Journal of Crustacean Biology Advance Access published 18 May 2020

The Crustacean Society

Journal of
Crustacean Biology

Journal of Crustacean Biology 40(4), 462–470, 2020. doi:10.1093/jcbiol/ruaa023

Aerial and aquatic respiration in littoral Oniscidea (Isopoda) from Southern California, USA

Jonathan C. Wright and Zechariah C. Harris

Department of Biology, Pomona College, Claremont, CA 91711, USA

Correspondence: Z.C. Harris; e-mail: zch02014@mymail.pomona.edu

(Received 7 March 2020; accepted 29 April 2020)

ABSTRACT

The large radiation of terrestrial isopods (suborder Oniscidea) includes several families that are represented primarily in marine-littoral or riparian habitats. Among these are members of Ligiidae and Tylidae as well as several basal families within the section Crinocheta. Structural and physiological evidence supports a marine-littoral ancestry of the Oniscidea. We examined aerial and aquatic respiration (measured as VCO_2) in six species of marine-littoral Oniscidea representing five families, as well as one riparian and one endogean species. Complimentary data were collected for immersion tolerance and whole-animal permeability in air, and structural specialization of the respiratory pleopods was examined using SEM. *Ligia occidentalis* Dana, 1853 (marine, littoral) and *Ligidium lapetum* Mulaik & Mulaik, 1942 (riparian) showed similar VCO_2 in air and water. VCO_2 in air for the other species was significantly higher than in water. Compared across species, aerial VCO_2 scaled with mass in accordance with Kleiber's law ($\beta = 0.774$) while aquatic VCO_2 increased in approximate proportion to mass ($\beta = 0.957$). At least some specimens of the six marine-littoral species survived over 24 h immersion. *Ligidium lapetum* and the endogean trichoniscid *Brackenridgia heroldi* (Arcangeli, 1932) also tolerated prolonged immersion in freshwater but did not survive beyond 5–6 h, probably due to limited capacity for hyper-regulation. The upper shore sand-burrowers, *Tylos punctatus* Holmes & Gay, 1909 and *Alloniscus perconvexus* Dana, 1856 had the lowest permeability among the study species and are the only representatives with elaborated pleopodal respiratory fields (*Alloniscus*) and lungs (*Tylos*). The ventral lung spiracles of *T. punctatus* are surrounded by an extensive cuticle meshwork and we propose that this functions as a plastron field to enhance aquatic gas exchange. Collectively, the results show that littoral species tolerate significant periods of immersion, allowing them to withstand habitat inundation during spring high tides, storm swells and, in riparian species, rainstorms and snowmelt.

Key Words: cuticular permeability, Kleiber's law, plastron, respiratory adaptations, terrestrial isopods, water immersion

INTRODUCTION

It is generally accepted that the terrestrial isopods (suborder Oniscidea) are derived from marine ancestors and have gone through many anatomical and physiological changes to adapt to the land environment (Edney, 1968; Little, 1983; Warburg, 1993; Carefoot & Taylor, 1995; Hornung, 2011, Broly *et al.*, 2013). Evidence for marine ancestry includes the extensive representation in marine-littoral habitats of the basal Ligiidae and Tylidae, as well as most of the more basal families within the large section Crinocheta (Erhardt, 1995; Schmidt, 2008). Structural and physiological evidence includes the externally filled "amphibious" marsupium in Ligiidae, Trichoniscidae, and Tylidae (Hoese, 1984), relatively high hemolymph osmolality in fully terrestrial Oniscidea (Little 1983), a capacity for salt-secretion and hyporegulation in *Ligia* and *Tylos* spp. (Parry, 1953; Wright *et al.*, 2003), and ionic composition of hemolymph similar to that of seawater and typical of marine Crustacea (Parry, 1953; Little, 1983; Gondko *et al.*, 1984; Wright *et al.*, 2003).

Consistent with a marine-littoral origin of Oniscidea, many of the basal families associated with the marine-littoral include species that tolerate regular immersion in seawater. These must be able to undergo efficient gas exchange in both air and water to prevent lethal anoxia when tissue oxygen demand exceeds respiratory oxygen uptake (physiological hypoxia). In some species, gas exchange occurs across pleopodal gills fundamentally similar to those of aquatic isopods, whereas other species have developed elaborated "respiratory fields" and diffusion lungs located within pleopodal exopodites (Unwin, 1932; Hoese, 1982a, 1983; Schmidt & Wägele, 2002; Paoli *et al.*, 2002; Hornung, 2011). A significant proportion of gas exchange also occurs across the general cuticle surface in more permeable species (Edney & Spencer, 1955).

Despite the littoral and supra-littoral habitats of many oniscidean families, and the documented ability of several *Ligia* species to survive prolonged or indefinite immersion in seawater (Parry, 1953; Todd, 1963; Carefoot & Taylor, 1995; Taylor & Carefoot, 1990, 1993), there are very few studies comparing aquatic and aerial metabolism in Oniscidea. The most substantive study is that of Taylor & Carefoot (1993) who examined underwater survival and metabolism of *Ligia pallasii* Brandt, 1833 and three terrestrial species, *Oniscus asellus* Linnaeus, 1758, *Porcellio scaber* Latreille, 1804, and *Armadillidium vulgare* Latreille, 1804. Their study showed prolonged survival (> 3 d) and sustained VO_2 in seawater-immersed *L. pallasii* while the terrestrial species, although surviving 15−18h (LT_{50}) in aerated freshwater or isosmotic saline, showed declining VO_2 over time. VO_2 in submerged *L. pallasii* was approximately 50% higher than for animals in air, whereas all three terrestrial species showed significantly reduced VO_2 when immersed (44−60% of aerial rates).

The goal of the present study was to build on this work by comparing aerial and aquatic metabolism and underwater survival in a selection of marine-littoral Oniscidea as well as one riparian and one endogean species. Respiratory specializations of the pleopodal exopodites were examined using SEM. We also measured water loss rates of the different species under standard conditions to derive standardized water flux (j_w, nl h^{-1} cm^{-2} Pa^{-1}). The latter is dimensionally equivalent to diffusional permeability (P_d, μm s^{-1}) in a given vapor pressure gradient. Since the permeability of the integument to water and oxygen is proportional to their respective diffusion coefficients (D, μm^2 s^{-1}), standardized water fluxes are predicted to vary in proportion to oxygen permeability across the general integument and its significance in meeting the gas-exchange demands of the whole animal.

We studied six marine littoral or supra-littoral species, representing five families, from Southern California. Four of the species are inhabitants of the spray zone on rocky beaches: *Ligia occidentalis* Dana, 1853 (Ligiidae), *Littorophiloscia richardsonae* Holmes & Gay, 1909 (Philosciidae), *Armadilloniscus lindahli* Richardson, 1905, and *Armadilloniscus holmesi* Arcangeli, 1933 (Detonidae). All four species are common on boulder beaches where they feed on beached algae and surfgrass *Phyllospadix scouleri* Hooker, 1838 (Menzies, 1950; Morris *et al.*, 1980). The two other marine species, *Tylos punctatus* Holmes & Gay, 1909 (Tylidae) and *Alloniscus perconvexus* Dana, 1856 (Alloniscidae), are inhabitants of sand beaches. These isopods burrow by day near the most recent high-high water mark and emerge onto the sand surface 1–2 h following sunset, again exploiting beached algae as their primary food source (Hayes, 1974; Holanov & Hendricksen, 1980; Morris *et al.* 1980; Hamner *et al.*, 1969; Garthwaite & Lawson, 1992). In the less disturbed Southern California beaches where they still occur, *T. punctatus* and *A. perconvexus* are commonly found together, and typically co-occur with the talitrid amphipod *Megalorchestia californica* (Brandt, 1851) with which they would appear to have considerable niche overlap (Hubbard *et al.*, 2014; personal observation). *Tylos punctatus* and *A. perconvexus* do not emerge during daylight and may therefore be submerged during the high-high mixed tides when these occur during the day and the isopod burrows are flooded to the sand surface.

We also studied two species native to inland riparian (stream-edge) woodland: *Ligidium lapetum* Mulaik & Mulaik, 1942 (Ligiidae) and *Brackenridgia heroldi* (Arcangeli, 1932) (Trichoniscidae). *Ligidium lapetum* is restricted to damp humic soil and litter on the riparian fringe, and readily enters water when disturbed. *Brackenridgia heroldi* is a true endogean species and is found near the soil surface only following winter rains. Both of these species will experience occasional immersion during winter rains and creek flooding associated with snowmelt. We hypothesized that, as for the marine-littoral species, they would be capable of sustained metabolism when immersed and would tolerate prolonged immersion in aerated water.

MATERIALS AND METHODS

The boulder beach marine species were collected at the southern end of Malaga Cove Beach, Redondo Beach, CA, USA (33°48′12.4″N 118°23′44.2″W). *Tylos punctatus* and *Alloniscus perconvexus* were collected approximately 2 h after sunset from sandy beaches in Malibu, CA, USA. *Ligidium lapetum* and *Brackenridgia heroldi* were collected from riparian oak woodland at Big Dalton Canyon, Glendora, CA, USA (34°09′46.9″N 117°49′04.8″W). *Ligia occidentalis* were maintained in a 20 l covered glass terrarium with a graded sand/gravel floor and rocks and containing a small volume of artificial seawater (Instant Ocean™, Blacksburg, VA, USA). *Tylos punctatus* and *A. perconvexus* were kept in a 10 l Plexiglas tank filled to approximately 20 cm with beach sand into which they burrowed freely. The sand was dampened with a small volume of artificial seawater and replaced weekly with clean, washed sand. The other marine-littoral species were housed in small plastic jars with moistened filter paper and a few rocks as substrate. All marine species were fed *ad libitum* with dried marine algae *Macrocystis pyrifera*. The riparian species *L. lapetum* and *B. heroldi* were maintained in 200 ml glass jars containing damp soil and oak litter.

Respirometry

Mass-corrected metabolic rates were recorded as VCO_2 (μl min^{-1} g^{-1}) using a Sable Systems CD10 CO_2 analyzer (Sable Systems Inc., Henderson, NV, USA). Individual isopods were placed in a 10 ml respirometry vial and perfused with dry, CO_2-free outside air. Water vapor and CO_2 were removed by passing the upstream air supply through respective columns of calcium sulphate (Drierite™) and soda lime. From the specimen chamber, air was passed in turn through a Sable Systems RH-300 humidity sensor to monitor water losses, an additional desiccant column, the CD-10 CO_2 sensor, and a Sable Systems SS4 subsampler pump. All components were connected using PE tubing. Vented air from the subsampler was passed through a Gilmont GF-1160 flow meter (Gilmont Instruments, Barrington, IL, USA) to give an independent measure of air flow rate. The output from the CO_2 sensor was recorded and analyzed using Sable Systems Expedata software.

Steady state CO_2 output (ppm) was averaged over a stable period of 5–10 min for the large-size species. Baseline-corrected mean CO_2 was then multiplied by air flow to derive VCO_2. For the small-size species (*Armadilloniscus* spp., *Littorophiloscia richardsonae*, *Brackenridgia heroldi*) we measured VCO_2 using a stop-flow method. This used two two-way valves to allow for a by-pass of the specimen chamber for specimen isolation and baselining. CO_2-free air was initially passed through the chamber for a minimum of 10 min to flush CO_2 and the chamber then isolated for 15–30 min, allowing respiratory CO_2 to accumulate. The chamber flow was then re-opened, yielding a transient CO_2 peak. The integral of the resulting peak (ppm CO_2 min. 10^6) was multiplied by the airflow (ml min^{-1}) to yield the total CO_2 production (ml) during the isolation period.

The same basic protocol was followed to measure the immersed VCO_2 except that individual isopods were immersed in a small measured droplet (20−200 μl) of seawater added to the respirometry vial. A small wire cage (1 mm mesh) was placed over the animal and used to confine it within the water droplet. Individuals were immersed in artificial seawater in the marine-littoral species. *Ligidium lapetum* and *Brackenridgia heroldi* were immersed in deionized water. Independent observations confirmed that both species tolerated immersion in a similar water droplet for at least 50 min.

Because all steady state runs with immersed individuals required a period of at least 15 min for the CO_2 output to stabilize, transient decreases in the measured ppm CO_2 owing to reaction with water (forming bicarbonate) were assumed to have negligible effect on the measured data. To further check for artifacts due to CO_2 exchanging with the aqueous bicarbonate pool, we compared mean ppm CO_2 at the beginning and end of each run period and observed no significant difference.

For the stop-flow trials with immersed individuals, it was critical to ensure that dissolved CO_2 was purged from the water droplet, and also to ensure that no CO_2 was leaking in/out of the chamber during isolation. Controls were run using the identical set-up and water droplet, but without an animal. These were flushed with air for 10 min then isolated for 30 min and the chamber then flushed once more.

Evaporative water loss

Evaporative water loss was measured for the species we studied by allowing individuals to desiccate in laboratory humidity and monitoring mass-losses gravimetrically. Any fecal losses were subtracted. Because oniscideans are well known to lose more water in the initial stages of drying, probably owing to drying of the integument and evaporation from the pleural capillary system (Quinlan & Hadley, 1983), mass losses were plotted over time, and loss rates for each animal determined from the slope once this had linearized.

Water loss rates were then standardized for animal surface area and vapor pressure deficit to derive standardized fluxes ($\text{nl h}^{-1}\text{ cm}^{-2}\text{ Pa}^{-1}$; Edney, 1977; Machin & Lampert, 1985). Surface area (SA) was calculated using the formula of Edney (1977):

$$\text{SA}\left(\text{cm}^2\right) = 12 \times \text{M}^{0.67}$$

Vapor pressure deficit was calculated as the difference between the vapor pressure of the laboratory air and that of the animal tissues. The saturation vapor pressure of water (SVP) at the lab temperature of 22 °C is 2.64 kPa. Based on the mole fraction of water to solutes (see Edney, 1977), the vapor pressure of the animal's tissues was assumed to be 0.99.SVP for the riparian species, and 0.98.SVP for littoral species. The vapor pressure of the laboratory air = SVP × relative humidity (RH). Laboratory RH was measured during desiccation trials using a VWR Traceable Hygrometer (VWR International, Radnor, PA, USA).

Immersion tolerance

Immersion tolerance was studied by submerging individuals in covered Petri dishes at lab temperature (22 ± 1 °C) and monitoring viability over time. Sufficient water was added just to immerse the animal. The marine littoral species were immersed in artificial seawater (Instant Ocean™, Instant Ocean Spectrum Brands, Blacksburg, VA, USA), and riparian species were immersed in deionized water. Cumulative mortality values were plotted against time and straightened using a probit transformation (Sokal & Rohlf, 1988). Linear regressions of the transformed data were then used to estimate the time for 50% mortality (probit value of 5.00), or LT_{50}.

Scanning electron microscopy

For SEM, specimens of *Armadilloniscus* spp., *Littorophiloscia richardsonae*, *Tylos punctatus* and *Alloniscus perconvexus* were fixed in 80% ethanol overnight and whole pleons or individual pleopods critical-point dried, mounted on stubs, and gold sputter-coated. Specimens were examined using a Hitachi SU 3500 (Hitachi, Tokyo, Japan) SEM at the Rancho Santa Ana Botanic Gardens, Claremont, CA, USA.

Statistical analysis

Statistical analyses were performed using IBM SPSS, Version 23, and Microsoft Excel. 2-sample t-tests were used to test for significance between mean VCO_2 values in water *versus* air for all of the species. For scaling VCO_2 with body mass we conducted independent regression analyses and then calculated the test statistic z in order to compare the slopes of the aerial *versus* aquatic runs (see Kleinbaum & Kupper, 1978).

RESULTS

Aerial and immersed metabolic rate

Most of the study species displayed a regular metachronal beating of the pleopods when immersed as documented by Taylor & Carefoot (1993); the one exception was *B. heroldi*. Measurements of VCO_2 in air and water are plotted in Figure 1 and fall into three general categories. The two ligiid species, *Ligidium lapetum* and *Ligia occidentalis*, each show similar VCO_2 in air and water, with no significant difference between mean rates. The two sand-burrowing species, *Tylos punctatus* and *Alloniscus perconvexus*, show rather similar mass-specific metabolic rates in air to the ligiids, but show significantly lower metabolism when immersed, with aerial VCO_2 exceeding aquatic rates 1.6– to 1.8–fold. The third category comprises *Armadilloniscus holmesi*, *A. lindahli*, and *Littorophiloscia richardsonae*, small-size species 3−6 mm in length. These show the highest mass-specific metabolism, and the greatest aquatic-aerial disparity, with aerial VCO_2 exceeding aquatic rates 1.8- to 2.8-fold.

The interspecific scaling of aerial and aquatic VCO_2 and body mass among the study species was studied by plotting the log-transformed data (Fig. 2). Aerial VCO_2 shows a tight log-linear scaling ($R^2 = 0.923$) with an exponent of 0.774, closely conforming to Kleiber's law ($\beta = 0.738$ or 0.75) (Kleiber, 1932, 1947). By contrast, immersed VCO_2 scales with a higher exponent, more nearly in proportion to animal mass ($\beta = 0.957$, $R^2 = 0.930$). To compare the two slopes, we conducted independent regression analyses and then calculated the test statistic z (Kleinbaum & Kupper, 1978):

$$z = \beta_1 - \beta_2/\sqrt{\left(S^2{}_{\beta 1} - S^2{}_{\beta 2}\right)},$$

where β_1 and β_2 are the slopes of the linear regressions in air and water, respectively, and $S_{\beta 1}$ and $S_{\beta 2}$ are the standard errors calculated with the regression models in Excel. The derived $z = 4.46$ yielding $p < 10^{-4}$ and thus a highly significant difference between the scaling exponents for aerial and immersed VCO_2.

Immersion tolerance

The two riparian species, *Ligidium lapetum* and *Brackenridgia heroldi*, survived for 3−6 h in deionized water, whereas one or more specimens of the marine littoral species survived immersion in seawater for at least 24 h (Table 1). These data exclude pre-molt individuals, with sternal calcium deposits (see Ziegler & Miller, 1997), which showed considerably lower tolerance of immersion, often succumbing within 1 h.

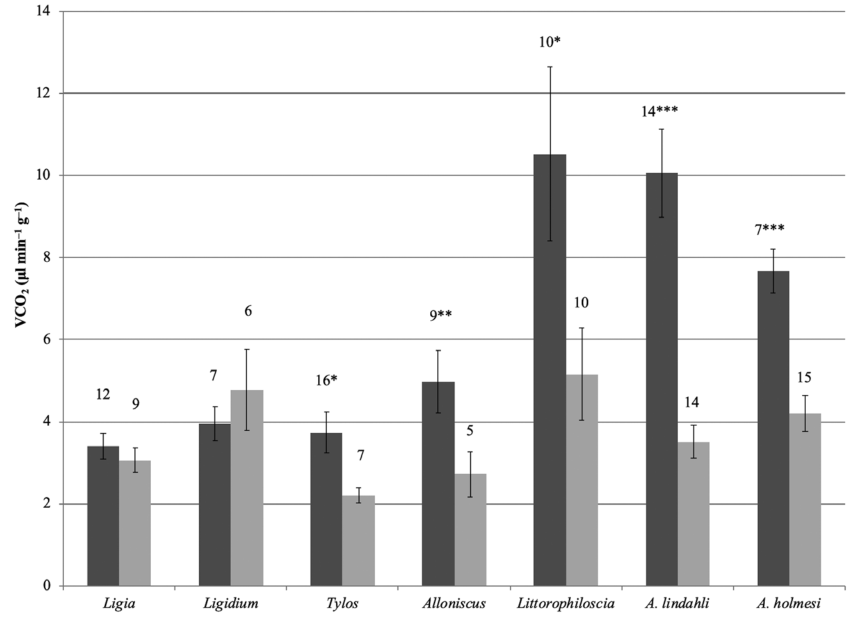

Figure 1. Mass-corrected VCO_2 (µl min^{-1} g^{-1}) for the seven species studied in air (light gray) and immersed (dark gray) in seawater or freshwater (*Ligidium*). Asterisks denote significant differences between immersed and aerial rates (*$P < 0.05$, **$P < 0.005$, ***$P < 0.0005$).

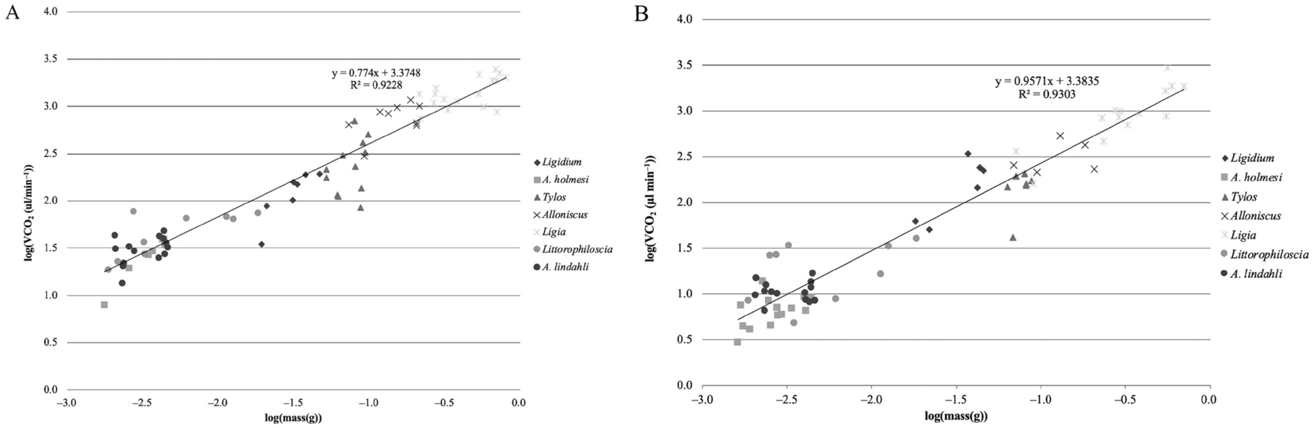

Figure 2. Log-log plots of VCO_2 against animal masses for the seven species studied. Individuals in normoxic air (**A**); individuals submerged in seawater or freshwater (*Ligidium lapetum*) (**B**). The corresponding metabolic scaling relationships are VCO_2 (µl min^{-1}) = 3.37 × $M^{0.774}$ for air and VCO_2 (µl min^{-1}) = 3.38 × $M^{0.957}$ for submerged metabolism. The metabolic scaling exponents differ significantly from one another.

Water flux

Standardized water fluxes for the study species are given in Table 2.

Scanning electron microscopy

The pleopodal exopodites of the ligiids, and those of *Armadilloniscus* spp. and *Littorophiloscia richardsonae*, are flattened and unmodified, resembling the pleopodal gills of fully aquatic species (Fig. 3A, B). These contrast sharply with the thicker and elaborated exopodites of *Alloniscus perconvexus* and *Tylos puncatus*. In *A. perconvexus* (Fig. 4), the ventral exopodite surface is smooth but the postero-lateral region of the dorsal surface is modified to form a complex respiratory field (Schmidt & Wägele, 2002). This consists of elaborately branching tubes originating from the outer hemolymph sinus (Fig. 4A) and forming a tridimensional honeycomb. The primary branches are approximately 30 µm in diameter and divide further, giving rise to an underlying network of smaller branches of about 10 µm diameter; these ultimately anastomose and terminate in the medial sinus. There is a sharp boundary between the respiratory field and the antero-medial region of the pleopod where the cuticle is unmodified. In exopodites 3–5, this region is overlain by the sac-like endopodites.

Tylos punctatus, like other Tylidae, lacks the first pair of pleopods (Hoese, 1983; Brown & Odendaal, 1994; Schmidt & Wägele, 2002). The exopodites of the four posterior pairs bear invaginated pleopodal lungs, with the lung cavity opening to the exterior via 7 or 8 antero-posterior slit-like spiracles or stigmata (Fig 5A). The cuticle surrounding the stigmata is highly modified, comprising dense, raised polygons undergirded by a reticulate meshwork of cuticular struts (Fig. 5B, C). Individual polygons are irregular in shape but typically 5–7 µm long by 1.5–2.5 µm wide. This modified structure extends over approximately 37% of the ventral surface area of the exopodite, forming an apparent plastron field.

DISCUSSION

The eight littoral Oniscidea examined here, including one riparian species (*Ligidium lapetum*) and one endogean species (*Brackenridgia heroldi*), all showed tolerance of prolonged immersion

Table 1. Tolerance of immersion in seawater or freshwater (*Ligidium lapetum*, *Brackenridgia heroldi*).

	Maximum time (min)	24 h survival	LT$_{50}$	*N*
Ligia occidentalis	> 24 h	12	> 24 h	12
Ligidium lapetum	300 min	0	–	14
Brackenridgia heroldi	350 min	0	300 min	8
Tylos punctatus	> 24 h	9	> 24 h	15
Alloniscus perconvexus	> 16 h	9 (> 16 h)	> 24 h	9
Littorophiloscia richardsonae	> 24 h	2	18 h	15
Armadilloniscus holmesi	> 24 h	6	14 h	24
Armadilloniscus lindahli	> 24 h	2	–	4

Table 2. Standardized water flux measurements for the study species in air at 20 °C. Units are nl h^{-1} cm^{-2} Pa^{-1}.

	Mean mass (mg)	Flux	SEM	*N*
Ligia occidentalis	388.7	2.22	0.103	8
Ligidium lapetum	19.2	3.40	0.077	8
Brackenridgia heroldi	0.674	1.33	0.306	5
Tylos punctatus	56.2	0.453	0.0302	16
Alloniscus perconvexus	117.0	0.710	0.0266	16
Littorophiloscia richardsonae	3.97	2.71	0.277	7
Armadilloniscus holmesi	2.72	4.84	0.425	10
Armadilloniscus lindahli	4.62	1.16	–	2

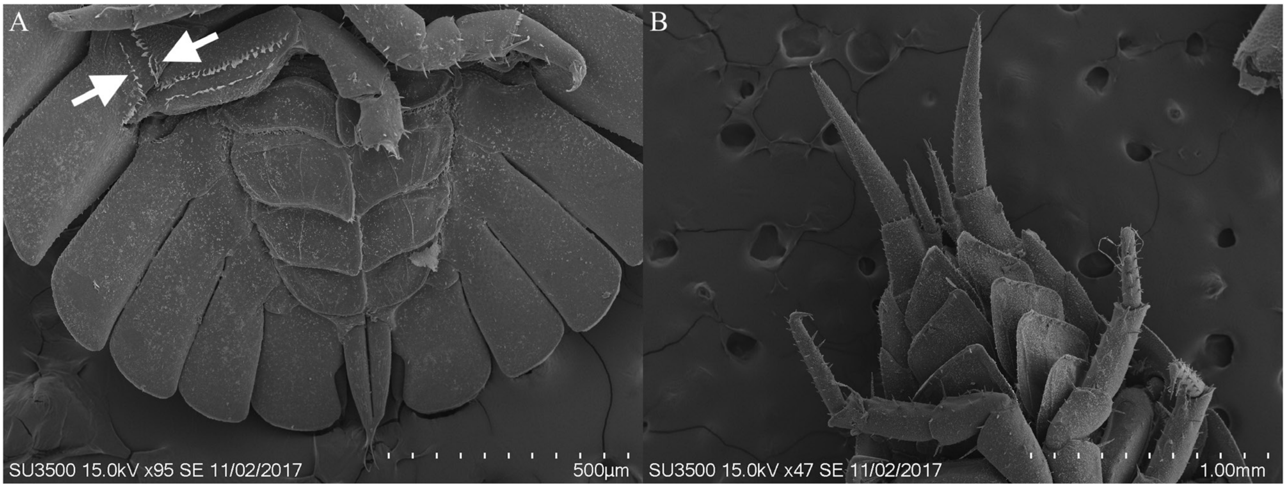

Figure 3. Ventral view of the pleon of *Armadilloniscus holmesi* showing the simple, flattened pleopodal exopodites (**A**). The ventro-lateral water capillary system (arrows) extends onto, and terminates, at the distal end of the ventral side of the basipodite of the seventh pereopods. Ventral view of the pleon of *Littorophiloscia richardsonae* (**B**). As with *Armadilloniscus* spp., this species possesses simple, flap-like pleopodal exopodites. The number in the bottom right of each image indicates the width of the entire scale (i.e., from the leftmost hash mark to the rightmost hash mark).

in freshwater (*L. lapetum* and *B. heroldi*) or seawater (six marine littoral species). The two sand-burrowing species, *Tylos punctatus* and *Alloniscus perconvexus*, had LT$_{50}$ values exceeding 24 h, and we have observed *Ligia occidentalis* to tolerate immersion in aerated seawater for at least five days. The two *Armadilloniscus* spp. and *Littorophiloscia richardsonae* were less tolerant of prolonged immersion, but a few individuals of all three species survived over 24 h. Not surprisingly, tolerance of freshwater immersion in the two riparian species was more modest, and probably limited by cumulative ion-loss given that oniscideans lack external ion-absorbing epithelia. The ability of all the study species to survive immersion for over 4 h nevertheless demonstrates efficient respiratory gas exchange in water as well as in air at 22 °C, and this is corroborated by the VCO_2 data.

Our results are in broad agreement with those of Taylor & Carefoot (1993). Aquatic respiration in the littoral Crinocheta was significantly reduced relative to aerial VCO_2, and resembles the corresponding VO_2 data presented for the three fully terrestrial crinochete species studied by Taylor & Carefoot (1993). In contrast to their findings, however, the two ligiids studied here showed no significant difference in aquatic and aerial VCO_2 whereas Taylor & Carefoot reported significantly increased VO_2 in seawater-immersed *Ligia pallasii*. Although *L. pallasii* is fundamentally a rock-dwelling wrack-feeder like *L. occidentalis* (Carefoot, 1989), its proficient aquatic metabolism may indicate a greater proclivity to aquatic foraging or adaptation to more frequent immersion than *L. occidentalis*. We also obtained significantly shorter survival times of *L. lapetum* and *B. heroldi* in freshwater compared to the crinochete species studied by Taylor & Carefoot (1993). A likely explanation for this is the lower experimental temperatures used by Taylor & Carefoot (–15 °C), compared to 22 °C

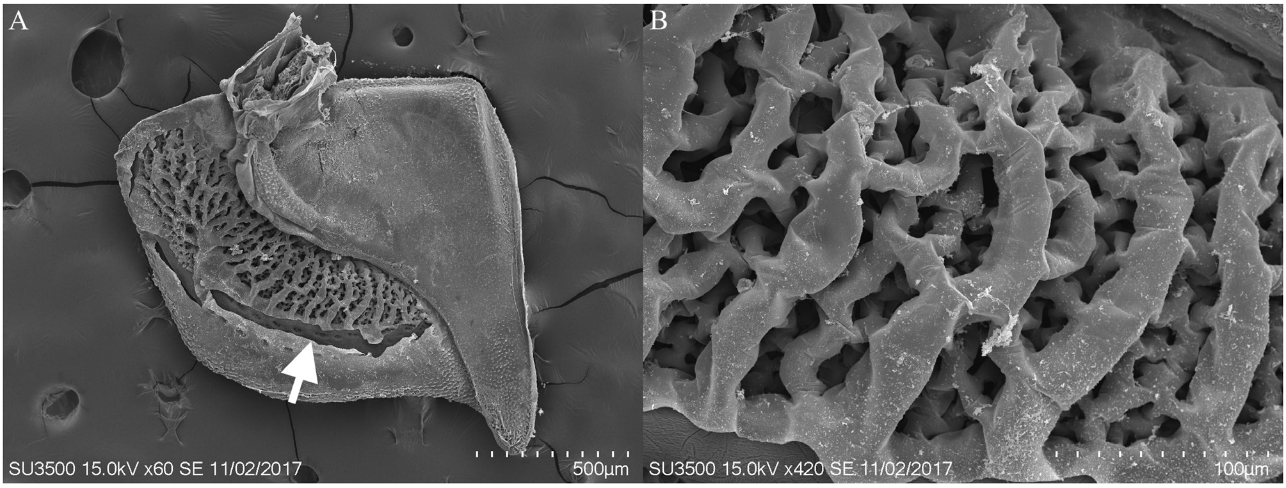

Figure 4. Dorsal surface of the third pleopodal exopodite of *Alloniscus perconvexus* showing the branching respiratory field (arrow) (**A**). The cuticle has torn along the postero-lateral hemolymph sinus (open arrow). Enlarged view of the respiratory field showing the branching from the main sinus (torn open; bottom) and layered 3-dimensional structure (**B**).

used in our study. Our independent observations have shown that *Armadillidium vulgare* usually dies within 2 h when immersed in deionized water at 22 °C (unpublished data).

Tolerance of prolonged immersion in the marine-littoral species studied here is likely to be a plesiomorphic trait. All belong to families that are phylogenetically quite basal with respect to the large, fully terrestrial radiations of Oniscidea (Fig. 6). The Ligiidae and Tylidae comprise primarily marine littoral species, while the Trichoniscidae are primarily endogean or riparian. Within the large radiation of Crinocheta, the basal families are again predominantly marine littoral or supra-littoral, including Alloniscidae, Detonidae and Scyphacidae. Independent terrestrial radiations outside the Crinocheta and Synocheta (Trichoniscidae) are seen in the genus *Ligidium* (Ligiidae), in at least seven terrestrial species of *Ligia* (Taiti *et al.*, 2003), and in the aberrant Mediterranean tylid *Helleria brevicornis* Ebner 1868 (Vandel, 1960; Michel-Salzat & Bouchon, 2000; Gentile *et al.*, 2010).

Scaling metabolism with mass is commonly done in animal physiology to correct for body size. From our results, the significant difference between the allometric scaling of aerial and aquatic VCO_2 with body mass is noteworthy and not readily explicable. The difference persists if the data for *L. occidentalis* are excluded, and in fact the scaling exponents barely change, so it is apparently not an artifact of the relatively high submerged metabolic rate in this species. The difference also persists if data for *Tylos* and *Alloniscus*, the two lung-bearing genera, are excluded. The scaling exponent of aerial metabolism ($\beta = 0.77$) closely conforms to the value of 0.738 (or approximately 0.75) predicted from Kleiber's law (Kleiber, 1932, 1947), but the exponent for aquatic VCO_2 ($\beta = 0.96$) indicates a mass-proportional scaling ($\beta = 1.0$). More recent work attempting to synthesize the huge body of work on metabolic scaling into one or more unifying, deterministic theories (e.g., West *et al.*, 1997, 1999; O'Connor *et al.*, 2007; Glazier, 2010, 2014) have focused primarily on the effects of surface, shape, and resource transport, but say little about differences in aquatic *versus* terrestrial animals *per se*, and there is very little work comparing aerial and aquatic metabolic scaling in truly amphibious taxa (i.e., able to breathe in air and water). Further work is required to assess whether the higher scaling exponent for aquatic metabolism observed here is applicable to a broader representation of littoral Oniscidea, or to other amphibious Decapoda and Amphipoda.

An intriguing observation in the present study was the ventral, seta-lined capillary channel on the basipodite of the seventh pereopod in *Armadilloniscus holmesi*. This channel adjoins the posterior terminus of the water capillary system. Ligiids possess apposable setal fields along the sixth and seventh pereopods, extending to the propodite, which function in water uptake (Hoese, 1981, 1982b; Horiguchi *et al.*, 2007). No comparable setal fields were observed on the sixth pereopods of *A. holmesi*, and a water-uptake function of the short and simple setal field on the seventh pereopodal basipodite seems unlikely. The ventral location also argues against these structures serving to convey water from the water capillary system to the pleoventral chamber. Their function is presently unknown but they may facilitate deposition of excess surface liquid onto the ground surface, a function more typically assumed by the uropods (Drobne & Fajgelj, 1993; Hopkin, 1991).

The anatomy and respiratory adaptations of the pleopodal exopodites in oniscidean families have been studied by Hoese (1982a, 1983) and Schmidt & Wägele (2002). The simple, thin pleopodal exopodites of *Armadilloniscus* spp. and *Littorophiloscia richardsonae* lack clear modification for a terrestrial environment and superficially resemble those of ligiids. Such minimally modified exopodites have a thin ventral cuticle to facilitate gas exchange (Schmidt & Wägele, 2002). By contrast, the pleopodal exopodites of *Alloniscus perconvexus* and *Tylos punctatus* are elaborated, possessing dorsal invaginated respiratory fields (*Alloniscus*) or pleopodal lungs opening via ventral stigmata (*Tylos*). Both of these species are sand-burrowers, and possess thick cuticle and short pereopods bearing massive digging spines (Vandel, 1960; Schultz, 1984; Brown & Odendaal, 1994). The dorsal location of the respiratory fields in *Alloniscus* spp. may help to protect them from sand abrasion. The pleopods of *Tylos* spp. are overlain by the peculiar operculate uropods, characteristic of the Tylidae, and by the ventrally extended epimera ("phylacomeres") of the fourth and fifth pleonal tergites (Brown & Odendaal, 1994; Erhard, 1995; Schmidt, 2008). Both structures are heavily calcified and rigid and will help to shield the pleopods from sand abrasion during burrowing. The epimera also laterally displace the sixth and seventh pairs of pereopods during conglobation (rolling up), preventing possible damage to the pleopods from the sturdy leg spines. When individuals are immersed, the operculate uropods frequently trap an air bubble against the surface of the pleopods and this may function as a physical gill.

A probably more significant respiratory adaptation in *T. punctatus* is the remarkable reticulate structure of the pleopodal cuticle, extending over approximately the anterior 40% of the ventral exopodite surface. This is contiguous with the stigmata and we propose that it functions as a plastron field to facilitate gas exchange in water. The following lines of evidence support this idea:

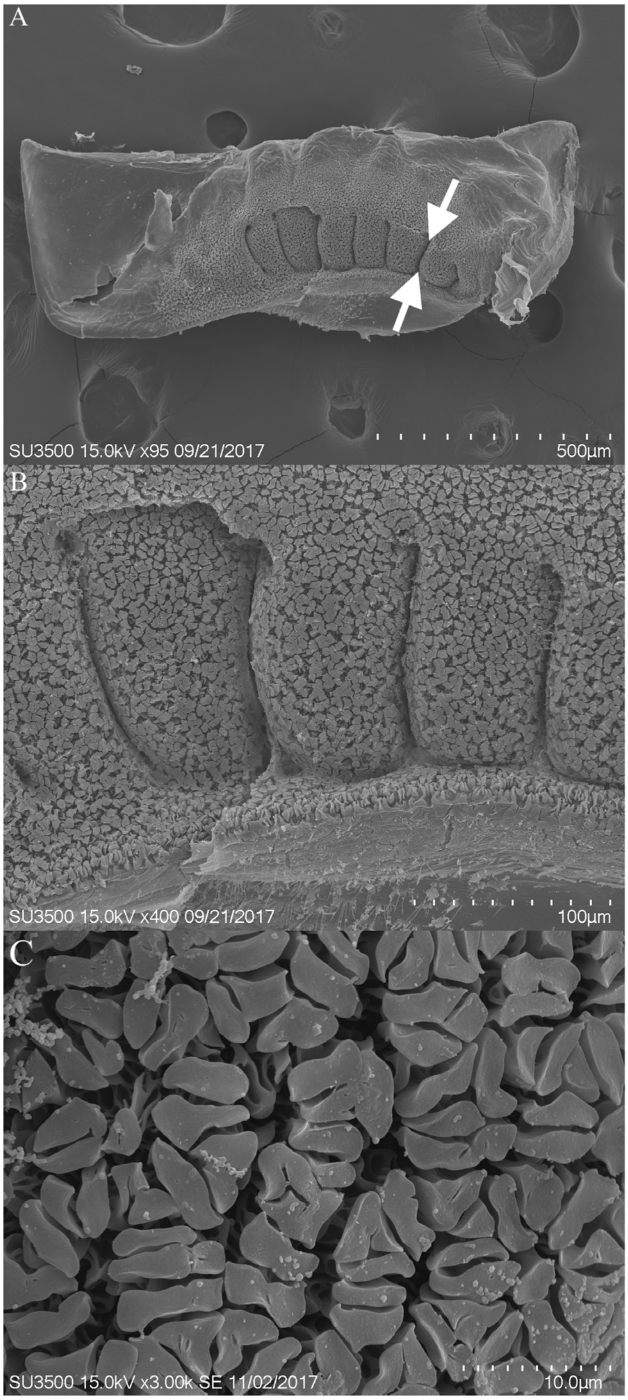

Figure 5. Ventral surface of the second pleopodal exopodite of *Tylos punctatus*. Whole exopodite showing the multiple slit-like stigmata (arrows) surrounded by the extensive plastron field (**A**); close-up of the spiracular region (**B**); high magnification image of the plastron with closely arrayed polygons and the dense underlying meshwork of cuticular struts (**C**).

- The overall area-corrected permeability in *T. punctatus* is low (comparable to that of *Armadillidium vulgare*) arguing against a regionally high permeability of the pleopods.
- Despite its low permeability, *T. punctatus* maintains a significant VCO_2 when immersed, approximately 60% of the aerial rate.
- The other marine littoral species studied here with unmodified pleopods (*L. richardsonae, Armadilloniscus* spp.) have higher permeability but show a greater reduction in VCO_2 when immersed.
- *Tylos punctatus* tolerates prolonged (> 24h) immersion in aerated seawater
- Other lung-bearing species, including *A. vulgare* and *Porcellio scaber*, show only modest survival when immersed in freshwater or in isotonic saline (Taylor & Carefoot, 1993; personal observation).
- Strikingly similar plastron structures have been described in the respiratory horns of the eggs of several insects, including the flies *Scopeuma stercorarium* (Linnaeus, 1758) and *Drosophila melanogaster* Meigen, 1830, and have evolved convergently in a number of families (Hinton, 1969).
- Other *Tylos* species studied (*T. latreillii, T. spinulosus, T. granulatus, T. capensis*) possess similarly modified cuticle surrounding the respiratory stigmata (Hoese, 1983).
- A comparable cuticular structure is lacking in the terrestrial tylid *Helleria brevicornis* Ebner, 1868, where the pleopodal lungs open into paired, ventral spiracles (Hoese, 1983).

Such a plastron field would serve to maintain an air-water interface with an appreciable surface area for gas exchange when *T. punctatus* is submerged in its sand burrow during diurnal high tides. Since the plastron is contiguous with the lung, oxygen would readily diffuse from the plastron surface via the stigmata into the lung cavity, and from there into the hemolymph. In this way, its function would be analogous to that of the ventral plastron fields of elmid beetles (Harpster, 1944; Hinton, 1976; Kodada *et al.*, 2014) and the aquatic heteropteran bug *Aphelocheirus aestivalis* (Fabricius, 1794) (Thorpe & Crisp, 1947a, b; Jones *et al.*, 2017), which are contiguous with the tracheal system.

A plastron services the animal in oxygen uptake only as long as the PO_2 of the surrounding air remains elevated above that of the tissues. Once this situation is reversed, the plastron functions equally efficiently to transfer oxygen out of the animal, as noted by Hinton (1969). This will preclude *T. punctatus* from inhabiting sands with low PO_2. *Tylos punctatus*, however, is a proficient metabolic regulator in air, maintaining undiminished VO_2 in oxygen partial pressures as low as 2% (Wright & Ting, 2006) and indicating highly efficient convective transfer of oxygen from hemolymph to the tissues. Given that this species migrates vertically in sand burrows according to the tidal height (Hamner *et al.*, 1969; Holanov & Hendricksen, 1980), and burrows close to the previous high-water mark (Hamner *et al.*, 1969), animals are probably only commonly immersed when very close to the sand surface where PO_2 values will allow the plastron fields to sustain typical metabolic rates.

We attempted to examine the effects of removing the plastron air film by immersing animals in 20, 50, and 70% ethanol for 5 min and then substituting this with seawater while keeping the animal submerged. Subsequent monitoring of VCO_2 showed that metabolism was unaffected and the pleopodal lungs maintained their white coloration showing air retention. The sustained high VCO_2 suggests that lung air readily replenishes the plastron field in seawater, even if this can be temporarily removed with ethanol. More prolonged immersion in 70% ethanol (10 min) was lethal before the lung air could be removed.

The lung spiracles of other Crinocheta that we have studied (*Venezillo arizonicus* (Mulaik & Mulaik, 1942), *Armadillidium vulgare,* and *Porcellio dilatatus* Brandt, 1833) possess a similarly elaborated cuticle structure immediately adjacent to the spiracle opening. These elaborations have been noted by other workers and assumed to have a water-repellant function (Hoese, 1982a, b;Schmidt & Wägele, 2002; Hornung, 2011), trapping air and preventing water from invading the lung cavity if the animal is inadvertently submerged. The more extensive perispiracular areas seen in the *Eubelum*-type and *Somaloniscus*-type polyspiracular lungs in Eubelidae probably serve a similar function (Paoli *et al.*, 2002). As with *Tylos punctatus*, the unwettable nature of the lung cavity in lung-bearing Oniscidea is generally readily seen from

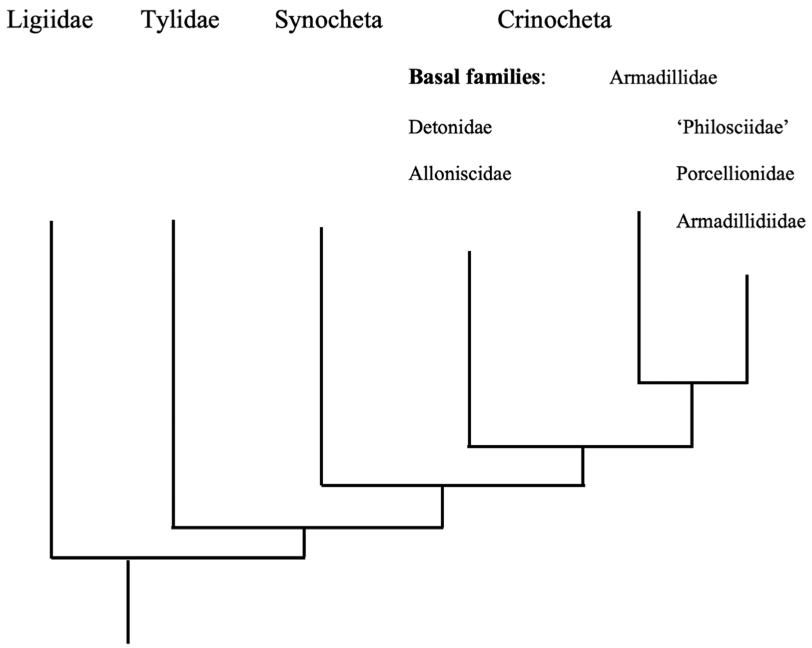

Figure 6. Phylogeny of the Oniscidea based on various sources (Erhard, 1995; Mattern & Schlegel, 2001; Schmidt, 2008). The Ligiidae, Tylidae and basal Crinocheta comprise primarily littoral and riparian species, while the Synocheta, comprising the two superfamilies Styloniscoidea and Trichoniscoidea (Schmalfuss, 2008), includes mostly endogean, troglobitic and secondarily aquatic (freshwater) species.

the persistent white color of the lungs in submerged individuals. The fact that such hydrofuge, perispiracular modifications of the cuticle are seen in the convergently evolved lungs of Tylidae, Eubelidae, and other Crinocheta (see Mattern & Schlegel, 1981; Ferrara *et al.*, 1994; Taiti *et al.*, 1998; Schmidt, 2008) attests to their general functional importance and suggests a probable evolutionary precursor for the expanded plastron fields in *Tylos* spp.

ACKNOWLEDGEMENTS

Special thanks are extended to Loraine Washburn and Lucinda McDade of the Rancho Santa Ana Botanic Gardens for SEM use and support, and to Pomona College for funding to ZCH. A special thanks is extended to Wallace "Marty" Meyer for essential help completing and publishing this paper and our anonymous reviewers for their input.

REFERENCES

Broly, P., Deville, P. and Maillet, S. 2013. The origin of terrestrial isopods (Crustacea: Isopoda: Oniscidea). *Evolutionary Ecology*, **27**: 461–476.

Brown, A.C. and Odendaal, F.J. 1994. The biology of oniscid Isopoda of the genus. *Tylos. Advances in Marine Biology*, **30**: 89–153.

Carefoot, T.H. 1989. Diet and its effect on oxygen consumption in the semiterrestrial isopod. *Ligia. Monografia, Monitore Zoologico Italiano*, **4**: 193–210.

Carefoot, T. H. & Taylor, B. E. 1995. Ligia: a prototypal terrestrial isopod. In: *Terrestrial isopod biology* (M.A. Alikhan, ed.). *Crustacean Issues*, **9**: 47–60.

Drobne, D. & Fajgelj, A. 1993. Use of Tc-99m-Pertechnetate to follow liquid water uptake by *Porcellio scaber*. *Journal of Experimental Biology*, **178**: 275–279.

Edney, E.B. 1968. Transition from water to land in isopod crustaceans. *American Zoologist*, **8**: 309–326.

Edney, E.B. 1977. *Water balance in land arthropods*. Springer-Verlag, Berlin.

Edney, E.B. and Spencer, J.O. 1955. Cutaneous respiration in woodlice. *Journal of Experimental Biology*, **32**: 256–269.

Erhard, F. 1995. Untersuchungen am Skelet-Muskel-System des Landassel-Pleon (Isopoda, Oniscidea). Ein Beitrag zur phylogenetisch-systematischen Stellung der Familie Mesoniscidae. *Verhandlungen der Deutschen Zoologischen Gesellschaft*, **88**: 144.

Ferrara, F., Paoli, P. & Taiti, S. 1994. Philosciids with pleopodal lungs? The case of the genus *Aphiloscia* Budde-Lund, 1908 (Crustacea: Isopoda: Oniscidea), with a description of six new species. *Journal of Natural History*, **28**: 1231–1264.

Garthwaite, R.L. & Lawson, R. 1992. Oniscidea (Isopoda) of the San Francisco Bay area. *Proceedings of the California Academy of Sciences*, **47**: 303–328.

Gentile, G., Campanaro, A., Carosi, M., Sbordoni, V. & Argano, W. 2010. Phylogeography of *Helleria brevicornis* Ebner 1868 (Crustacea, Oniscidea): old and recent differentiations of an ancient lineage. *Molecular Phylogenetics and* Evolution, **54**: 640–646.

Glazier, D.S. 2010. A unifying explanation for diverse metabolic scaling in animals and plants. *Biological Reviews*, **85**: 111–138.

Glazier, D.S. 2014. Metabolic scaling in complex living systems. Systems, **2**: 451–540.

Gondko, R., Helszer, Z. & Serafin, E. 1984. Protein and cation content of the haemolymph of some Oniscoidea. *Comparative Biochemistry and Physiology A*, **78**: 367–369.

Quinlan, M.C. & Hadley, N.F. 1983. Water relations of the terrestrial isopods *Porcellio laevis* and *Porcellionides pruinosus* (Crustacea, Oniscoidea). *Journal of Comparative Physiology*, **151**: 155–161.

Hamner, W.M., Smith, M. & Mulford, E.D. 1969. The behavior and life history of a sand-beach isopod, *Tylos punctatus*. *Ecology*, **50**: 442–453.

Harpster, H.T. 1944. The gaseous plastron as a respiratory mechanism in *Stenelmis quadrimaculata* Horn Dryopidae. *Transactions of the American Microscopical Society*, **63**: 1–26.

Hayes, W. 1974. Sand-beach energetics: importance of the isopod *Tylos punctatus*. *Ecology*, **55**: 838–847.

Hinton, H.E. 1969. Respiratory systems of insect egg shells. *Annual Review of Entomology*, **14**: 343–368.

Hinton, H.E. 1976. Plastron respiration in bugs and beetles. *Journal of Insect Physiology*, **22**: 1529–1550.

Hoese, B. 1981. Morphologie und Funktion des Wasserleitungssystems der terrestrichen Isopoden (Crustacea, Isopoda, Oniscoidea). *Zoomorphology*, **98**: 135–167.

Hoese, B. 1982a. Morphologie und Evolution der Lungen bei den terrestrischen Isopoden (Crustacea, Isopoda, Oniscoidea). *Zoologische Jahrbücher Abteilung für Anatomie und Ontogenie der Tiere*, **107**: 396–422.

Hoese, B. 1982b. Der Ligia-Typ des Wasserleitungssystems bei terrestrichen Isopoden und seine Entwicklung in der Familie Ligiidae

(Crustacea, Isopoda, Oniscoidea). *Zoologische Jahrbücher Abteilung für Anatomie und Ontogenie der Tiere*, **108**: 225–261.

Hoese, B. 1983. Struktur und Entwicklung der Lungen der Tylidae (Crustacea, Isopoda, Oniscoidea). *Zoologische Jahrbücher Abteilung für Anatomie und Ontogenie der Tiere*, **109**: 487–501.

Hoese, B. 1984. The marsupium in terrestrial isopods. In: *The biology of terrestrial isopods* (S.L. Sutton & D. Holdich, eds.), pp. 65–76. Clarendon Press, Oxford.

Holanov, S.H. & Hendricksen, J.R. 1980. The relationship of sand moisture to burrowing depth of the sand-beach isopod *Tylos punctatus* Holmes and Gay. *Journal of Experimental Marine Biology and Ecology*, **46**: 81–88.

Hopkin, S.P. 1991. *A key to the woodlice of Britain and Ireland*. Field Studies Council, Montford Bridge, Shrewsbury, UK.

Horiguchi, H., Hironaka, M., Meyer-Rochow, V.B. & Hariyama, T. 2007. Water uptake via two pairs of specialized legs in Ligia exotica (Crustacea, Isopoda). *Biological Bulletin*, **213**: 196–203.

Hornung, E. 2011. Evolutionary adaptation of oniscidean isopods to terrestrial life: Structure, physiology and behavior. *Terrestrial Arthropod Reviews*, **4**: 95–130.

Hubbard, D.M., Dugan, J.E., Schooler, N.K. & Viola, S.M. 2014. Local extirpations and regional declines of endemic upper-beach invertebrates in Southern California. *Estuarine, Coastal and Shelf Science*, **150**: 67–75.

Jones, K.K., Hetz, S.K. & Seymour, R.S. 2017. The effects of temperature, activity and convection on the plastron PO_2 of the aquatic bug *Aphelocheirus aestivalis* (Hemiptera, Aphelocheiridae). *Journal of Insect Physiology*, **106**: 55–62.

Kleiber, M. 1932. Body size and animal metabolism. *Hilgardia*, **6**: 11–541.

Kleiber, M. 1947. Body size and metabolic rate. *Physiological Reviews*, **27**: 511–541.

Kleinbaum, D.G. & Kupper, L.L. 1978. *Applied regression analysis and other multivariable methods*. Duxbury, Boston, MA, USA.

Kodada, J., Jäch, M.A. & Ciampor, F. 2014. *Acronyx reticulatus* and *A. pulcherrimus*, two new riffle beetle species from Borneo, and discussion about elmid plastron structures. *Zootaxa*, **3760**: 383–395.

Little, C. 1983. *The colonization of land*. Cambridge University Press, Cambridge, UK.

Machin, J. & Lampert, G.J. 1985. A passive two layer permeability-water content model for *Periplaneta* cuticle. *Journal of Experimental Biology*, **117**: 171–179.

Mattern, D., & Schlegel, M. 2001. Molecular evolution of the small subunit ribosomal DNA in woodlice (Crustacea, Isopoda, Oniscidea) and implications for Oniscidean phylogeny. *Molecular Phylogenetics and Evolution*, **18**: 54–65.

Menzies, R.J. 1950. The taxonomy, ecology and distribution of Northern Californian isopods of the genus *Armadilloniscus*, with description of *Armadilloniscus coronacapitalis* n. sp. *Proceedings American Academy of Arts and Sciences*, **26**: 467–480.

Michel-Salzat, A. & Bouchon, 2000. Phylogenetic analysis of LSU rRNA in oniscids. *Comptes rendus de l'Académie des Sciences, Série III, Sciences de la Vie*, **323**: 827–837.

Morris, R.H., Abbott, D.P. & Haderlie, E.C. 1980. *Intertidal invertebrates of California*. Stanford University Press, Stanford, CA, USA.

O'Connor, M.P., Kemp, S.J., Agosta, S.J., Hansen, F., Sieg, A.E., Wallace, B.P., McNair, J.N. & Dunham, A.E. 2007. Reconsidering the mechanistic basis of the metabolic theory of ecology. *Oikos*, **116**: 1058–1072.

Paoli, P., Ferrara, F. & Taiti, S. 2002. Morphology and evolution of the respiratory apparatus in the family Eubelidae (Crustacea, Isopoda, Oniscidea). *Journal of Morphology*, **253**: 272–289.

Parry, G. 1953. Osmotic and ionic regulation in the isopod crustacean *Ligia oceanica*. *Journal of Experimental Biology*, **30**: 567–574.

Schmalfuss, H. 2003: World catalog of terrestrial isopods (Isopoda: Oniscidea). *Stuttgarter Beiträge zur Naturkunde*, Serie A, **654**: 1–341.

Schmidt, C. 2008. Phylogeny of the terrestrial Isopoda (Oniscidea): a review. *Arthropod Systematics and Phylogeny*, **66**: 191–226.

Schmidt, C. & Wägele, J.W. 2002. Morphology and evolution of respiratory structures in the pleopodal exopodites of terrestrial Isopoda (Crustacea, Isopoda, Oniscidea). *Acta Zoologica*, **82**: 315–330.

Schultz, G.A. 1984. Four species of *Alloniscus* Dana, 1854, from the west coast of North America and Hawaii (Isopoda, Oniscoidea). *Crustaceana*, **47**: 49–67.

Sokal, R.R. & Rohlf, F.J. 1995. *Biometry*, Edn. 3. W.H. Freeman, New York.

Taiti, S., Paoli, P. & Ferrara, F. 1998. Morphology, biogeography and ecology of the family Armadillidae (Crustacea, Oniscidea). *Israel Journal of Zoology*, **44**: 291–301.

Taiti, S., Arnedo, M.A., Lew, S.E. & Roderick, G.K. 2003. Evolution of terrestriality in Hawaiian species of the genus *Ligia* (Isopoda, Oniscidea). In: *Biology of terrestrial isopods* (S. Sfentourakis, ed.), pp. 85–102. Brill, Leiden, The Netherlands.

Taylor, B.E. & Carefoot, T.H. 1990. Underwater survival, orientation, and locomotory ability in the semiterrestrial Japanese "Funamushi" *Ligia exotica* (Roux). In: *The biology of terrestrial isopods III: Proceedings of the third International Symposium on the Biology of Terrestrial Isopods*, pp. 121–135. Poitiers, France.

Taylor, B.E. & Carefoot, T.H. 1993. Terrestrial life in isopods: evolutionary loss of gas-exchange and survival capability in water. *Canadian Journal of Zoology*, **71**: 1372–1378.

Thorpe, W.H. & Crisp, D.J. 1947a. Studies on plastron respiration I. The respiratory efficiency of the plastron in *Aphelocheirus*. *Journal of Experimental Biology*, **24**: 270–303.

Thorpe, W.H. & Crisp, D.J. 1947b. Studies on plastron respiration II. The biology of *Aphelocheirus* (Hemiptera, Aphelocheiridae (Naucoridae)) and the mechanism of plastron retention. *Journal of Experimental Biology*, **24**: 227–269.

Todd, M. 1963. Osmoregulation in *Ligia oceanica* and *Idotea granulosa*. *Journal of Experimental Biology*, **40**: 381–392.

Unwin, E.E. 1932. On the structure of the respiratory organs of terrestrial Isopoda. *Papers and Proceedings of the Royal Society of Tasmania*, **1931**: 37–104.

Vandel, A. 1960. Isopodes terrestres 1. *Faune de France*, **64**: 1–416.

Warburg, M.R. 1993. *Evolutionary biology of land isopods*. Springer-Verlag, Berlin.

West, G.B., Brown, J.H. & Enquist, B.J. 1997. A general model for the origin of allometric scaling laws in biology. *Science*, **276**: 122–126.

West, G.B., Brown, J.H. & Enquist, B.J. 1999. The fourth dimension of life: Fractal geometry and allometric scaling of organisms. *Science*, **284**: 1677–1679.

Wright, J.C., Carefoot, T.H. & Albers, M.A. 2003. Osmoregulation and salt excretion in the Ligiidae and Tylidae (Isopoda, Oniscidea). *Crustaceana Monographs*, **2**: 311–334.

Wright, J.C. & Ting, K. 2006. Respiratory physiology of the Oniscidea: aerobic capacity and the significance of pleopodal lungs. *Comparative Biochemistry and Physiology A*, **145**: 235–244.

Ziegler, A. & Miller, B. 1997. Ultrastructure of CaCO3 deposits of terrestrial isopods (Crustacea, Oniscidea). *Zoomorphology*, **117**: 181–187.