University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

Eileen Hebets Publications

Papers in the Biological Sciences

January 2000

Surviving the flood: plastron respiration in the non-tracheate arthropod *Phrynus marginemaculatus* (Amblypygi: Arachnida)

Eileen Hebets

University of Nebraska - Lincoln, ehebets2@unl.edu

Reginald F. Chapman

University of Arizona, Tucson, AZ

Follow this and additional works at: <https://digitalcommons.unl.edu/bioscihebets>

Part of the [Behavior and Ethology Commons](#)

Hebets, Eileen and Chapman, Reginald F., "Surviving the flood: plastron respiration in the non-tracheate arthropod *Phrynus marginemaculatus* (Amblypygi: Arachnida)" (2000). *Eileen Hebets Publications*. 20. <https://digitalcommons.unl.edu/bioscihebets/20>

This Article is brought to you for free and open access by the Papers in the Biological Sciences at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Eileen Hebets Publications by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Surviving the flood: plastron respiration in the non-tracheate arthropod *Phrynus marginemaculatus* (Amblypygi: Arachnida)

Eileen A. Hebets, Department of Ecology and Evolutionary Biology,
University of Arizona, Tucson, AZ 85721, USA (Corresponding author)

Reginald F. Chapman, Division of Neurobiology,
University of Arizona, Tucson, AZ 85721, USA

Abstract

Specimens of *Phrynus marginemaculatus* can remain responsive when submerged in water for more than 24 hours. Behavioral data indicate that *P. marginemaculatus* utilizes dissolved oxygen from the surrounding water. Scanning electron microscopy and light microscope sections show cuticular modifications for plastron respiration. All previous examples of plastron respiration have involved animals with tracheal systems, but amblypygids respire through the use of two pairs of book lungs. This study provides the first example of plastron respiration not only in the order Amblypygi, but also, in any non-tracheate arthropod.

Keywords: amblypygid, arachnids, plastron respiration, book lungs, *Phrynus*

1. Introduction

In climates with heavy rainfall and consequent flooding, the ability of terrestrial arthropods to survive submersion for extended periods of time is a critical element of survival. Many insects survive in habitats subject to inundation through the possession of various behaviors and structures that enable them to respire underwater. Among the physical structures permitting survival underwater, plastron respiration has evolved independently a number of times among the eggs, larvae, pupae, and adults of terrestrial insects (Hinton, 1960, 1961).

A plastron is defined as a gas film of constant volume that is held on the outside of the cuticle by hydrofuge hairs or cuticular projections that provide an extensive water-air interface (Crisp and Thorpe, 1948). Although plastrons have evolved many times in insects, the only other known examples of plastron respiration are in millipedes (Diplopoda) and some mites (Acari: Arachnida) where, as in insects, gas exchange underwater involves a plastron linked to a tracheal system (Hinton, 1971; Messner et al., 1992; Messner and Adis, 1995; Adis, 1997; Adis and Messner, 1997; Adis et al., 1997). All extant arachnids, with the exception of a few mites and spi-

ders, are terrestrial, and are subject to the same flooding hazards as terrestrial insects. However, apart from these mites and spiders, no adaptations for survival underwater comparable with those found in insects have yet been discovered within the class Arachnida.

Certain habitat types and geographic locations are more or less likely to experience severe flooding. The Florida Keys consist of a 210 Km island arc located a few degrees North of the Tropic of Cancer and are considered tropical. Key West (one of the lower keys) receives an annual precipitation of 100 cm, with approximately 70% of that rainfall concentrated into only six months of the year (mid May–mid November) (Ross et al., 1992). The Keys are also often subject to severe tropical storms and Big Pine Key was one of the worst hit by Hurricane George in the summer of 1998. Coupled with the fact that most of the land is less than 2 m above sea level and the highest point reaches only 5.5 m, these conditions often result in severe inundation by the sea (Ross et al., 1992). In response to the resulting periods of submergence, many of the plant species present on the Keys are adapted to survive periods of inundation (Ross et al., 1992) and it might be expected that this would also be true of the arthropods present on the islands.

Amongst these island arthropods is the amblypygid *Phrynus marginemaculatus* C. L. Koch. Amblypygids, whip spiders, comprise the lesser known order of arachnids, Amblypygi. Although the order itself is small, containing less than 150 species, amblypygids are commonly found inhabiting tropical and subtropical habitats around the world. They are morphologically distinct animals with elongate antenniform first legs, enlarged spined pedipalps, and dorso-ventrally flattened bodies. *Phrynus marginemaculatus* is a species commonly found underneath limestone rocks on Big Pine Key, FL. These rocks are in fairly open habitats with little vertical surface upon which to climb, and thus, it would not be surprising if this amblypygid species has some adaptation to avoid drowning. In this paper, we describe the ability of *P. marginemaculatus* to survive underwater by means of a plastron connected with its two pairs of book lungs.

2. Materials and methods

2.1. Specimens

Individuals were collected during the day from underneath rocks on Big Pine Key, FL on December 31, 1997 and January 1, 1998. They were brought back to the laboratory at the University of Arizona where they were housed individually in 20 oz Dixie cups. They were kept on a 12:12 L:D light cycle at $26 \pm 2^\circ\text{C}$ and ambient humidity. They were fed two to three crickets approximately every two weeks.

2.2. Responses underwater

To determine how long *Phrynus marginemaculatus* would voluntarily remain underwater, individuals were submerged and allowed to re-surface voluntarily. Five plastic containers measuring 21 cm in diameter and 7.5 cm in height were filled with distilled water at $\sim 21^\circ\text{C}$ to approximately 4 cm deep. The water in these containers was not aerated in any way, but dissolved gases were presumably in equilibrium with air. A large rock was placed in the center of each plastic container so that only the very top of the rock was exposed to air, creating a moat around the rock. Individuals were then placed upon the rock in each of these containers and gently pushed with soft forceps into the water until completely submerged. The time until each individual voluntarily re-surfaced was recorded.

Two individuals were also placed in 600 ml beakers containing 200 ml of distilled water and allowed to remain there for over 24 hours. In these trials, air was bubbled into the beakers through a Pasteur pipet attached to an aquarium pump and individuals had no means of climbing out of the water and thus could not voluntarily re-surface.

In order to determine whether the animals could utilize oxygen from the surrounding water, we conducted a series of trials examining the responses of individuals over time in deoxygenated water. Two 600 ml beakers containing 200 ml of distilled water at $\sim 21^\circ\text{C}$ were each covered tightly with parafilm. A continuous flow of nitrogen was bubbled through the water of the test beaker via a Pasteur pipet passing through the parafilm; air was bubbled through the other beaker which acted as the control. After two hours, during which time the water in the test beaker was assumed to be completely deoxygenated, an individual was placed into the water of each beaker. The individuals were paired for size. Every two minutes, the response to mechanical stimulation with forceps of each individual was determined. If it moved location, it was scored as a positive, while if no movement was observed, it was scored as a negative. Trials lasted 10 minutes, during which time the gas flow was continued to ensure that oxygen was not replenished in the test beaker. After 10 minutes, individuals were removed from the water and placed on their backs. If an individual was able to right itself within two minutes, it was scored as a positive and if not it was scored as a negative with the assumption that it was oxygen deprived. Fourteen pairs of individuals were tested. A Cochran's Q test was conducted to determine if individual response was independent of time submerged.

In order to verify the action of a plastron as the means by which amblypygids obtain oxygen underwater, we tested the responses of individuals underwater with a wetted, and thus disfunctional, plastron. The plastron was wetted with water of low surface tension placed on the ventral side of the opisthosoma. When viewed through a dissecting microscope, the wetting of the plastron can be seen as a darkening of the ventral surface. When the plastron is not wetted, the water cannot penetrate the cuticular structure and thus remains intact as a water droplet on the surface of the opisthosoma. Batches of water with different surface tensions were created by adding various amounts of the detergent NP40 to 200 ml of distilled water. The surface tension of the experimental water was subsequently measured through capillary action (Denny, 1993).

For experimental trials, a new batch of low surface tension water that was successful in wetting the plastron was produced using one drop of NP40, dead crickets, dirty paper towels, and dead cabbage looper caterpillars in water that was allowed to sit for one week to simulate a naturally produced reduction in surface tension. The wetting of the plastron with this dirty water did not in any visible way affect the behavior of the individuals when out of the water. The individuals with wetted plastrons were then placed into a 600 ml beaker and submerged in 200 ml of aerated distilled water at $\sim 21^\circ\text{C}$. These same individuals were also submerged

Figure 1. Duration of submergence by animals in normally aerated water. Over 50% voluntarily remained underwater for more than 2.5 hours without ever coming to the water's surface ($n=14$). Two individuals voluntarily remained underwater for more than eight hours.

underwater after placing a drop of distilled water on the ventral surface of their abdomens which did not wet the plastron. Every two minutes, individuals were mechanically stimulated with forceps and their responsiveness was scored in the same manner as described for the deoxygenated versus oxygenated trials. Once again, a Cochran's Q test was used to analyze individual responsiveness over time.

2.3. Plastron structure

For scanning electron microscopy, specimens were cleaned in a 50:50 bleach solution and sonicated for 1.5 minutes after which time they were cleaned with chloroform, mounted and sputter coated with gold, and viewed with a scanning electron microscope.

For sectioning, individuals were fixed in Bouin's fixative, embedded in paraplast, and sectioned longitudinally at 6 μm . Sections were then stained with a solution after Streba-Schobess (Schoenfeld, 1980).

Ventral plastron surface area was calculated from SEM photographs. The area of the water-air interface of the plastron was determined by tracing a known area of cuticular ridges from a photograph similar to Figure 3c. The interface was assumed to extend as a flat plane between the tops of the cuticular columns. This area was cut from the tracing and its relative size determined by weighing the paper.

3. Results

3.1. Response underwater

When submerged in normally aerated water, individuals of *Phrynos marginemaculatus* remained submerged voluntarily for periods of 30 minutes to more than eight hours without coming to the surface (Figure 1). They re-

Figure 2. Responses of individuals submerged in oxygenated and deoxygenated water. In deoxygenated water, they rapidly stopped responding over time ($n=14$) (Cochran's $Q=98.5$, $df=5$, $P<0.01$). In oxygenated water, all animals remained responsive for 10 minutes ($n=14$) ($Q=0.00$, $df=5$, $P>0.05$).

mained active underwater even though they did not carry a visible air bubble and periodically walked along the underside of the rock. Immediately following emergence, their activity levels appeared normal. The two individuals that were placed in aerated water for more than 24 hours remained responsive the entire time, responding positively to periodic mechanical stimulation. After ~26 hours underwater, they were removed from the water and appeared to have normal activity levels.

After 10 minutes in oxygen-free water, only one of the fourteen individuals tested remained responsive, while all individuals placed in aerated water for the same time remained responsive (Figure 2). All of the individuals from oxygenated water were able to right themselves within two minutes when placed on their backs upon conclusion of the trial, while none of those from oxygen-free water was able to similarly do so, often taking up to 10 minutes to regain movement. When animals were submerged, they did not carry visible air bubbles with them.

The plastron of *P. marginemaculatus* was not wetted by water with a surface tension of 0.39 mN cm^{-1} , but was wetted when the surface tension was reduced to $\sim 0.21 \text{ mN cm}^{-1}$, which is below the level they would normally encounter in their natural environment. None of the animals with a wetted plastron, when submerged in clean oxygenated water, responded to mechanical stimulation after 14 minutes ($n=5$; Cochran's $Q=26$, $df=8$, $P<0.01$), while all of the control individuals did respond ($Q=0$, $df=8$, $P>0.05$). After emergence from the deoxygenated water, all individuals regained responsiveness after ~10 minutes.

3.2. Structure

Scanning electron micrographs showed that the cuticle around the openings of the book lungs forms a series of

Figure 3. Scanning electron micrographs of the plastron on the ventral surface of *Phrynus marginemaculatus* (a) Ventral view of the opisthosoma showing the extent of the plastron as a series of transverse stripes (e) connected longitudinally by lateral bands of plastron (c). The arrows indicate the book lung openings. Boxes with letters correspond to the remaining photos. (b) Opening to the left book lung. The normal (non-plastron) cuticle can be seen at the top of the picture. Immediately above (anterior to) the book-lung opening, the cuticle is developed into a series of sharp projections while below it (posterior to), the projections are blunt with buttresses. The bulk of the plastron is formed from the latter type. (c) A close-up of the cuticular plastron showing the buttressed projections found laterally on the ventral surface of the opisthosoma. The arrowhead indicates the opening of a gland of unknown function. (d) A closer look at the cuticular projections at the opening of the book lung. (e) Cuticular plastron located in strips between the sternites on the ventral surface of the opisthosoma. (f) Normal smooth (non-plastron) cuticle found on the ventral sternites.

buttressed projections virtually identical to those forming the plastron of larvae of the fly *Geranomyia unicolor* (Tipulidae) (Figure 3b, d) (Hinton, 1966). Such a series of cuticular structures, if not readily wetted, is known to

be able to retain a layer of gas in their interstices. These cuticular structures were found to continue from both book lung openings down the sides of the opisthosoma and across the ventral opisthosoma between the sternites

(Figure 3a–e). Similar strips of cuticle occur dorsally between the tergites as well. Elsewhere, the cuticle is relatively smooth and flattened (Figure 3f). The cuticular projections are taller and nearer together approaching the entrance of the book lungs. At the narrowest section of the opening, the cuticular spikes extend across almost the entire width of the opening (Figure 4), thus, because of their hydrofuge surface properties, potentially preventing any water from entering the book lung itself.

The entire ventral opisthosomal surface area of *P. marginemaculatus* covered with plastron is 27.30 mm² in an individual weighing approximately 100 mg. This comprises approximately 58% of the surface of the opisthosoma. Sixty-three percent of the total plastron area (17.20 mm²) is air-water interface, providing approximately $1.7 \times 10^5 \mu\text{m}^2 \text{mg}^{-1}$ surface area available for gas exchange.

4. Discussion

Our results show that individuals of *Phrynus marginemaculatus* can remain underwater for periods of more than 24 hours. Since arachnid cuticle is not usually very permeable (Hadley, 1994), we explored the possibility of plastron respiration. We determined that animals were not able to remain responsive in deoxygenated water and thus, to remain active while submerged, they must be obtaining oxygen from the surrounding water. Structural observations indicate cuticular buttressing on the ventral surface of the opisthosoma which acts as a cuticular plastron. When this plastron was wetted with water of low surface tension, and thus made dysfunctional, animals quickly became unresponsive. Thus, through a series of experimental techniques and structural observations, we were able to confirm plastron respiration in *P. marginemaculatus*.

Figure 4. A longitudinal section through the opisthosoma, showing the opening of a booklung (anterior to left). Notice the spines at the opening of the booklung which are refractory to staining and which extend across most of the opening. Typical plastron can be seen to the right of the opening. At the top left is a leaf in the book lung in surface section.

An essential feature of a plastron is that it is made up of a hydrofuge structure that resists wetting. Because floodwater is contaminated by organic materials, its surface tension is usually lower than that of clean water and an effective plastron must be able to resist wetting under these conditions. The surface tension of distilled water at 20°C is 0.73 mN cm⁻¹ while that of stream water may be around 0.7 mN cm⁻¹ (Hinton, 1960). By contrast, standing water on the surface of cow dung may have a surface tension as low as 0.50 mN cm⁻¹ and on decaying meat, 0.40 mN cm⁻¹. Muddy water associated with flooding will have a surface tension value between these two extremes and the resistance to wetting of the amblypygid plastron, which was not wetted by water with a surface tension of 0.39 mN cm⁻¹, is clearly adequate to resist wetting even under these conditions.

In determining the functionality of a plastron, it is important to consider the area available for gas exchange in relation to the size and respiratory rate of the organism. In most plastron-bearing insects, the plastron air-water interface ranges from 1.5×10^4 to $2.5 \times 10^6 \mu\text{m}^2 \text{mg}^{-1}$ and that of the mite, *Platyseius*, is 2.1 – $2.8 \times 10^5 \mu\text{m}^2 \text{mg}^{-1}$ (Hinton, 1966, 1971) while in *P. marginemaculatus* it is $1.7 \times 10^5 \mu\text{m}^2 \text{mg}^{-1}$ on the ventral surface alone. The rate of oxygen consumption of *P. marginemaculatus* at 20°C is about 0.02 ml g⁻¹ h⁻¹ (Anderson, 1970) which is an order of magnitude lower than the average resting level of about 0.2 ml g⁻¹ h⁻¹ for most insects; however, it is comparable to that of other arachnids (Anderson, 1970). Thus, the plastron area of *P. marginemaculatus* appears more than adequate to meet the aerobic needs of this organism.

In insects respiring with a plastron, oxygen tension within the plastron varies with the distance from the spiracles. Hinton (1969) has calculated values for insect eggs indicating that the distant parts of the plastron may not be effective in gas exchange with the surrounding environment. Since *P. marginemaculatus* is a relatively large animal with the book lungs restricted to the second two opisthosomal segments, some parts of the plastron are remote from the site of oxygen uptake into the organism and the question of efficiency of the plastron is critical. For a plastron to function effectively, the pressure difference across the air-water interface must be maintained through the entire plastron. Thorpe and Crisp (1947) and Crisp (1964) show that the pressure difference varies with distance from the spiracles in insects according to the function:

$$nx_1 = (i_0 x_1^2 / Dh)^{0.5}$$

where i_0 is the invasion coefficient of oxygen (5×10^{-4} ml cm⁻² atm⁻¹ sec⁻¹), x_1 is the maximum distance from the respiratory organ (i.e. the spiracles in insects; the booklung in amblypygids), D is the diffusion constant of oxygen in plastron space (0.18 ml cm⁻² sec⁻¹), and h is the

thickness of the plastron, which in *P. marginemaculatus* is about 6.5 μm . For a small individual of *P. marginemaculatus* weighing about 100 mg and with an opisthosomal ventral surface area of 47.26 mm², x_1 is about 5 mm and the calculated value for $nx_1=1.03$. The curve generated from this value for the change in pressure $(\Delta p)_x$ does not deviate greatly from the average (Δp) , thus providing evidence that oxygen is drawn relatively uniformly into the plastron across its entire surface (Crisp, 1964). For larger individuals, however, it might be expected that only the parts of the plastron closer to the book lungs would normally be used and this would almost certainly be true of much of the plastron on the dorsal surface.

Although aquatic respiration is not uncommon among terrestrial insects, it is uncommon among the terrestrial arachnids. The hairs located on the abdomens of some spiders are known to act as air stores, thus enhancing survival in flood situations (Rovner, 1986 and Messner) and there is one example of a spider that can walk and swim underwater through the use of a diving bell (*Argyroneta aquatica*) (Messner and Adis, 1995; Foelix, 1996). However, in all of these examples, the spiders are utilizing oxygen from trapped air that they bring down with them, not dissolved oxygen from the surrounding water. Respiration through a plastron enables organisms to remain active underwater indefinitely as opposed to simply prolonging survival for several more minutes as is commonly true for insects that carry air bubbles. Certain other arachnid groups commonly found in tropical habitats subject to inundation respond to flooding by migrating up onto tree trunks (Pseudoscorpions and Araneae) (Adis, 1997). However, the strategy of migration could potentially increase the risk of predation to these typically nocturnal, cryptozoic creatures. The lack of a flood survival strategy in certain arachnid groups has likely affected their ability to successfully colonize particular habitats. Palpigrads and ricinulids, both of which are common inhabitants of dry-land forests, are not found in the Central Amazonian floodplain forests (Adis, 1997), possibly due to their inability to survive flooding. Thus, adaptations to environmental hazards such as flooding are important factors to examine when discussing issues of species diversity and distribution.

All of our results, including behavioral, morphological, and physiological data, demonstrate that *Phrynus marginemaculatus* is able to respire underwater via a cuticular plastron. *Phrynus marginemaculatus* represents the first example of plastron respiration in any order of arachnid other than the semi-aquatic mites and represents the first example of plastron respiration in an organism with a non-tracheate respiratory system. The evolutionary innovation of plastron respiration in *P. marginemaculatus* may have allowed this species

to invade habitats unavailable to non-plastron-bearing arachnids and could have some implications for adaptive radiations or major lifestyle and/or behavioral changes within the order.

Plastrons have evolved independently a number of times within Insecta and it is possible that the same pattern will hold for Arachnida as well. The two arachnid groups that are currently known to have species with plastrons, Acari and Amblypygi, are fairly distantly related according to cladistic analyses (Schultz, 1990). Unfortunately, many of the lesser known arachnid orders have not been examined to the extent that would enable one to discover the presence, or absence, of a plastron. Future work within these smaller groups as well as within the order Amblypygi itself will shed more light on the evolutionary history of plastron respiration in arachnids.

Acknowledgements

We would like to thank W. Hebets and N. Hebets for aiding in the collection of specimens. We also thank H. Alonso-Pimentel, E. Jockusch, K. Ober, W. Maddison, G. Binford, G. Bodner, S. Masta, M. Hedin, P. Gerba, E. Dyreson, J. O'Brien, M. Conklin, D. Bentley, B. Daley and S. Redella for their help in experimental procedures/design and their constant encouragement. E. Bernays and C. Martinez del Rio kindly commented on the manuscript. We would also like to acknowledge the United States Department of the Interior, Fish and Wildlife Service, National Key Deer Refuge for providing EAH with a special use permit for collecting animals used in this study. This research was supported by a grant to EAH from the Research Training Group, Department of Ecology and Evolutionary Biology, University of Arizona.

References

- Adis, J., 1997. Terrestrial invertebrates: survival strategies, group spectrum, dominance and activity patterns. In: W.J. Junk, Editor, 1997. *The Central Amazon Floodplain — Ecology of a Pulsing System*, pp. 299–314.
- Adis, J., and Messner, B., 1997. Adaptations to life under water: tiger beetles and millipedes. In: W. J. Junk, Editor, 1997. *The Central Amazon Floodplain — Ecology of a Pulsing System*, pp. 319–324.
- Adis, J., Caoduro, G., Messner, B., and Enghoff, H., 1997. On the semiaquatic behaviour of a new troglobitic millipede from northern Italy (Diplopoda Polydesmida: Polydesmidae). *Entomologica Scandinavica* 51, p. 301.
- Anderson, J. F., 1970. Metabolic rates of spiders. *Comparative Biochemistry and Physiology* 33, pp. 51–72.
- Crisp, D. J., 1964. Plastron respiration. *Recent Progress in Surface Science* 2, pp. 377–425.
- Crisp, D. J., and Thorpe, W. H., 1948. The water-protecting properties of insect hairs. *Discussions of the Faraday So-*

- ciety* **3**, pp. 210–220.
- Denny, M. H., 1993. *Air and Water: The Biology and Physics of Life's Media*. Princeton University Press, Princeton, NJ.
- Foelix, R. F., 1996. *Biology of Spiders*. (2nd ed.), Oxford University Press, New York.
- Hadley, N. F., 1994. *Water relations of terrestrial arthropods*. Academic Press, San Diego.
- Hinton, H. E., 1960. The structure and function of the respiratory horns of the eggs of some flies. *Philosophical Transactions of the Royal Society (London)* **243**, pp. 45–73.
- Hinton, H. E., 1961. How some insects, especially the egg stages, avoid drowning when it rains. Proceedings of the South London Entomological and Natural History Society 1960, pp. 138–154.
- Hinton, H. E., 1966. Plastron respiration in marine insects. *Nature* **209**, pp. 220–221.
- Hinton, H. E., 1969. Respiratory systems of insect egg shells. *Annual Review of Entomology* **14**, pp. 343–368.
- Hinton, H. E., 1971. Plastron respiration in the mite *Platyseius italicus*. *Journal of Insect Physiology* **17**, pp. 1185–1199.
- Messner, B., and Adis, J., 1995. There is only facultative plastron respiration in diving web spiders (Araneae). *Deutsche Entomologische Zeitschrift* **42** (2), pp. 453–459.
- Messner, B., Adis, J., and Ribeiro, E. F., 1992. A comparative study on plastron structures in mites. *Deutsche Entomologische Zeitschrift* **39**, pp. 159–176.
- Ross, M. S., O'Brien, J. J., and Flynn, L. J., 1992. Ecological site classification of Florida Keys terrestrial habitats. *Biotropica* **24** (4), pp. 488–502.
- Rovner, J. S., 1986. Spider hairiness: air stores and low activity enhance flooding survival in inland terrestrial species. Proceedings of the 10th International Arachnological Congress Jaca (España), vol. 1, p. 123.
- Schoenfeld, C., 1980. Die Kernechtrot–Faerbung nach Sterba–Schobess. *Mikrokosmos* **68**, pp. 253–254.
- Schultz, J. W., 1990. Evolutionary morphology and phylogeny of arachnida. *Cladistics* **6**, pp. 1–38.
- Thorpe, W. H., and Crisp, D. J., 1947. Studies on plastron respiration. I. The biology of *Aphelocheirus* (Hemiptera, Aphelocheiridae) (Naucoridae), and the mechanism of plastron retention. *Journal of Experimental Biology* **24**, pp. 227–269.