held in hand without touching the insect's legs. (B2) – The water strider is being inside the holder. (B3) – A water strider inside of the "pipe" of the holder with its forelegs sticking out; the toothpick is now used to properly arrange the midlegs of the water strider in the holder. (B4) – The opened part of the holder was closed around the forelegs and the head of the water strider. (B5) – The closed part was fixed with tape leaving the midlegs available for shaving. (C) – The midleg of the water strider inside the holder was being shaved by a razor. The lower-left panel shows a close-up view highlighting the importance of avoiding contact between the experimenter's hand and the water strider's wetted legs in order to avoid contamination that may affect hydrophobicity.



Fig. 5-S3 The polynomial quadratic equation of fitting. (**A**, **B**, **D**, **E**) – Examples of polynomial quadratic equations fitted to describe the x, y coordinates of body center as functions of time. (**C**, **F**) – Examples of polynomial quadratic equations fitted to describe the femur angle as functions of time. Red lines indicate the fitted lines. Blue, green, and orange dots indicate body center, left femur angle, and right femur angle, respectively. Interpolation was reasonably matched with raw data in both shaved (**A**, **B**, **C**) and unshaved (**D**, **E**, **F**) groups.

Table 5-S1. The results from the linear mixed model analysis for maximum body speed as a function of maximum average leg angular velocity and experimental treatment. Results of the linear mixed model: (Maximum body speed) ~ (Maximum leg angular velocity) + (Treatment) + (Maximum leg angular velocity):(Treatment) + (1 | Individual), No. of observations: 25, No. of Individuals: 10. The reference treatment is unshaved group. Results are shown in Fig. 5-5B.

	Estimate	df	<i>t</i> -value	Pr(> t)
(Intercept)	-2.853e-01	1.990e+01	-3.405	0.00283
(Shaved)	3.182e-01	1.988e+01	2.290	0.03312
(Max. Ang. Vel.)	9.526e-04	1.462e+01	14.126	6.38e-10
(Max. Ang. Vel.):(Shaved)	-3.857e-04	1.742e+01	-2.989	0.00808

Table 5-S2. The results from the linear mixed model analysis for maximum force as a function of maximum *contributing leg velocity* and experimental treatment. Results of the linear mixed model: ln(Maximum body force) ~ (Maximum contributing leg velocity) + (Treatment) + (1 | Individual), No. of observations: 25, No. of Individuals: 10. The reference treatment is unshaved group. Results are shown in Fig. 5-5C, F.

	Estimate	df	<i>t</i> -value	Pr(> t)
(Intercept)	-6.8622	20.2632	-25.730	< 2e-16
(Shaved)	-0.7814	8.2439	-4.098	0.00324
(Max. Cont. Vel.)	4.1085	21.5122	4.431	0.00022

Table 5-S3. The results from the linear mixed model analysis for time difference between moment reaching maximum force and moment reaching maximum *contributing leg velocity* as a function experimental treatment. Results of the linear mixed model: (Time difference) ~ (Treatment) + (1 | Individual), No. of observations: 25, No. of Individuals: 10. The reference treatment is unshaved group. Results are shown in Fig. 5-5D.

	Estimate	df	<i>t</i> -value	Pr(> t)
(Intercept)	1.923	7.368	0.681	0.517
(Shaved)	-1.326	7.899	-0.358	0.730

Table 5-S4. Descript data set of striding.

					Maximum	
					body	
			Body mass	Moving	velocity	Maximum
Movie name	Individual	Sex	(mg)	direction	(m/s)	force (mN)
EVT10 125fps	2	Male	0.000113	Forward	0.554892999	1.342559
EVT10 125fps	2	Male	0.000113	Backward	0.498616474	1.122
EVT10 125fps	2	Male	0.000113	Forward	0.575723354	1.137213
EVT10 125fps	2	Male	0.000113	Backward	0.4218113	0.816077
EVT12 125fps	2	Male	0.000113	Forward	0.667186743	1.278281
EVT12 125fps	2	Male	0.000113	Backward	0.563635067	1.41383
EVT12 125fps	2	Male	0.000113	Forward	0.605178515	1.396325
EVT27	5	Famale	0.000108	Backward	0.477563561	1.414127
EVT27	5	Famale	0.000108	Forward	0.469074997	1.968556
EVT27	5	Famale	0.000108	Backward	0.453584834	2.083377
EVT27	5	Famale	0.000108	Forward	0.496660457	1.36445
EVT37	6	Male	0.000143	Backward	0.945256406	5.251079
EVT37	6	Male	0.000143	Forward	1.134205674	6.897415
EVT37	6	Male	0.000143	Backward	1.055794615	5.951236
EVT37	6	Male	0.000143	Forward	1.040015636	5.56745
EVT41	6	Male	0.000143	Backward	0.690093011	4.330292
EVT41	6	Male	0.000143	Forward	0.960299095	4.550157
EVT41	6	Male	0.000143	Backward	0.763942797	3.28382
EVT41	6	Male	0.000143	Forward	0.551412893	2.517551
EVT18 125 fps	3	Male	0.000119	Forward	0.761782945	1.997369
EVT18 125 fps	3	Male	0.000119	Backward	0.744871372	2.018588
EVT18 125 fps	3	Male	0.000119	Forward	0.911659916	2.854106
EVT18 125 fps	3	Male	0.000119	Backward	0.704819753	2.099775
EVT20 125fps	3	Male	0.000119	Forward	0.854305184	2.415806
EVT20 125fps	3	Male	0.000119	Backward	0.635392175	1.623807
EVT20 125fps	3	Male	0.000119	Forward	0.77913879	1.938766
EVT20 125fps	3	Male	0.000119	Backward	0.599045016	1.703545
EVT29	5	Female	0.000108	Forward	0.721531001	1.862073
EVT29	5	Female	0.000108	Backward	0.654100091	2.058114
EVT29	5	Female	0.000108	Forward	0.756787643	2.223823
EVT29	5	Female	0.000108	Backward	0.629026518	1.624002
EVT31	5	Female	0.000108	Forward	0.932371902	2.714887
EVT31	5	Female	0.000108	Backward	0.575790931	1.764515
EVT31	5	Female	0.000108	Forward	0.719151997	1.942918
EVT31	5	Female	0.000108	Backward	0.539827754	2.254868
EVT32	5	Female	0.000108	Forward	0.91185899	2.377555
EVT32	5	Female	0.000108	Backward	0.61827791	2.595827
EVT32	5	Female	0.000108	Forward	1.015913145	3.042005
EVT32	5	Female	0.000108	Backward	0.573522044	2.222232
EVT39	6	Male	0.000143	Backward	1.339517081	7.909042
EVT39	6	Male	0.000143	Forward	2.217747915	16.9713
EVT39	6	Male	0.000143	Backward	1.400934787	7.915711
EVT39	6	Male	0.000143	Forward	1.975717343	12.54987

Table 5-S6. Data set of shaving experimentary	iments.
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Indi vidu al	Bod y mas s (mg)	Treatme nt	Video name	Max. leg angular velocity (degrees/s)	Max. body speed (m/s)	Max. contributing leg velocity (m/s)	Max. body force (N)	Time difference (ms)	Wetted leg speed at Max. cont. vel. (m/s)
6	123	Control	C0049	859.54	0.649	0.192	0.00177	0.00	0.225
6	123	Control	C0054	841.27	0.598	0.218	0.00267	8.34	0.232
14	144	Control	C0128	1342.95	0.847	0.326	0.00432	4.17	0.411
14	144	Control	C0131	1158.99	0.699	0.271	0.00293	11.47	0.340
17	138	Control	C0283	1017.25	0.694	0.172	0.00192	-14.60	0.247
17	138	Control	C0285	1169.54	0.821	0.221	0.00319	3.13	0.314
17	138	Control	C0287	1210.51	0.888	0.162	0.00335	-2.09	0.327
19	123	Control	C0002 (2)	956.91	0.641	0.201	0.00181	-3.13	0.321
19	123	Control	C0003 (2)	1255.89	0.867	0.278	0.00298	-1.04	0.380
19	123	Control	C0004 (2)	983.76	0.705	0.238	0.00221	-1.04	0.304
19	123	Control	C0005 (2)	1555.51	1.275	0.407	0.00702	12.51	0.474
5	90	Shaved	C0032	1300.16	0.713	0.423	0.00220	3.13	0.457
5	90	Shaved	C0033	1154.49	0.637	0.354	0.00185	1.04	0.361
5	90	Shaved	C0034	1124.44	0.612	0.351	0.00194	2.09	0.381
7	95	Shaved	C0065	777.50	0.415	0.195	0.00064	-18.77	0.226
7	95	Shaved	C0066	1082.15	0.603	0.339	0.00146	2.09	0.354
7	95	Shaved	C0067	822.88	0.502	0.216	0.00062	-10.43	0.254
13	142	Shaved	C0105 (2)	996.81	0.662	0.390	0.00275	13.56	0.327
13	142	Shaved	C0108 (2)	1033.04	0.699	0.197	0.00262	4.17	0.390
15	143	Shaved	C0148	811.60	0.578	0.293	0.00188	2.09	0.336
15	143	Shaved	C0150	912.91	0.609	0.318	0.00227	4.17	0.341
18	100	Shaved	C0305	769.91	0.463	0.175	0.00121	-3.13	0.195
18	100	Shaved	C0307	860.08	0.543	0.233	0.00131	1.04	0.234
23	118	Shaved	C0079 (3)	956.61	0.466	0.277	0.00085	3.13	0.282
23	118	Shaved	C0080 (3)	1074.59	0.630	0.269	0.00182	0.00	0.312

Chapter 6. Locomotion and flow speed preferences in natural habitats by large water striders, *Ptilomera tigrina*, with micromorphological adaptations for rowing

Abstract

Water strider (Gerridae) morphology and behavior have become the focus of interdisciplinary research in biological diversification and bio-inspired technology. However, the diversity of behaviors and morphology of the large-sized Gerridae have not been intensely studied. Here, we provide locomotory behaviors and legs' micro-morphology of the large South-East Asian water strider, *Ptilomera tigrina*. Using high-speed videography and experiments in natural habitats, as well as scanning electron microscopy of midlegs, we have determined that (1) *P. tigrina* individuals prefer relatively high flow speeds of 0.15-0.30 m/s, compared to other water striders previously studied, and they are also observed in very high flow speeds of up to 0.6 m/s; (2) they avoid stagnant water, but when on still and very slow flowing water they perform constant back-and-forth rowing using their midlegs; (3) their antipredatory reaction involves repetitive and very fast "protean" movements propelled by the midlegs; (4) their midleg tarsi and tibiae are equipped with brushes of ribbon-like hairs, which are used as paddles for rowing. As the locomotory behaviors and flow-speed preferences by *P. tigrina* require constant use of midlegs for rowing, the presence of special paddle structures on midlegs illustrates a hypothetical adaptive match between midlegs' locomotory function and their micro-morphology.

Keywords: water strider, habitat preference, locomotion, Gerridae, morphology, setae, water flow speed, *Ptilomera tigrina*

6.1. Introduction

Semiaquatic bugs (Gerromorpha), including water striders (Gerridae), inhabit different semiaquatic niches^{17,18,22–26} such as stagnant or flowing waters of streams and creeks, to which water striders have adapted over 60 million years of evolution^{90,91,186,191}. A basic general view of the evolution that produces a match between various macro and micro morphological adaptations and the requirements of diverse aquatic habitats of Gerridae has been presented ~ 40 years ago^{17,23,24,40}, and was recently revived with a focus on the evolutionary developmental genetics of macro-morphological¹⁸ and micro-morphological^{19,192} traits. However, apart from general categorizations of habitat types^{17,23,24,40}, the relevant quantitative statistical and experimental approaches to habitat selection concerned mostly stagnant and slow-flowing water habitats^{79–85}, and did not consider comparative evaluations of a hypothetical match between the locomotion required in a given aquatic habitat and detailed descriptions of micro-morphological adaptations to locomotion of a variety of water strider taxa in the diversity of aquatic habitats.

Some morphological studies concerned morphology of the "typical" Palearctic and Nearctic water striders from genera *Gerris* and *Aquarius* that live in still or relatively slow-flowing waters. For example, already 40 years ago it was known⁴⁰ that the midleg tarsi and tibiae (parts that interact with water surface) of these "typical" water striders are covered with diverse hairs (formally called setae but as the less formal term "hairs" has also been used we use it too). At least five different types of hairs varying in length, diameter, and cross-section shape are present in densities from hundreds (larger hair) to several thousand (smaller hairs) per mm² with asymmetrical distribution consisting of especially diversified hair layers on the ventral side of tibiae and tarsi (surfaces that interact with water surface). Andersen¹² also noted that the hair's surface consists of small grooves running along a hair. It has also been known for a long time that all these morphological characteristics contribute to the hydrophobicity/superhydrophobicity of water strider legs, and that the special hair types on legs play a role in the rowing function of midlegs^{17,40,167–169}. All the aforementioned studies focus on the morphology of mostly the two genera, *Gerris* and *Aquarius*. However, it has also been noted that some taxonomic groups of water striders such as the small marine water striders *Halobatinae*^{17,20} or the large water striders *Ptilomerinae*^{35,37} have special structures on the midleg tarsi and tibiae functioning as paddles.

The recent revival of interest in water striders by scientists focusing on bio-mimicry expanded this already existing knowledge^{28,40,41,169,188,193,194} and resulted in a series of novel studies determining the physics of the water striders' hydrophobicity^{71–76} and locomotion behavior^{16,18,49,67,195,30,31,33,34,38,43,44,48}. However, none of these new studies have explored the hypothetical adaptive functions of each of the several types of hairs, and their uneven distribution on the leg's cuticle, as described by Andersen⁴⁰ for *Gerris*. Additionally, none of these studies even considered large water striders with specialized "paddles" on their midlegs, such as the genus *Ptilomera*^{35–37}.

The rarely studied (except for taxonomy-focused papers^{37,77,78,145,196,197} large water striders from the subfamily Ptilomerinae, such as genus *Ptilomera*, inhabit streams and creeks of subtropical and tropical SE Asian regions^{17,21,37}. Considering basic information in the literature^{17,35–37}, it is expected that these water striders differ from the "typical" water striders in the following aspects. Unlike in the well-studied genera, such as *Gerris* or

Aquarius, the body of *Ptilomera* is supported only on four legs, the hindlegs' tibiae and tarsi and the forelegs' tarsi, leaving midlegs free for rowing. The midlegs have a "brush" of setae along the ventral edge of the midleg tibiae and tarsi. Therefore, we predicted that *Ptilomera*'s natural behavior in typical habitats may involve the frequent use of such a specialized midleg's structure for rowing.

The "typical" water striders genus *Aquarius* are able to optimally choose their foraging positions in a stream by finding a tradeoff between the increase in the energy intake from the larger number of food items brought by the faster-flowing water and the considerable energy needed for striding against the fast current in order to maintain their foraging position⁸⁸. They maintain a specific position in flowing water by using midlegs for rowing against the current and by relying on visual information about the surroundings^{86,87}. However, except for Fairbairn & Brassard's research⁸⁹ on *Aquarius remigis*, detailed quantitative tests of naturally preferred water flow speeds by water striders have not been conducted. The basic literature on *Ptilomera*'s biology^{17,21,36} suggests without any quantitative statistical assessments that within the typical creeks inhabited by *Ptilomera* the water striders may prefer sites with relatively fast water flow.

The aim of the study was to present observations of *Ptilomera tigrina's* locomotion and to quantitatively test their preferences for water flow speed in their typical natural habitats. We also aimed to provide basic micro-imaging of micro-morphological structures on their midlegs used for rowing. Based on the results, we suggest that the main function of the row of ribbon-like setae on ventral midleg tibiae and tarsi is not the support of insect body on the surface but providing thrust through rowing during striding locomotion in their preferred relatively high flow speeds and during their typical locomotory behaviors.

6.2. Materials and Methods

6.2.1. Study sites and species

We conducted the study at two sites: the Melinh Station for Biodiversity (21°23'01.9"N 105°42'44.2"E = Google map: 21.383870, 105.712264; Fig. 6-1a; Fig. 6-S1a), Vinh Phuc Province, Vietnam, and "May waterfalls" (Thac May; 20°21'51.4"N 105°26'51.6"E= Google map: 20.364275, 105.447665), in the vicinity of the Cuc Phuong National Park, Vietnam, (Fig. 6-S1a in Supplementary Materials Part 2). Examples of habitats studied are in Fig. 6-S1b. At both locations, we studied *Ptilomera tigrina* (Fig. 6-2a), one of the common large water striders in Vietnam¹⁹⁶. All research was conducted in accordance with the laws of Vietnam. The Melinh Station for Biodiversity, IEBR, allowed us to collect specimens, and the Vietnam National Museum of Nature, VAST, provided the specimens loan permits.

6.2.2. Morphology and SEM imaging

We weighed and photographed each individual water strider (GEM20 High Precision Digital Milligram Jewelry Scale, Smart Weigh, 0.001 g) in the manner that allowed extraction of morphological measurements from the photographs using ImageJ. We used Scanning Electron Microscopy (SEM (JSM-6390LV, JEOL, Japan) to examine the morphology of the brush of setae on the midleg tibia and tarsi, which were dried in a vacuum chamber, attached to the aluminum mounts by a carbon tape, and coated with gold by a sputter coater (Cressington 108auto, Cressington Scientific Instruments, UK) for ~ 200 s using a current of 30 mA. We used the same procedure to obtain SEM images of midleg tarsi of *Aquarius paludum* for comparison with the main study species, *P. tigrina*.

6.2.3. Observations of locomotion in the natural habitat

We conducted observations of locomotion of individual water striders in October, 2014, June 2016, and June 2018 in a creek near the Melinh Station for Biodiversity. We filmed (high speed and standard movies using Casio Exilim Z-1000 and Sony RX10-III cameras) the locomotion of water striders in various situations in natural conditions in order to obtain a full spectrum of natural locomotory behaviors of this species in its natural habitat. We used some of the collected movie clips to compare the stroke frequency by *P. tigrina* between the sites with relatively slow-flowing water and those with fast-flowing water. The categorization into slow-flowing and fast-flowing water sites was made without quantitative assessment of water flow but rather based on the visual evaluation and choice of movies that clearly represent relatively slow and those that clearly represent the relatively fast water flow (the data set is presented in Supplementary Materials Part 5).

6.2.4. Evaluation of water flow speed preferences in the natural habitat

In March 2016, we conducted observations of flow speed preferences in a stream near the Melinh Station for Biodiversity (Fig. 6-1a) at locations where *Ptilomera* was present in the creek (n = 63), and compared them with the velocities at locations in the creek where *Ptilomera* was absent (n = 65). This resulted in the total 128 locations. The strategy for choosing the locations is shown in an example in Fig. 6-1a. At each location, we measured the velocity of water at the surface by throwing small pieces of paper (5x5 mm) on the surface, filming their movement using a camera located directly above the water (120 fps), digitizing the movement of the center of each piece of paper from the video during a total of 17-1974 frames per movie to extract their speed, and finally calculating water flow speed at a location as an average value from speeds of the pieces of papers at each location. (2-3 pieces of paper at 110 locations), or using the value for one piece of paper if only one was digitized at a location (18 locations). We used quadratic logistic regression for statistical analysis of the effect of the flow speed (continuous explanatory variable) on the use of the habitat (binary variable with two values indicating water strider "present" or "absent' at a sampled

location) in R version 3.6.1. The regression model assumes that the relationship follows a specific type of function chosen by the researcher, and we used the quadratic regression. We additionally run an alternative test that does not have any assumptions about the nature of the differences between the water strider "present" and "absent" distributions: the classical non-parametric Kolmogorov-Smirnoff D test for differences in flow speed distribution between water strider present and absent locations.

For graphical purposes, we presented the distribution (with the frequency expressed as % of all observations) of *Ptilomera*-present and *Ptilomera*-absent speeds (n = 63 and n = 65 respectively) in 5 cm/s bins over the range from 0 to 30 cm/s and in 10cm/s bins for the range of speeds 30-90 cm/s. Due to the small sample size for the very high speeds, the bin width was larger for faster speeds. Next, we created a smoothed figure using the Excel's chart type "Scatter with Smooth Lines" and x-axis data comprising the values of mid-range for each bin and y-axis comprising % of observations in a bin. We also calculated the traditional Chi-square test for statistical comparison of water speed distributions between *Ptilomera*-present and *Ptilomera*-absent categories. As the test does not provide reliable conclusions if cell counts are near zero, we calculated the Chi-square tests for two alternative contingency tables: the 4x2 and 5x2 tables (Fig. 6-S3c), in which the range of observed speeds was divided into either four (0-10, 10-20, 20-40, >40 cm/s) or five classes (0-10, 10-15, 15-20, 20-40, >40 cm/s). The results from these additional Chi-square tests are presented only in Supplementary Materials Part 4. The raw data set is presented in the Supplementary Materials Part 6.

6.2.5. Experiments: comparison of behavior in flowing versus non-flowing water

As the observations in the natural habitat revealed that *Ptilomera tigrina* appears to avoid non-flowing water, we conducted experiments in 2019 in order to compare their behavior on flowing and non-flowing water. We used two plastic bowls (Fig. 6-1b, c; diameter ~50 and depth ~15 cm) placed side by side in the middle of a creek. The bowls were freshly filled with water from the creek each time before a trial. One bowl imitated still water and the other bowl imitated flowing water conditions. We put holes on the bottom of the still water bowl to keep the water inside the bowl constantly blending with the creek water without disturbance of the water surface in the bowl. We cut two openings in the flowing water bowl's opposite walls to allow water to flow through the bowl (Fig. 6-1b). To prevent water striders from escaping through these openings we covered the openings with plastic mesh (mesh size ~5 mm) such that water can flow but water striders cannot go through. The two bowls were located next to each other in a shady location, and experiments in the two treatments were conducted simultaneously so that the time of day and lighting do not bias the comparison between the two treatments (Fig. 6-1c).

A trial consisted of putting a freshly captured *Ptilomera* into a bowl and observing (and filming; Fig. 6-1c) its behavior. We counted the number of jumps out of the bowl per minute during the first 5 minutes as an indicator of rejection of the situation. A 20cm high wall around the bowl perimeter (Fig. 6-1b) around the bowl prevented water striders from escaping during a jump, except for relatively high jumps. We conducted 15 tests in still water bowls (10 at "May waterfall" and 5 at Melinh Station for Biodiversity) and 14 tests in flowing water bowls (10 at "May

waterfall" and 4 at Melinh Station for Biodiversity, where one individual escaped before data were collected). As the relative contributions of the two locations to the number of tests in still and flowing water treatments were similar (10:5 and 10:4 for waterfall:station in still and flowing treatment respectively), we believe that we avoided the potential bias due to different contributions of the two sites to each treatment. Therefore, we analyzed the pooled data (an alternative analysis of each site separately led to the same conclusions). Each individual was tested once. Based on earlier observations in the natural habitats, we expected that *Ptilomera* may jump more in bowls with still water, and we used the Mann-Whitney test to evaluate this prediction. The raw data are presented in the Supplementary Materials Part 7.

6.3. Results

6.3.1. Observations of locomotion and midleg's micro-morphology

Ptilomera tigrina is a relatively large water strider with the average body mass of 115 ± 30 mg and 107 ± 18 mg for males and females respectively (Fig. 6-2a, and Supplementary Materials Part 1: Table 6-S1 and Fig. 6-S2a). While only males possess the fringe of hairs on their femur (compare femur of a male in Fig. S2a with that of a female in Fig. 6-2a), both sexes have a "brush" of ribbon-like setae on the ventral (water-facing, i.e., interacting with water surface) side of tibiae and tarsi of midlegs (Fig. 6-2b-e; Fig. 6-S2b, d). Those structures are absent from the commonly studied water striders such as *A. paludum* (Fig. 6-S2c). The ribbons in the "brush" reach the length of about 250 µm and therefore the brush adds considerably to the surface area that pushes the water surface dimple during rowing (typical rowing is without surface breaking). The "brushes" are the most developed on the distal tibia and proximal tarsus: the leg's sections (near the tibio-tarsal joint; rectangle in Fig. 6-2a, b) that actively push the water meniscus during locomotion (see below). Forelegs and hindlegs do not have this structure.

Observations of *Ptilomera tigrina* in the natural habitat show that their midlegs are not used for support on the water surface. They are used for rowing against the current (Fig. 6-3a, b), apparently adjusting the strikes' strength and frequency to the flow speed such that an individual can remain in approximately the same location for an extended time period resulting in high stroke frequency (4.24 ± 0.79 strokes per second; mean \pm SD, n = 6) in fast-flowing water and a relatively low stroke frequency (1.96 ± 0.35 strokes per second; mean \pm SD on slow-flowing water (Fig. 6-3c). The main section of midlegs that produces the most prominent meniscus during rowing is the section comprising distal tibia and proximal tarsus. While midlegs are normally not used to support the body (e.g., Fig. 6-2a), they may serve this function when the water strider uses forelegs to handle captured prey (Fig. 6-3d). When a potential food item is brought on the surface with the flowing current then the individual makes extra strides to examine the potential food item and returns if the item is not edible. When a food item is captured, a water strider holds it between the femur and tibia of both forelegs making it impossible for the forelegs to support the body. In this situation, water striders use midlegs to support the body or to push the body forward using an asymmetric mode of striding when one midleg pushes the insect while another is extended forward to provide support on the surface.

On very slow-flowing water and still water surface, the undisturbed water striders use midlegs to slowly push their body back and forth (Fig. 6-3e), which results in almost constant movements of the insect body on the water surface (an example of striding frequency was about 1 back-and-forth cycles /sec). However, when disturbed by visual or tactile (through water waves) stimuli indicating potential danger, the species performs super-fast back and forth, as well as diagonal, movements powered by the midleg's brushes in an apparent antipredatory "protean"⁶² type of behavior with back-and-forth strides occurring in various directions at a relatively high frequency (up to 4.3 strides/sec). This behavior occurs in both flowing and still water and it is triggered only by stimuli indicating potential danger.

6.3.2. Preferences for water flow speed in natural habitats

During the survey of water flow speed preferences, water striders were only observed at locations with water flow ranging from ~0.1 to ~0.6 m/s, while the full range of recorded surface water flow speeds ranged from 0 (still water in small "bays" at the edge of the stream, separated by rocks/plants from the main current) to ~0.8 m/s (in the middle of rapid flow between rocks). Individuals of *Ptilomera tigrina* were observed especially often, relative to the observed frequency of flow speeds at sites without water striders, in the flow speed range from ~0.15 to ~0.30 m/s (Fig. 6-4a-c; see also Figure 6-S3a in Supplementary Materials Part 4). The frequency distribution of flow speed for "*Ptilomera* present" sites was statistically significantly different (Kolmogorov-Smirnov test, D = 0.29133, *p*-value = 0.008761; see Supplementary Material Part 4 for additional Chi-square tests), and the effect of water flow velocity on the probability of the water strider's occurrence can be described by quadratic logistic regression (*p*<0.0001; Fig. 6-4d; Table S2 in Supplementary Materials Part 4). In summary, the results illustrate that water striders avoid still water and prefer water flow of ~0.15 to ~0.30 m/s, albeit they are also observed maintaining their positions in faster water flows of up to ~0.6 m/s.

6.3.3. Behavior on still and flowing water in experimental containers

The water striders tried to escape from the container with still water significantly more often than from the container with flowing water (Fig. 6-5; Mann-Whitney test, two-tailed, U = 27.5, $U_{critical p<0.05} = 59$; z = 3.36056, p = 0.00078). Their locomotory behavior differed between the two types of containers in a manner consistent with field observations. They pushed their bodies back-and-forth in the containers with still water, and they performed forward strides against the current in the containers with flowing water (Supplemental Movie 6)

6.4. Discussion

All the results are consistent with the idea that *Ptilomera tigrina* water striders' water flow preferences and their typical locomotory striding behaviors involve constant rowing performed by midlegs, and that midlegs are normally

not used for support on the water surface. The preferred range of water flow speeds (0.15-0.3 m/s) observed in our study is higher than the preferred range by *A. remigis* (~0.1 m/s⁸⁹), the species that also lives in streams and represents the differentiation of hairs on the midlegs typical for water striders in the most commonly studies genera, *Gerris* and *Aquarius*⁴⁰. As we did not observe midlegs being used for support on the water surface (except in special situations involving prey handling with forelegs), we propose that, unlike in the "typical" water striders (e.g., genera *Gerris, Aquarius*), who use midlegs for support on the unbroken water surface as well as for rowing, the main function of midlegs in *P. tigrina* is just rowing. Therefore, we hypothesize that the unique taxon-specific brush of ribbon-like hairs on *Ptilomera*'s midleg tibiae and tarsi^{35–37,145} (Fig. 6-2b-e; also in Chapter 5 of this thesis) is an adaptation to consistent rowing against relatively fast water flow (without water surface breaking), rather than to supporting insect body on the water surface.

Consistent with this interpretation is the absence of similar structures in A. paludum⁷³ (Fig. 6-S2c) and other species of Aquarius or Gerris^{17,40,71,72,190}, which are the "typical" water striders who use midlegs for two functions: supporting the insect body on the unbroken water surface and rowing 17,28,40,41 . Also, the lack of these structures on P. *tigrina's* (Chapter 5 of this thesis) as well as other Ptilomerinae's 36,37,145 fore and hind legs, which do not provide thrust during rowing (but are used for support on the water surface), is consistent with this hypothesis. Finally, these structures appear to be the most developed in *Ptilomera* on the distal tibia and proximal tarsus near tibio-tarsal joint (our observations and information in Cheng & Fernando³⁶ and Jehamalar et al.³⁷) i.e., along the ventral edge of the midleg's section that actively pushes that water meniscus during locomotion. Therefore, their adaptive function clearly seems to be related to the crucial role of midlegs in constant rowing against the relatively fast and moderate water flow speeds preferred by *P. tigrina* in their natural habitats, and also in slow constant rowing on still water as well as super-fast "protean" locomotion¹⁸⁹ in response to danger. This fast antipredatory locomotion is different from antipredatory vertical jumps/leaps in water striders of genus Gerris or Aquarius because the antipredatory escape locomotion observed in A. nebularis and A. conformis^{80,107} involves a few seconds of several chaotic jumps that quickly take the water strider away from danger, while Ptilomera's locomotory response to danger involves constantly repeated and extremely fast horizontal back-and-forth movements at approximately the same location over an extended period. This suggests the importance of persistent rowing for survival. Although an experimental laboratory-based comparison of striding performance by individuals with the brushes removed and with brushes intact is needed to completely evaluate this hypothesis, our observations in the natural habitat suggest an adaptive match between the preferences for the situations requiring frequent fast movements powered by rowing on the water surface and the presence of the specialized midleg "brushes" of ribbon-like setae that function as paddles for rowing (and are not used for support on the water surface) by P. tigrina.

Although the preferred flow speeds are larger than in the other quantitatively studied water striders⁸⁹, they are intermediate considering all ranges of flow speeds present in the *Ptilomera's* habitat. We did not observe individuals of *P. tigrina* in areas of still water and near-stagnant (near-zero water flow speed) even though those areas are more likely to be located near vegetation on the creek's edge where shadows are more prevalent and might provide protection from the sun (*Ptilomera* tend to occur in more shady spots; T.A. Duc, personal observations). *Ptilomera's*

avoidance of the fastest flowing water is consistent with the optimal foraging reasoning by Rubenstein⁸⁸ who observed that *A. remigis* individuals chose to spend time in the water current that is sufficiently fast to bring food items at a relatively high rate to the vicinity of an individual, but sufficiently slow to permit individuals to stay in it for an extended period using frequent rowing against the current. As we have not measured the rate of food items' arrival in various flow speeds, we are unable to fully evaluate this hypothesis, except for direct video evidence that the rowing strike rate is obviously faster in faster currents. Additionally, we cannot evaluate the likely hypothesis that a hungry individual chooses a faster flow that provides more resources and when satiated or exhausted by rowing it moves to the slower flowing parts of the creek. A bimodal shape of "*Ptilomera* present" flow speeds' distribution (Fig. 6-4b) is consistent with, but does not fully confirm this hypothesis. It is also possible, albeit never studied, that faster flow brings more micro-debris that can clog the setae on the water striders' legs and this may be an additional cost of staying in fast-flowing water. Naturally, we cannot exclude the opposite scenario, that stagnant and slow water at the edges of creeks accumulates debris that can clog the micro-structures on water striders' legs, and faster water flow cleans the legs from this debris. *Ptilomera tigrina* seems to be a good species to test those hypotheses in the future.

Hair fringes generally similar to the "brushes" in *Ptilomera* are also present in another subfamily of large stream-dwelling water striders, Cylindrostethinae (T.A. Duc, personal observation), as well as in a subfamily of small water striders (Halobatinae) with a body mass of only a few milligrams^{20,40} (Kim W, Jablonski PG. personal observations). They apparently need powerful rowing performance as some of the species live in relatively fast freshwater creeks (e.g., genus *Austrobates*¹⁹⁸), and others on the surface of oceanic waters (e.g., genus *Halobates*^{17,20,40}) where requirements of the physical habitat may create the need for powerful rowing performance by these small-bodied insects. Similar to Ptilomerinae, the small marine water striders, Halobatinae, also do not seem to use midlegs for the main support of their body on the water surface⁴⁰ but only touch the surface during resting position (Kim W, Jablonski PG. personal observations on *Asclepios*). As the typical habitats in the subfamily of Ptilomerinae appear to be fast-flowing creeks and streams^{17,21,36,37}, we predict that similar local preferences for water flow speed and similar locomotory behaviors may be discovered in future quantitative studies of other species of Ptilomerinae, and maybe also in other water strider species from fast-flowing creeks. We predict that the main function of their midleg's microstructures on tarsi/tibiae may turn out to be rowing rather than support on the water surface.

Our observations of *P. tigrina* in its natural habitats appear to challenge some of the recently promoted research paradigms in modern interdisciplinary science of new hydrophobic materials and water walking devices, which are inspired by truly simplified knowledge about behavior and midleg morphology of "typical" water striders, *Aquarius* and *Gerris*^{16,30,49,71–76,195,31,33,34,38,43,44,46,48}. The paradigm accepts a simplified notion of a midleg as an elastic rod/tube, covered with a relatively non-differentiated layer of hairs (with nano-grooves) that simultaneously serve two functions: providing general hydrophobicity for the support of the insect's body on the unbroken water surface and pushing the unbroken meniscus of water surface backward during rowing locomotion. Although those new studies bring a crucial new level of quantitative understanding of the physics of water strider's hydrophobicity and

locomotion, they ignore the already known diversity among water striders. Our study reminds the scientists that this simplified approach has a narrow empirical basis, and that a shift of paradigm is needed to incorporate not only the long-existing knowledge on hair differentiation on the power-providing midlegs in the two "typical" genera⁴⁰. More importantly, we argue that scientists and engineers need to incorporate the view that many water strider taxa have legs with diverse sophisticated and specialized micro and nano structures (as revealed in numerous taxonomic studies) that very likely serve different specific functions required for life and locomotion in their species-specific semi-aquatic habitats. The current focus of engineers on the largely simplified view of midlegs (of *Gerris/Aquarius*) that likely represent an adaptive trade-off between adaptations to supporting insect's body on the unbroken water surface and adaptations to propelling the insect in striding locomotion does not allow to reveal the micro-designs of legs optimized specifically for support on water surface separately from the micro-structures optimized specifically for rowing locomotion. We propose that a collaboration between taxonomists and ethologists during intense field studies in natural habitats, and a full consideration of microstructural complexity of fore, mid and hind legs can provide new discoveries and ideas for novel morphological designs for bio-inspired technology of water walking devices.



Fig. 6-1. Observational and experimental methods. (a) – An example of flow speed sampling. The "*Ptilomera* present" sites (red) were chosen based on the presence of insects upon arrival of the observer who evaluated the presence of insects from a distance without disturbing their normal behavior. The "*Ptilomera* absent" sites (light gray) were chosen semi-randomly taking into account that they should include a full range of visually estimated flow speeds from still/nearly stagnant to high-speed flow, and that together with "*Ptilomera* present" sites they should cover the full creek surface area without a noticeable bias. (b) – photo of the experimental bowl. (c) – an example of an experimental set-up in a creek with two bowls representing two treatments: "Still water" and "Flowing water", and with cameras set on tripods to film the experiment. All photos by W. Kim & P.G. Jablonski



Fig. 6-2. The study species, *Ptilomera tigrina*, and brushes of ribbon-like hairs on its midlegs interacting with water surface during locomotion. (a) – a female of *Ptilomera tigrina* in a natural habitat with the tibia-tarsus joint marked with a white rectangle; notice that midlegs are not used to support the insect's body on the water surface; (b) – zoomed-in view on the tibia-tarsus joint; notice the presence of "hair brushes" on both the distal tibia and proximal tarsus; (c, d, e) – Scanning Electron Micrographs of the brush of ribbon-like hairs on midleg tibiae and tarsi shown at different magnifications (separate scale bars shown in each panel). Notice nano-groves on the surface of the ribbons in (e). Photos in a, c by WK & PGJ; photo in b by NPD; SEM images in d, e by PGJ, SIL



Fig. 6-3. The behavior of *Ptilomera tigrina* in natural situations based on screenshots from the slow-motion movie frames and photos. (a) – a water strider is pushed back by the water current and it regularly moves forward pushed by the midlegs' stroke (two images were combined to illustrate the two positions of the insect); (b) – a composite image illustrating a typical sequence of midleg positions during striding against current; (c) – comparison of stroke frequency on slow and fast water; (d) – a photo illustrating that midlegs are used for support when forelegs are used for prey handling; (e) – a photo-based schematics of typical back-and-forth slow striding on still and near-stagnant water surface. (a) and (b) are composite images created from several frames in movies. Photos by W. Kim and P.G. Jablonski



Fig. 6-4. Water flow preferences of *Ptilomera tigrina* water striders and their behavior on still water. (a) – An example of natural habitat with water flow speed measured at *Ptilomera* present and *Ptilomera* absent sites; (b) – distribution (% of locations) of flow speeds at *Ptilomera* present (solid line, circle markers) and *Ptilomera* absent (broken line, square markers) locations; (c) – degree of preferences calculated as % for *Ptilomera* present *minus* % of *Ptilomera* absent. The gray area in b and c indicates the range of flow speeds that are preferred by water striders; (d) – Results of quadratic logistic regression of presence/absence of *P. tigrina* as a function of water flow speed; red and gray circles represent empirical data of *Ptilomera* present and *Ptilomera* absent sites; gray vertical arrows and gray box along the x-axis indicate the range for which probability of *P. tigrina's* presence is larger than 0.5. Data file and a basic histogram of the frequencies of flow speeds for *Ptilomera* absent and *Ptilomera* present sites, as well as details of statistical analyses are presented in Supplementary Materials Part 4. Photo in (a) by P.G. Jablonski



Fig. 6-5. The behavior of *Ptilomera tigrina* water striders in artificial containers with still and flowing water located in the natural habitat. The effect of water flow on the tendency to get away from an experimental container: number of jump attempts/min in the first 5 minutes of test in two treatments: still (stagnant) water and flowing water. The circles indicate data points, the circle's diameter represents the number of data points of the same value, the horizontal thick line indicates the median value. Sample sizes are n = 15 and n = 14 for stagnant and flowing treatment respectively. The data file is provided in Supplementary Materials Part 7.

SUPPLEMENTARY MATERIALS PART 1:

Morphology of P. tigrina

Table 6-S1. Morphological measurements of the study species, *Ptilomera tigrina*,captured near Melinh Station for Biodiversity. Mean \pm S.D. was calculatedfor each sex. Hindlegs' wetted length is a total length of tibia and tarsus.

	Sex	Male (n=13)	Female (n=12)
Body mass (r	ng)	114.8 ± 29.5	106.8 ± 17.7
Body length (mm) (from abdomen tip to the top of the head)		17.4 ± 1.0	17.1 ± 0.6
Foreleg	femur length (mm)	10.0 ± 0.5	9.3 ± 0.5
	tibia length (mm)	6.7 ± 0.7	6.4 ± 0.4
	tarsus length (mm)	6.8 ± 0.4	6.6 ± 0.3
Midleg	femur length (mm)	25.3 ± 0.9	23.3 ± 0.6
	tibia length (mm)	15.6 ± 0.6	14.3 ± 0.5
	tarsus length (mm)	8.3 ± 0.5	7.9 ± 0.4
Hindleg	femur length (mm)	29.8 ± 1.5	26.0 ± 0.8
	wetted length (mm)	19.2 ± 1.4	16.6 ± 0.8

SUPPLEMENTARY MATERIALS PART 2:

Study sites and examples of the natural habitats of Ptilomera tigrina



Fig. 6-S1 Study sites and examples of the natural habitats of *Ptilomera tigrina* (a) - Location of the main study site (Melinh Station for Biodiversity) and the additional study site ("Thac May"); (b) - examples of sections of the creek comprising natural habitats of *Ptilomera tigrina* near the Melinh Station for Biodiversity. Photos in (b) show fragments of the natural habitat of *Ptilomera tigrina* near Melinh Station for Biodiversity. In each photo in (b) various specific locations on the water surface within an image have different water flow speeds. An example of a typical distribution of different water flow speeds within the creek is shown in the main text's Fig. 6-4a. Photos in (b) are by P. G. Jablonski; the map in (a) – courtesy of E. Sterling (New York) and K. Koy (Berkeley) to HTP

SUPPLEMENTARY MATERIALS PART 3:

Additional images of P. tigrina and A. paludum



Fig. 6-S2 A male of *Ptilomera tigrina* shown to illustrate the species' size (**a**) and midlegs of *Ptilomera tigrina* with brushes of ribbon-like hairs (**b**, **d**) compared the midleg of *A. paludum* (**c**) without brushes. Unlike *P. tigrina*, *A. paludum* uses midlegs both for support on the water surface and for rowing, and does not have the brush of ribbon-like setae. The images of (**b**) and (**c**) are scaled to make the leg vertical radius of relatively similar size in the two images to compare the relative length of the ventral setae between the two species. In (**d**), the SEM image shows the cross-section of tarsus of *Ptilomera tigrina* midleg with the brush of ribbon-like setae visible extending downwards towards the water surface

SUPPLEMENTARY MATERIALS PART 4:

Water flow speed preferences



Fig. 6-S3 Comparison of distributions of water flow speed for *Ptilomera* present and *Ptilomera* absent sites in the natural habitats at Melinh Station for Biodiversity in September 2016. (a) Histogram of the distributions; (b) Results of quadratic logistic regression: Presence probability = $1/(1+\exp(-(-2.305+28.452*Vel-57.581*Vel^2))))$, where "Vel" indicates water velocity (m/s); (c) Frequency tables: Table S3 is based on 4 categories of water flow speed and the corresponding Chi-square test for comparison between *Pitlomera* present and *Ptilomera* absent distributions is shown below the table; Table S4 is based on 5 categories of water flow speed and the corresponding Chi-square test for speed and *Ptilomera* absent distributions are shown below the table; Table S5 is based on the same categories of water flow speed that are shown in Fig. 6-S3a. We also run the Kolmogorov-Smirnoff test on the raw data and it resulted in Kolmogorov-Smirnoff test statistic, D=0.29133, p=0.008761 (in R version 3.6.1).

SUPPLEMENTARY MATERIALS PART 5:

Data set of stroke frequency

Video	Flow speed	Stroke frequency (st/s)	Strokes	Duration (s)
C0194	Fast	3.764706	8	2.125
C0195	Fast	3.294118	7	2.125
C0197	Fast	4.235294	9	2.125
C0223	Fast	5.647059	12	2.125
C0226	Fast	4.235294	9	2.125
C0229	Fast	4.235294	9	2.125
C0198	Slow	2.352941	5	2.125
C0200	Slow	2.352941	5	2.125
C0201	Slow	1.882353	4	2.125
C0202	Slow	1.882353	4	2.125
C0216	Slow	1.882353	4	2.125
C0231	Slow	1.411765	3	2.125

SUPPLEMENTARY MATERIALS PART 6:

Data set on flow speed preferences

Ptilomera Present/Absent	Flow speed m/s	Ptilomera Present/Absent	Flow speed m/s	Ptilomera Present/Absent	Flow speed m/s
Present	0.0868	Present	0.2533	Absent	0.0555
Present	0.1954	Present	0.2064	Absent	0.0095
Present	0.0872	Present	0.146	Absent	0.1591
Present	0.225	Present	0.0731	Absent	0.1228
Present	0.2589	Present	0.1184	Absent	0.3876
Present	0.0819	Present	0.0996	Absent	0.037
Present	0.0695	Present	0.0854	Absent	0.0728
Present	0.099	Present	0.1086	Absent	0.0507
Present	0.1964	Present	0.4119	Absent	0.0653
Present	0.2495	Present	0.3589	Absent	0.0732
Present	0.3069	Present	0.5702	Absent	0.0507
Present	0.2319	Present	0.4923	Absent	0.0653
Present	0.2784	Present	0.2357	Absent	0.2847
Present	0.1852	Present	0.3022	Absent	0.228
Present	0.2753	Present	0.1989	Absent	0.3921
Present	0.2152	Present	0.1415	Absent	0.4202
Present	0.132	Present	0.2162	Absent	0.0442
Present	0.1504	Present	0.1205	Absent	0.1322
Present	0.1846	Present	0.1385	Absent	0.1273
Present	0.0966	Present	0.0908	Absent	0.0422
Present	0.0578	Absent	0.4324	Absent	0.5995
Present	0.2282	Absent	0.4369	Absent	0.4273
Present	0.2414	Absent	0.1654	Absent	0.4213
Present	0.235	Absent	0.6432	Absent	0.3554
Present	0.2523	Absent	0.5864	Absent	0.2057
Present	0.0864	Absent	0.2816	Absent	0.0185
Present	0.1751	Absent	0.6902	Absent	0.4085
1		1		1	

Present	0.0893	Absent	0.1027	Absent	0.4573
Present	0.2473	Absent	0.0569	Absent	0.0389
Present	0.301	Absent	0.73	Absent	0.0844
Present	0.215	Absent	0.4665	Absent	0.0312
Present	0.154	Absent	0.151	Absent	0.0477
Present	0.1058	Absent	0.4128	Absent	0.0261
Present	0.2164	Absent	0.0548	Absent	0.087
Present	0.4353	Absent	0.0647	Absent	0.128
Present	0.2844	Absent	0.0289	Absent	0.3921
Present	0.2177	Absent	0.0701	Absent	0.1081
Present	0.103	Absent	0.1696	Absent	0.4728
Present	0.3122	Absent	0.0172	Absent	0.4649
Present	0.2826	Absent	0.2262	Absent	0.6594
Present	0.1811	Absent	0.4363	Absent	0.199
Present	0.1207	Absent	0.1996	Absent	0.104
Present	0.1912	Absent	0.5525		

SUPPLEMENTARY MATERIALS PART 7:

	N / .		
Date	Location	Flowing/Still Water	Ni Jumps/min
JUN/23/19	May Waterfall	F	0.00
JUN/23/19	May Waterfall	F	0.00
JUN/23/19	May Waterfall	F	0.00
JUN/23/19	May Waterfall	F	0.00
JUN/23/19	May Waterfall	F	0.00
JUN/23/19	May Waterfall	F	0.00
JUN/23/19	May Waterfall	F	0.00
NOV/16/19	Melinh Station	F	0.00
NOV/16/19	Melinh Station	F	0.00
NOV/16/19	Melinh Station	F	0.00
NOV/16/19	Melinh Station	F	0.00
JUN/23/19	May Waterfall	F	0.20
JUN/23/19	May Waterfall	F	0.40
JUN/23/19	May Waterfall	F	1.20
JUN/23/19	May Waterfall	S	0.00
JUN/23/19	May Waterfall	S	0.00
JUN/23/19	May Waterfall	S	0.00
JUN/23/19	May Waterfall	S	0.73
JUN/23/19	May Waterfall	S	1.00
JUN/23/19	May Waterfall	S	1.40
NOV/16/19	Melinh Station	S	1.40
NOV/16/19	Melinh Station	S	1.60
JUN/23/19	May Waterfall	S	2.05
JUN/23/19	May Waterfall	S	3.64
NOV/16/19	Melinh Station	S	3.80
JUN/23/19	May Waterfall	S	4.00
JUN/23/19	May Waterfall	S	10.38
NOV/16/19	Melinh Station	S	12.00
NOV/16/19	Melinh Station	S	13.60

Data set from the experiments with still and flowing water in plastic bowls

Chapter 7.

General discussion

Semiaquatic habitats have shaped various adaptive traits of water striders, Gerridae¹⁷. Locomotion of water striders on the water surface is constrained by the laws of physics^{27–32,34}. Morphological traits of hairs provide hydrophobicity of the leg and body of water striders^{71–76}, which is crucial for their semiaquatic lifestyle. However, locomotion, morphological traits, and habitat characteristics of water striders have been studied separately. In this thesis, I attempted to provide examples of links between behavioral (i.e., locomotion) and morphological adaptation under the requirements given by the habitat condition (i.e., flow speed) in large-sized water strider species: *Gigantometra gigas* and *Ptilomera tigrina*. In this chapter, I first present an overview of all the results in the thesis, and then I discuss links among the thesis chapters and how my results relate to the existing literature.

7.1. Locomotive behavior and leg geometry

First, I focused on the behaviors of the large water striders, and then I looked into the morphological details of the legs used in these behaviors. Hence, I started by examining jumping and striding: two basic locomotion modes of water striders. I found that the two large-sized water strider species jump with surface breaking, which is not covered by the currently existing theory of surface-tension-dominant jumping in water striders³⁴. The surface-breaking jump was divided into three phases: a surface tension phase, a transition phase, and a drag phase. In the surface tension phase, water striders get thrust from the surface tension, until their legs start to break the water surface. After the start of surface breaking, in the transition phase, midlegs penetrate the water surface until they are fully submerged. In the drag phase, submerged midlegs still descend, and provide upward thrust from drag with air sheathes around the legs. Hindlegs maintain a certain depth of dimples and provide continuous surface tension without surface breaking. I also observed that the two main study species differ from the smaller species in striding: *G. gigas* performs asymmetric striding whereas *P. tigrina* performs symmetric striding using the midlegs during which extremely long forelegs (tarsus) provides the support on the water surface.

In order to understand the differences between these large species and the "typical" small/medium size species, I applied mathematical modeling of the hydrodynamics of their locomotion. By modifying the surface-dominant jumping model from the precedent study³⁴, I built a mathematical model based on my observations of surface-breaking jumps. I also built a mathematical model of symmetric and asymmetric²¹ striding. From the observations of behavior and morphology of six study species, combined with the literature data from over 200 species I largely confirmed predictions from these two models. Both models predicted that when the body size increases from the typical size of *A. paludum* to the size of *P. tigrina* (hence in the approximate range of 50-80 mg) there is an allometric switch in behavioral and morphological adaptations to locomotion.

For jumping, the model predicted and empirical observations confirmed, that as the body of a species becomes heavier the water strider switch from the typical surface-tension-dominant jumps to drag-utilizing jumps with their midlegs penetrating the water surface (Chapter 2 in this thesis). In order to use the model of striding on the water to predict the adaptations to striding by large-bodied water striders across the full variety of Gerridae, I proposed a concept of "wetted leg geometry" and incorporated it in the model (Chapter 3 in this thesis). I proposed that the Gerridae species can be divided into three categories: intermediate-foreleg proportions observed in typical commonly studied small/medium species of Gerrinae, long-foreleg geometry involving elongated wetted foreleg to support the heavy body, and short-foreleg geometry with very small (or none) support from forelegs and relatively longer hindlegs. The model predicted and observations confirmed that the small/medium-sized water striders have intermediate-foreleg geometry and use symmetric striding comprising simultaneous backward movements of the two midlegs. However, for the larger species, the model predicted and observations confirmed, that the water striders either develop long-forelegs geometry and use symmetric striding observed in P. tigrina, or they develop short-forelegs geometry and use asymmetric striding observed in G. gigas, who propel their strides with one midleg (with additional help from the opposite side hindleg). As *P. tigrina* prefers relatively fast-flowing water where frequent and strong rowing is required and G. gigas lives in slower-flowing streams where frequent rowing is relatively less important than fast current, I hypothesize that habitat characteristics (flow speed) affect which of the two strategies develop in the large-bodied species: long foreleg/symmetric striding in fast current and short foreleg/asymmetric striding in slowly flowing waters.

The discovery of allometric shifts between different mechanisms of locomotion in semi-aquatic insects presents a novel contribution to the existing knowledge. As these allometric shifts have been observed across genera or families of insects that inhabit the water surface, the discovery emphasizes the importance of different forces used in locomotion depending on the semiaquatic animal body size and mass. In fluid dynamics, dimensionless numbers are used to evaluate relative contributions of different types of forces utilized by various species for locomotion on the water surface²⁹. For instance, the Bond number represents the ratio between buoyancy and surface tension, while the Weber number represents the ratio between drag and surface tension. Dolphins jumping up in the air, with Bond and Weber numbers around 10⁵, rely about 100,000 times more on buoyancy and drag than on surface tension, indicating that surface tension is negligible for them. Basilisk lizards running on water are characterized by Bond and Weber numbers of about 100. They employ buoyancy, added mass, and drag by slapping the water surface when running on the surface¹⁰⁹. Fishing spiders galloping on the water surface are characterized by Bond and Weber numbers of about 1, and use both surface tension and drag¹⁷⁸. These comparisons based on the previous studies primarily focus on allometric comparisons across wide range of body sizes and across large diversity of animals from different families, orders, and even phyla, where significant differences in body mass and locomotion are clearly expected. While there have been detailed studies on specialized gaits in fishing spiders¹⁹⁹, these differences appear between families, not species. In contrast to these previous research, my study demonstrated that even within the same family (Gerridae), or even at the subfamily level, such as in Gerrinae, there can be significant body size difference that lead to the evolution of different locomotion strategies and the use of different forces in locomotion.

Predation avoidance and habitat physical characteristics contribute to these body-size dependent allometric changes in the locomotion mechanisms. It was previously believed that water strider species had Weber numbers below 1²⁹. However, my results indicate that the jumping locomotion of two large water strider species, *G. gigas* and *P. tigrina*, is characterized by Weber number values over 1. This finding suggests the previously unreported relative importance of drag in the jumping of water striders. Additionally, I discovered that medium-sized species like *A. paludum*, with body sizes near the range where the allometric switch between locomotion strategies is predicted, exhibit behavioral plasticity and employ various types of striding locomotion, including symmetric and asymmetric striding, as well as leaping over the water surface. They switch between these striding types to optimize their sliding performance by avoiding situations of high resistance. This adaptive plasticity of locomotion behavior has not been previously reported despite extensive studies on the striding behaviors of this species world-wide. Hence, I think that my study highlights the importance of detailed observations and experiments to unravel the locomotive mechanisms of semiaquatic organisms traditionally used as study subjects.

7.2. Micro-scale morphology: hair structure

After the empirical observations and theoretical models provided insights into the behavioral and macromorphological adaptations of large water striders to locomotion on the water surface in their natural habitats, I examined the details of legs' micro and nano structures relevant to locomotion and support of heavy body on the water surface. I found that several special hair structures that are present in the two large species are absent or less developed in the "typical" small/medium size species from genera *Gerris* and *Aquarius* (including *A. paludum*).

First, in Chapter 4, I established that the giant water strider, *G. gigas*, has in general more differentiated and more developed setae on their wetted segments of midlegs and hindlegs than those on the legs of water striders in genus *Gerris* and *Aquarius* (including *A. paludum*). Second, the giant water striders' midlegs used in rowing and jumping, have especially long and bent setae, and their hindlegs have a beam-like structure consisting of overlapped leaf-shaped setae and associated several types of setae. Third, the midlegs of *P. tigrina* have ribbon-like hairs on their ventral wetted segments, which are used for rowing but not for the support on the surface (Chapters 5, 6). Ribbon-like setae are very long and together with several associated types of setae, they form a functional oar which importantly contributes to thrust as demonstrated in the ribbons removal experiments (Chapter 5).

7.3. The link between behavior, leg geometry and micro-morphology

Striding and jumping of the world-largest water striders, *Gigantometra gigas*, that are different from the "typical" small/medium size species, are closely related to the micro-morphological traits observed on their legs. The theoretical model of jumping showed that surface breaking by midlegs during a jump is crucial in heavy water striders for creating upward force sufficiently strong to power the jump that provides escape from predators (Chapter

2 in Fig. 7-1). Without the upward drag force from the midlegs moving downward in the body of water (after surface breaking) the jump would be too slow to escape from attacking predators.

From the force profiles in the simulation model, it can be determined that midlegs provide a drastic increase of upward force in the early stage of a jump that includes surface tension phase followed by drag force phase, while hindlegs provide a continuous stable upward force during the whole jump. The large projected area of a thrusting leg is important to produce high drag force, and the long setae on the midlegs of *G. gigas* provide such a large projected area by capturing air sheath around the midlegs (Chapter 4 in Fig. 7-1). During upward jumping, the hindlegs of *G. gigas* do not have long setae but a specialized beam structure consisting of leaf-shaped setae to maintain stable menisci on the water surface. The smooth outline made by leaf-shaped setae with their aligned nano-grooves (parallel to the direction of sliding) may reduce resistance when hindlegs slide out from the water surface in the last stage of a jump (Chapter 4 in Fig. 7-1).

Another distinct locomotion of *G. gigas* is asymmetric striding (Chapter 3 in Fig. 7-1). The mathmatical model for striding shows that the short forelegs of *G. gigas* cannot provide enough force to support the anterior part of their heavy body. Therefore, *G. gigas* use one midleg extended forward to support their body while the second midleg (together with a contralateral hindleg) thrusts. One midleg creates a lower thrust than two asymmetrically working midlegs. Therefore, the long and highly inclined setae on the ventral side of a thrusting midleg could improve this one-midleg-stroke, by providing large vertical leg diameter extension (Chapter 4 in Fig. 7-1). Large vertical leg diameter is advantageous in creating thrust for locomotion by using the drag force^{30,49} and from the curvature force on the surface¹⁶. Leaf-shaped setae on hindlegs can also provide a large vertical leg diameter (Chapter 4 in Fig. 7-1). The orthogonal direction of nano-grooves to the thrusting direction may also contribute to increasing the role of the viscous force in rowing. Two rows of thorn-shaped setae beside the beam-like structure of leaf-shaped setae indicate they may support the beam structure during asymmetric thrusting.

During striding, sliding resistance is important as it affects the deceleration of sliding and determines the distance and speed of sliding. Asymmetric striding is advantageous from the viewpoint of sliding resistance. The direction of nano-grooves on leaf-shaped setae is parallel to the sliding direction and the beam-like structure makes a smooth air-water interface, suggesting that it may help in reducing resistance during the sliding of *G. gigas*. Another noticeable characteristic is the bending of the tip of long macrosetae on midlegs. The contact force is larger when the leg is sliding in the opposite direction of the tilted setae^{179,180}. However, the midlegs of *G. gigas* should slide on the water surface in both longitudinal directions: they should be pulled out of the water body during jumping and they should advance forward in asymmetric striding. The long macrosetae provide better chances that the tip of a macrosetae may reduce sliding resistance by being bent naturally according to the sliding direction of a midleg in jumping and striding.

Ptilomera tigrina exhibits another example of the link between locomotive and micro-morphological traits. *P. tigrina* has long forelegs to support its heavy body while two midlegs provide the thrust (Chapter 3 in Fig. 7-1).

Symmetric stroke of the midlegs and the subsequent sliding with hindlegs and forelegs creates higher resistance than asymmetric striding, but these insects can use two midlegs to provide high thrust sufficient for pushing the heavy body forward on the typically fast-flowing current in *P. tigrina*'s habitats where strong and very frequent strides are needed. I hypothesize that in this condition, natural selection has favored the development of special hair brushes consisting of ribbon-like setae that substantially increase thrust (Chapter. 5 in Fig. 7-1). Midlegs equipped with the hairbrushes created extreme anteroposterior asymmetric dimples (the surface of water almost folded in the crossectional view) when they thrust, suggesting that they can fully exploit the surface tension to produce their horizontal thrust, without wasting it in the vertical direction. This apparent ability to produce high thrust allows this species (Chapter 5 in Fig. 7-1), as well as other members of the whole subfamily Ptilomerinae with similar morphological traits, to occupy the fast-flowing waters. Fast-flowing water may bring more food particles than slow-flowing stream does²³ and this benefit may outweigh the energetic costs of frequent striding especially when hair brushes increase stroke efficiency, associated with small deceleration from their heavy mass and long-foreleg geometry.

They also use special anti-predatory movements, 'protean'¹⁸⁹ locomotion (Chapter 6 in Fig. 7-1), in response to stimuli from above that indicate danger. This behavior comprises a series of super-fast chaotic back-and-forth striding, that is not observed in either G. gigas or the smaller typical species from genus Gerris or Aquarius (e.g., A. *paludum*), and are powered by the midlegs equipped with hairbrushes that increase the thrust force needed in those antipredatory movements. Smaller species typically use upward jumps or series of jumps in this situation^{80,107}. However, as the theoretical model indicates, and observations confirm, the take-off delays during jumps by the large-sized water striders are longer than those of small/medium-sized water striders, suggesting that fast semirandom anti-predatory movements on the surface may be more favorable than upward jumping for P. tigrina considering their ability to obtain high thrust using their hair brushes. Additionally, the surface waves on the water created by their strides combined with the natural turbulence of fast-flowing streams make it difficult to for the predators that visually hunt from above and below the water surface to track the position of the water strider. We are not aware of this type of powerful protean antipredatory striding on the surface in other species of small (*Gerris*), medium (Aquarius), or even large body size (G. gigas), who do not have midleg brushes and typically use upward jumps or a series of upward jumps that take the insect away from danger. Hence, the presence of hair brushes might have promoted the evolution of this unique behavior, and once it evolved the predation avoidance context might have additionally contributed to the further evolution of the hair brushes to provide thrust for the protean striding. Ptilomera may use the drag-utilizing upward jumps only in a situation when the danger comes from below the water surface.

There have been several hypotheses regarding the role of different morphologies and leg elasticity in water striders. The elasticity of the legs may vary among species, depending on their jumping behaviors. Smaller water striders, which aim to avoid breaking the water surface³⁴, require flexible legs to prevent breaking the surface. In contrast, the case of larger water striders is more complex. As they utilize both capillary force and drag, they also benefit from maintaining an unbroken surface to create a deeper dimple. However, if the surface begins to break,

having stiff legs allows them to penetrate the surface and descend faster by utilizing the accumulated elastic potential energy stored by surface tension. This may contribute to high acceleration during the initial drag phase, which is crucial for generating larger drag since the downward velocity of the leg affects drag quadratically. These factors may play an important role to enhance the jumping performance of larger water striders.

The midlegs of G. gigas are used in the most versatile manner among the legs of water striders. While the midlegs of most water striders are primarily adapted for rowing, as seen in species like Halobates²⁰ and Ptilomera⁵³, the midleg of G. gigas serves multiple purposes. In addition to its role in jumping and thrusting, the midleg of G. gigas also needs to slide on the water surface in a direction opposite to the hair growth, due to its use of asymmetric striding. A. paludum also employs asymmetric striding but at a slower velocity of less than 0.5 m/s, unlike the fast striding exceeding 1 m/s of G. gigas. Furthermore, G. gigas does not employ leaping during fast striding, whereas A. paludum utilizes leaping at high speeds. As a result, the midleg of G. gigas has to slide rapidly on the water surface while also contributing to thrusting. In terms of jumping, the flexible long macrosetae on the midleg may easily get pressed when the leg descends, causing a change in the functional shape of the descending legs (including air bubble around the legs) and resulting in higher drag coefficients if the advancing part of the hairs gets pressed and flattened. Additionally, G. gigas has a greater variety of hair types on its midleg compared to typical water striders. The short and stiff hairs present on legs may interact with the water when the long flexible hairs are pressed enough during jumping, leading to a bumpy outline of the lower leg surface facing the water surface and advancing against the water during the drag phase of jumping. This, in turn, could contribute to increased drag during jumping. The long flexible macrosetae may prevent water or debris from penetrating between the hairs when G. gigas slides asymmetrically on the water surface, as they can bend in a direction not opposite to the advancing direction, may also potentially providing a self-cleaning or anti-fouling mechanism.

This putative self-cleaning or anti-fouling characteristic may be observed in their hindleg, reminiscent of the riblets found in sharks²⁰⁰ (also including drag reduction). Since the hindleg is constantly sliding on the water surface in their lotic habitats, it may be relatively more challenging to keep them clean compared to the midlegs, which can be cleaned using other legs such as the forelegs. In sliding, the hindleg should reduce sliding resistance, and the phenomenon of pulling the surface upward near the tip of the hindleg may help reduce drag by smoothly connecting the surface to the free surface at the end of the hindleg.

7.4. Contribution of the results to the understanding of allometry and plasticity of locomotory behavior of water striders

The two mathematical models of hydrodynamic processes provide theoretical predictions regarding the allometric shift between different mechanisms of locomotion as the body size of water strider species becomes larger. To my knowledge, this is the first comprehensive theoretical analysis of the sliding phase and drag-utilizing jump of water striders, and it is the first study of the large water strider species. Both models predict that physical constraints from the water surface properties cause the shift in behavior and morphology within the range of body
sizes between the size of *A. paludum* and that of *P. tigrina*. Two large-sized water strider species, *G. gigas* and *P. tigrina*, should use locomotive behaviors that are different from smaller species to survive on the water surface (Chapter 2, 3 in Fig. 7-1).

This hypothesis is consistent with the leg length data across Gerridae species from Matsuda⁵²: proportional length of forelegs increases in Ptilomerinae and decreases in Gerrinae when their body size increases. Asymmetric striding mode has the lowest sliding resistance with the disadvantage of low thrust, this mode would be favorable for long-distance sliding. Symmetric striding mode with long forelegs has moderately low sliding resistance with maintaining high thrust, this mode would be favorable for fast-flowing habitats. Living on the flowing water where the food items can be frequently encountered on the water surface⁸⁸ would be advantageous for large-sized water striders with high daily energy expenditure²⁰¹⁻²⁰³. Even though large-sized species should develop long forelegs or use asymmetric striding just to float on the water surface, both strategies (longer forelegs, or long midleg leg in front of the body during sliding) also reduce energy consumption to maintain a certain position on the flowing water (i.e., striding frequency⁸⁸) by reducing sliding resistance. The heavy mass of large-sized species also makes the effect of resistance less prominent on the deceleration of the sliding insect (by Newton's first law of motion). However, there are examples of the long-forelegs of small-sized taxa that leaves on fast current, *Metrocoris*⁵⁰, and on turbulent oceanic waters, *Halobates*^{20,40}. These taxa may indicate that regardless of body size in habitats of fast flowing and/or turbulent water that require frequent, strong, and efficient production of thrust by midleg strokes, natural selection for efficient locomotion may favor special "oar" structures on midlegs, which may lead to the use of midlegs to only row and no longer to support the body on the water surface and elongation of forelegs to help the reduction of sliding resistance. It may also be possible that after colonizing habitats that require frequent, strong, and efficient production of thrust for striding forward, miniaturization of body size could be beneficial, despite the increased slowing down effect of resistance, as it may increase the efficiency of thrust in producing higher velocity and acceleration of the body.

In contrast to the large species, the medium size species, *Aquarius paludum*, can 'choose' their striding type. The model predicts resistance of asymmetric sliding is smaller than that of symmetric sliding, suggesting that asymmetric striding is advantageous for long-distance sliding. Additionally, the resistance difference between the two striding types is large in fast striding. Accordingly, I observed that *A. paludum* actually 'choose' asymmetric striding in fast striding and the sliding distance and duration of asymmetric striding were longer than those of symmetric striding. This is the first demonstration that species of intermediate body size can switch between two striding modes (symmetrical and asymmetrical) in an adaptive manner. In addition, small/medium-sized species are able to use leaping to avoid high sliding resistance (also proven by the experiments in chapter 3) but large-sized species seem not to use leaping because of their large take-off delays.

Hence, the results suggest that selective pressure towards the ability to support the body on the water surface, to maintain position in fast-flowing waters, and towards decreasing resistance in locomotion might have caused evolutionary changes/switches in macro/micro-morphology and locomotor behavior as the body size increases

among the water strider species. Those changes include the switch to asymmetric gait associated with shortening of forelegs that are not used to support the body and the development of long setae on midlegs that require higher thrust-producing efficiency as only one midleg is used in a stroke. Those changes may alternatively include the elongation of forelegs for the symmetrically striding water striders with "intermediate-foreleg geometry", and a shift to using midlegs for only one function, the thrusting during locomotion, which is associated with preferences for fast-flowing water and with the evolution of special hairbrushes on midlegs. Recent discoveries in genetic backgrounds of leg elongation^{144,204} and setae morphology¹⁹ and distribution on water strider body²⁰⁴ indicate that natural selection on those aspects of morphology may easily result in adaptive changes in both macro and micro morphology that matches body size and habitat of a water strider. This selective pressure might have also maintained behavioral plasticity at medium body size, where water striders may optimize their locomotion by choosing either symmetric or asymmetric gait depending on the situation.

My theoretical models of diverse locomotion mechanisms and the observed variability of morphological and behavioral characteristics among species of Gerridae, along with their significant 100-fold differences in body sizes, highlight the previously unknown allometric shifts in locomotive mechanisms within this family compared to other taxa that live and move on the water surface²⁹. The findings from my research on several species form a solid ground for hypotheses and quantitative predictions regarding the evolutionary history of locomotive behaviors and macro (leg proportion "geometry") as well as micro (hairs) morphology used in locomotion by a variety of species within the family Gerridae. Those hypotheses can now be tested in phylogenetic comparative studies once morphological and behavioral data are compiled for variety of species across the full range of body sizes. Considering body sizes of water striders, it is reasonable to assume that the most recent common ancestor of Veliidae (a closely related clade) and Gerridae had a small body size, given that most Veliinae and many Gerrinae species reaching very large body sizes (exceeding 200 mg). Ptilomerinae is the second subfamily that includes relatively large species (exceeding 100 mg, but below 200 mg), but their phylogeny is not well studied.

Although it has been suggested that the ancestors of Gerrinae inhabited lentic water⁹⁰, it is possible that the common ancestor of these semi-aquatic Gerromorpha species lived in lotic habitats, considering that most Veliinae and many Gerrinae species reside in such habitats (Fig. 1-3). If we assume that these insects transitioned from lotic habitats to occupy the water surface habitat, it aligns with the idea that the common ancestor of Veliidae and Gerridae exhibited wing polymorphism, with some individuals having wings (macropterous) and others being wingless (apterous)⁹⁹. This is because, in lentic habitats, wings are crucial for moving to other water habitats when their current habitat becomes overpopulated or experiences drought. Conversely, in lotic habitats, being wingless is advantageous for conserving resources. While wings may still have some advantages in certain cases, the fact that the current anti-predatory behaviors of water striders are jumping (Chapter 2) and protean defense (Chapter 4, 5) indicates that wings are not helpful for rapid escape from the predator. Instead, wings primarily are used in long-distance travel to other habitats, which is unnecessary in lotic habitats such as creeks or oceans. Taken together, it appears that only Gerrinae and Ptilomerinae have evolved to increase in size (Fig. 3-6). As their body size increased,

they had to either develop elongated forelegs or adopt asymmetric striding (Chapter 3), and only some of Gerrinae shifted to lentic water habitats. Living in lentic water, Gerrinae needed to retain their wings for territorial movement, and G. gigas diverged early in from the adaptation to lentic water. It is worth noting that in Gerrinae, there seems to be a trend where species inhabiting lentic water have smaller body sizes compared to those living in both lentic and lotic water (Fig. 1-5). Furthermore, the large water strider species, G. gigas and P. tigrina, still occupy lotic habitats, suggesting a hypothesis that residing in lotic habitats is necessary to maintain their large body size due to the abundance of food supply⁸⁸ and reducing the visibility of their large bodies to predators through the turbulence of the water surface. However, it is still unclear what the advantage of having a large body size is, except for the reduction of deceleration in flowing water due to their heavy mass (Chapter 3). Two plausible explanations are that large body size is a result of sexual selection or selection for large body size to escape predators who cannot capture large water striders. In Gerrinae, both the extremely large species, G. $gigas^{21}$ and A. $elongatus^{205}$ exhibit sexual dimorphism in body size, with males being larger than females. This is in contrast to the dimorphism observed in typical water striders like A. paludum, where females are larger than males. It has been suggested that the long midlegs of male A. elongatus are for the purpose of defending territory and achieving higher mating success²⁰⁶. Therefore, the large size may not be advantageous solely for locomotive characteristics, but rather primarily for mating purposes. The locomotive behaviors, micromorphology, and habitat preferences observed can be adaptations for the maintenance of this large size. Future comparative analyses across phylogeny of Gerridae could be used to test some of these hypotheses.

7.5. General conclusion

The thesis concerned size-dependent adaptations of insects to life and locomotion on the water surface. It proposed new theoretical and empirical evidence for the link among the specific habitat, locomotion behavior, morphological traits, and size of the species. The results illustrate that natural selection optimizes the locomotion of water striders and produces different behaviors and macro- as well as micro-morphology depending on the species' habitat and species' body size. Hence, the study provided theoretical and empirical understanding that allows us to determine the range of body size where the allometric change/shift in locomotory behavior and in the associated macro/micro morphological structures occurs, and to suggest that the nature of the allometric shift depends on the habitat characteristics (i.e., flow speed/turbulence). The results also showed (for the first time to my knowledge) that species, like *A. paludum*, with body sizes located within the range where the allometric shift occurs, have evolved the behavioral plasticity that allows them to adaptively choose the locomotion mode depending on the situation. I believe that the study showed the importance of a comprehensive approach that combines theoretical modeling with detailed observations in natural habitats of a variety of water striders, including the large variety of species that may not be easily available. The results highlight the need to modify the current research paradigm in this field by shifting from the narrow focus on a few easily available small/medium size species to including the full range of body sizes, especially the large species that may face critical physical challenges to locomotion on the water surface.

These results open the possibility to formulate predictions for the next step of the research: comparative phylogenybased analyses of the evolutionary history of behavioral and morphological traits across Gerridae.

Many unsolved questions arise from the results of this thesis. I plan to address these questions in my future research. In this thesis, I suggested hypothetical functions of the micro-morphological traits of the studied species, and future experimental research has to be conducted to evaluate those hypotheses. This research should combine experiments with observations of locomotive/morphological traits against the existing literature on the hydrodynamic functions of hairs on water strider legs. It would be interesting to actually measure interacting force and test how the various types of hairs interact with flowing water. For example, the bending of midleg long setae, a directional viscous force on the hindleg's beam structure of *G. gigas*, and the thrusting mechanism involving the ribbon-like setae of *P. tigrina* are interesting subjects to be further studied. Additionally, as the large species such as *Gigantometra gigas* naturally experience small-body size at the nymphal stages of their lifecycle, I expect that they may show switching between locomotion mechanisms and behaviors as they grow.





hypothetical/observed phenomena and their connection between the size, locomotion, micro-morphology, and leg geometry of two large-sized water striders: *Ptilomera tigrina* and *Gigantometra gigas*. The contents of each chapter are enclosed by dotted lines. The effects of morphological traits/adaptations on the behavioral performance are marked with red arrows. The proposed effects of the physical properties of the substrate/habitat on adaptations to locomotion are marked with green broken line arrows. Effects on predation that exert natural selection on locomotory performance and morphological traits contributing to performance are marked with blue connecting lines with T-shaped end. Each species' locomotive/morphological characteristics are marked as colored capital letters: red G, orange P, and black T for *G. gigas*, *P. tigrina*, and typical water striders, respectively.

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Additional information

This thesis, supervised by Prof. Piotr G. Jablonski, is based on research manuscripts or published articles where I am the first author with other co-authors. Co-authors of the manuscripts on which each chapter is based (asterisks indicate corresponding authors, daggers indicate co-first authors) are as follows:

Chapter 2: Woojoo Kim⁺, Juliette Amauger⁺, Jungmoon Ha, Thai Hong Pham, Anh Duc Tran, Jae Hong Lee, Jinseok Park, Piotr G. Jablonski^{*}, Ho-Young Kim^{*}, Sang-im Lee^{*}

This chapter will be published in the Proceedings of the National Academy of Sciences (PNAS) in 2023 as Kim, W., Amauger, J., Ha, J., Pham, T. H., Tran, A. D., Jae, H. L., Park, J., Jablonski, P. G., Kim, H. Y., & Lee, S. I. (2023). Two different jumping mechanisms of water striders are determined by body size.

Chapter 3: Woojoo Kim⁺, Jae Hong Lee⁺, Thai Hong Pham, Anh Duc Tran, Jungmoon Ha, Sang Yun Bang, Piotr G. Jablonski^{*}, Ho-Young Kim^{*}, Sang-im Lee^{*}

Chapter 4: Woojoo Kim, Jungmoon Ha, Thai Hong Pham, Sang-im Lee*, Piotr G. Jablonski*

Chapter 5: Woojoo Kim, Thai Hong Pham, Nguyen Phuong Dung, Anh Duc Tran, Jungmoon Ha, Piotr G. Jablonski^{*}, Sang-im Lee^{*}

Chapter 6: Woojoo Kim, Thai Hong Pham, Nguyen Phuong Dung, Anh Duc Tran, Jungmoon Ha, Piotr G. Jablonski^{*}, Sang-im Lee^{*}

This chapter is published in the Journal of Ethology in 2022 as Kim, W., Pham, T. H., Nguyen, P. D., Tran, A. D., Ha, J., Jablonski, P. G., & Lee, S. I. (2022). Locomotion and flow speed preferences in natural habitats by large water striders, *Ptilomera tigrina*, with micro-morphological adaptations for rowing. *Journal of Ethology*, 1-11.

국문 초록

Allometry 는 서식지의 물리법칙과 유기체의 생물학적 특성으로 결정된 형태학 및 행동학적 특성이 몸 크기와 주고 받는 상호관계에 대한 연구이다. 소금쟁이과에 속하는 반수생 곤충들은 수표면이라는 특정한 환경에 살기 때문에 수면에서 활동하는 생명체의 운동을 관찰하고 수면의 물리법칙에 적응하여 습득한 형태학적 특징을 연구하기 위한 적합한 분류군이다. 소금쟁이의 수직도약과 수평이동에 대한 기존 연구들은 Gerris 및 Aquarius 와 같은 특정 분류군에 속하는 종을 대상으로 유체 역학 및 생체 역학적 원리에 초점을 맞춰왔다. 또한 몸체에 있는 미세모 구조의 유체 역학적 기능도 비교적 좁은 범위의 소금쟁이 좋에서 연구되었다. 본 연구는 동남아에서 서식하는 Gigantometra gigas 와 Ptilomera tigrina 를 관찰, 실험 및 이론적 모델링을 진행함으로서 소금쟁이과(Gerridae)의 적응을 행동, 형태 및 서식지 특성으로 설명하고자 하였다. 이 종들의 몸무게는 기존 연구에서 널리 다뤘던 종들보다 2 배에서 10 배 가량 무겁기 때문에 지금껏 보고되었던 연구 모델의 가용성은 베일에 쌓여 있었다. 소금쟁이의 수직도약을 예측하는 기존 연구 모델은 수면의 표면 장력이 도약의 주요 추진력을 제공하기 때문에 소금쟁이가 수면을 깨지 않고 도약하여 도약 속도를 향상시키고 수표면 탈출 지연시간을 줄이는 것으로 예측했다. 하지만 본 연구에서 다루는 두 대형 종은 기존의 예측과 달리 수면을 깨뜨리면서 도약하기 때문에 기존 모델에 항력과 표면장력을 모두 포함하도록 수정하였다. 이를 토대로 두 종의 수직도약을 예측해본 결과, 표면장력 외에도 항력을 추진력으로 활용해야만 수중 포식자로부터 탈출하기에 충분한 도약 성능을 낼 수 있었다. 이밖에도 소금쟁이가 수면 위에 떠있기 위한 조건과 수면 위에서 미끄러질 때의 저항을 예측하는 모델도 개발하였다. 그 결과로 무거운 소금쟁이 종은 좌우 비대칭 추진(앞으로 뻗은 하나의 중간 다리가 앞쪽 몸체를 떠받치고 반대편 중간다리와 뒤쪽의 뒷다리로 추진하는 방식)을 하거나 좌우 대칭 추진(양쪽 앞다리가 앞쪽 몸체를 떠받치고 양쪽 중간다리로 동시에 추진하는 방식)을 하되 다른 종들보다 긴 앞다리로 앞쪽 몸체를 지지해야만 수면에 떠있을 수 있었다. 본 연구에서 시행된 행동 관찰과 형태학적 측정 데이터 또한 모델 예측 결과와 일치했다. 두 종의 자세한 마이크로 털 구조체와 그 사용방식은 주사전자현미경, 광학현미경, X 선현미경, 고속영상촬영을 이용하여 관찰되었다. 흐르지 않는 물 위에 사는 일반적 크기의 소금쟁이 종인 A. paludum 과 비교했을 때, 두 거대한 소금쟁이 종의 다리 사용 방식과 털의 형태학적 특성의 일치는 두 종의 독특한 털 구조체의 적응에 대한 가설을 시사한다. *P.* tigrina 의 추진용 중간다리에 자라 있는 특수한 빗형 털 구조체는 이 연구에서 보여준 빠른 유속 서식지 선호도 및 고속 수평 이동과 관련이 있었으며 G. gigas 의 중간다리의 긴 미세모와 뒷다리의 특수한 미세모로 이루어진 빔형태의 구조체 또한 좌우 비대칭 추진과 관련이 있는 것으로 보인다. 본 연구는 이론적 모델링과 관찰 및 실험을 사용해 소금쟁이과(Gerridae)의 행동 및 형태학적 적응을 서식지 특성에 연결하여 설명한다.