

Adaptations for the maintenance of water balance by three species of Antarctic mites

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Abstract Three species of Antarctic mites, *Alaskozetes antarcticus*, *Hydrogamasellus antarcticus* and *Rhagidia gerlachei*, are abundant in the vicinity of Palmer Station, Antarctica. No single mechanism for reducing water stress was shared by all three species. *A. antarcticus* and *R. gerlachei* (both ca. 200 µg) are over twice as large as *H. antarcticus* (ca. 90 µg), but all had similar body water content (67%) and tolerated a loss of up to 35% of their body water before succumbing to dehydration. All imbibed free water and had the capacity to reduce water loss behaviorally by forming clusters. *Alaskozetes antarcticus* was distinct in that it relied heavily on water conservation (xerophilic classification) that was largely achieved by its thick cuticular armor, a feature shared by all members of this suborder (Oribatida), and abundant cuticular hydrocarbons. In comparison to the other two species, *A. antarcticus* was coated with 2–3× the amount of cuticular hydrocarbons, had a 20-fold reduction in net transpiration rate, and had a critical transition temperature (CTT) that indicates a pronounced suppression in activation energy (E_a) at temperatures below 25°C. In contrast, *H. antarcticus* and *R. gerlachei* lack a CTT, have lower amounts of cuticular hydrocarbons and have low E_a s and high net transpiration rates, classifying

them as hydrophilic. Only *H. antarcticus* was capable of utilizing water vapor to replenish its water stores, but it could do so only at relative humidities close to saturation (95–98 %RH). Thus, *H. antarcticus* and *R. gerlachei* require wet habitats and low temperature to counter water loss, and replace lost water behaviorally through predation. Compared to mites from the temperate zone, all three Antarctic species had a lower water content, a feature that commonly enhances cold tolerance.

Keywords Mite · Water balance · Cuticular hydrocarbons · Critical transition temperature · Antarctica

Introduction

Despite the paucity of the insect fauna in Antarctica, the abundance of mites is impressive. Three species, *Alaskozetes antarcticus* (Oribatida: Podacaridae), *Hydrogamasellus antarcticus* (Mesostigmata: Ologamasidae), and *Rhagidia gerlachei* (Prostigmata: Rhagidiidae) are especially prevalent under rocks on islands close to Palmer Station, Antarctica (Peckham 1967; Block and Convey 1995). *H. antarcticus* and *R. gerlachei* are primarily found in wet habitats and are voracious predators of other mites and springtails (Collembola) that co-exist in this moist habitat (Strong 1967; Gressitt 1967a). *A. antarcticus* is more ubiquitous, primarily acting as a scavenger, and is usually encountered in dense clusters of up to several hundred individuals. *A. antarcticus* is found in Antarctica and on several sub-Antarctic islands, but *H. antarcticus* and *R. gerlachei* are restricted to the Antarctic Peninsula (Gressitt 1967b). No studies, except those for identification and basic distribution, have been directed toward *H. antarcticus* and *R. gerlachei*, but *A. antarcticus* has been the

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focus of many studies describing their distribution, development and physiology (Peckham 1967; Block 1977; Block et al. 1978; Cannon 1986; Shimada et al. 1992; Worland and Block 2003).

In this study we construct water balance profiles for adult females of all three species to determine the properties exploited by these mites to maintain water balance in this extreme environment. We determined percentage body water content, dehydration tolerance (lowest amount of water that is needed to survive), net transpiration rate as a function of temperature, and the contribution of aggregation to water conservation. Water loss characteristics were evaluated as a function of the quantity of cuticular hydrocarbons. Mites were also tested for their ability to absorb water vapor from the air and their ability to drink free water. Samples from two different islands (Cormorant and Humble Islands) that are separated by a distance of approximately 3 km were also evaluated for potential population differences.

Materials and methods

Source of mites

Mites were collected from Cormorant and Humble Islands near Palmer Station, Anvers Island, Antarctica (64°04'S, 64°03'W) in January, 2006 and 2007. Individuals were found under rocks, collected using an aspirator, placed into 100 cc vials and transported back to the laboratory within a few hours. Mites were maintained at 4°C prior to all experiments. Primary collection sites for *A. antarcticus* were under rocks that were exceptionally dry, with moss or algae in the near vicinity. This species was consistently present in large aggregations of several hundred individuals, but was also collected individually. *Hydrogamasellus antarcticus* and *R. gerlachei* were also found under rocks, but for these species the rocks were in sites where water was readily available from standing pools or condensation, and usually the rocks harbored large Collembola colonies used as prey. Unlike *A. antarcticus*, *H. antarcticus* and *R. gerlachei* were solitary. Only adult females were used in this study to readily facilitate comparisons with other water balance literature. The exact age of the mites was not known, but individuals were acquired at the same time of year and underwent a starvation/desiccation pretreatment (described below) for standardization.

Determination of water requirements

Wharton's (1985) standard methods were followed with slight modifications as described in Benoit et al. (2005; 2007).

Briefly, mites were weighed individually without enclosure and without anesthesia using an electrobalance (precision of 0.2 µg SD and accuracy of ±0.6 µg at 1 mg; CAHN 26, Ventron Co., Cerritos, CA) to track changes in body water levels at varying relative humidities (% RH). Transfer to the weighing pan was accomplished using an aspirator, and the mites were weighed and returned to test conditions within 1 min. Select relative humidities (% RH, still air conditions) were obtained using saturated salt solutions (Winston and Bates 1960) or calcium sulfate (0% RH, $1.5 \times 10^{-2}\%$ RH; Toolson 1978) placed at the bases of 3,000 cc sealed glass desiccators. Each relative humidity was measured and varied less than 1% during the course of the experiment (hygrometer SD ± 0.5% RH; Thomas Scientific, Philadelphia, PA). Individual mites were placed within 1 cc mesh-covered chambers, and the chambers rested on a perforated porcelain plate placed inside the desiccator.

Observations were conducted at 4°C (ecologically relevant January temperature) and at 25°C (SD ± 0.5°C), 18 h:6 h, light:dark cycle. Before use in an experiment, the mites were placed at 98% RH, 4°C for 24 h and deprived of food to minimize the effects of ingestion, defecation and reproduction on mass changes (Arlian and Ekstrand 1975). Mites were subsequently transferred to 33% RH until they lost 6–8% body mass to remove passively adsorbed water from the cuticular surface. This standard pretreatment was performed so that changes in mass measurements represented changes in body water (Arlian and Ekstrand 1975). At the end of each experiment, mites were transferred to a drying oven (65°C, 0% RH) and weighed daily until mass remained constant; this was defined as the dry mass (d). The dry mass (d) was subtracted from each mass measurement so that values represented the amount of body water in the mite that was available for exchange, which was defined as the water mass (m).

Equations presented by Wharton (1985) were used for determining the water balance characteristics. Percentage body water content was determined using Eq. 1, where f is the fresh (initial) mass; dehydration tolerance was expressed as a percentage change in water mass as described by Eq. 2, where m_C was the critical mass defined as the mass measurement when the legs were curled and the mite was unable to right and crawl 10 body lengths at all test relative humidities; net transpiration rate (integumental plus respiratory water loss) was determined by five hourly mass measurements at 0% RH so that water loss was exponential as described by Eq. 3, where m_t is water mass at time t , m_0 is initial water mass, and $-k_t$ is water loss rate; and activation energy (E_a) for water loss was based upon Eq. 4, where R_{gas} is gas constant, T is absolute temperature, and A is steric factor, by measuring passive water loss (k) using freshly killed mites (−20°C freeze/thaw) as described by

Eq. 3 at different temperatures. A change in E_a was used to denote the temperature threshold of a particular rapid water loss known as the critical transition temperature (CTT). A spot of paint (Pactra, Van Nuys, CA) was used to mark individuals for net transpiration rate determination (Eq. 3) in the group effect experiment; paint had no effect on mass changes (data not shown).

$$\text{Percentage } m = 100(f - d)/f, \quad (1)$$

$$\text{Percentage change in } m = 100(f - m_C)/f, \quad (2)$$

$$m_t = m_0 e^{-kt} \text{ or } \ln(m_t/m_0) = -k_t, \quad (3)$$

and

$$\ln k = -E_a/(R_{\text{gas}}T) + \ln A. \quad (4)$$

Change in water mass (m) was tracked for mites held at 85, 93, 98 and 100% RHs over a period of 5 days by weighing the mites every 12 h. The relative humidity where water mass (m) remained constant, in dynamic equilibrium (thus, water gain = water loss), served as an indicator of the ability to absorb water vapor from the air as designated by the critical equilibrium humidity (CEH). Supplemental observations testing for free water drinking were carried out by exposing mites, 10 at a time, to 20 droplets of 1% Evans blue-stained water (varying sizes ranging from 5 to 20 μl) in 100 \times 15 mm Petri dishes for 12 h. Soaked filter paper disks (90 mm i.d.; Whatman No. 3; Whatman, Hillsboro, OR) in 1 ml of 1% Evans blue dye were run for comparison, using untreated filter paper disks as controls. After exposure, mites were rinsed with distilled water to remove residual dye, and the gut was dissected in 0.1% NaCl under 100 \times (light microscopy) to test for incorporation of the blue tracer.

Cuticular hydrocarbon extraction and quantification

Mites were killed by freezing at -70°C and then weighed. Non-polar lipids (hydrocarbons) were extracted by washing the mites in groups of 100 with 0.5 ml HPLC grade hexane (Sigma Chemical Co., St. Louis, MO) two times for 1 min using fresh solvent for each extraction. A third hexane rinse was analyzed separately, and only trace amounts of lipids were recovered, indicating a nearly complete recovery in the first two rinses (data not shown). The pooled extract was concentrated to 100 μl using a stream of N_2 gas and passed through a silica gel column (Fisher Scientific, Pittsburgh, PA). Hydrocarbons were eluted using column volumes of hexane as the solvent. Each sample was dried onto pre-weighed aluminum pans, thus, the mass change yielded the overall cuticular lipid content. This method recovers 97% of the cuticular lipids (Benoit and Denlinger 2007).

Samples size and statistics

Each water balance characteristic was based on 30 mites (10 per replicate, $n = 3$) from both Cormorant and Humble Islands. The lipid quantification was replicated three times with 100 mites per replicate. An analysis of variance (ANOVA) and Student's t -tests were used to compare data using arcsin transformation in the case of percentages. Sokal and Rohlf's (1995) test for the equality of slopes was used to compare data derived from regression lines. Probit analysis at 95% confidence interval was used to assess survival. Data are presented as the mean \pm SE.

Results

Body water content

Initial, dry and water mass values for the mites are presented in Table 1. Females of *A. antarcticus* and *R. gerlachei* were nearly the same size (ANOVA; $P > 0.05$, $df = 2, 58$, $F = 2.46$) and were approximately twice as large as *H. antarcticus* (ANOVA; $P < 0.05$, $df = 3, 87$, $F = 8.92$). In spite of this difference, all mite body water content averaged 67%, without any significant difference across species when water mass was expressed as a percentage. In all cases, water mass was a positive correlate of dry mass (combined for both populations, $r^2 \geq 0.87$ for *A. antarcticus*, $r^2 \geq 0.89$ for *H. antarcticus*, and $r^2 \geq 0.91$ for *R. gerlachei*; ANOVA; $P < 0.01$). No significant differences in any measured parameters were noted within a species between the two island populations (ANOVA; $P > 0.05$, $df = 3, 87$, $F = 5.22$). We conclude that water content correlates with body mass for the three species of mites. Although *H. antarcticus* is only half the size of *A. antarcticus* and *R. gerlachei*, it requires a similar proportion of body water.

Water loss

Survival (for 50% of mites) without food or free water, in complete dry air of 0% RH and 4°C exceeded 90 h for *A. antarcticus*, whereas *H. antarcticus* and *R. gerlachei* were able to survive for only 5–6 h under these conditions (Table 1). At a higher temperature of 25°C , survival times at 0% RH were shortened to 44.2 ± 0.8 h for *A. antarcticus* and <1 h for the other two species (t -test; $P < 0.05$, $df = 3, 87$, $F = 11.20$). For all species, mortality under these conditions of starvation and dehydration took place rather synchronously, and all individuals died quickly after mortality reached 50%. All three mite species became irreversibly dehydrated, with legs curled and unable to

Table 1 Comparison of water balance characteristics for female adults of *Alaskozetes antarcticus*, *Hydrogamasellus antarcticus* and *Rhagidia gerlachei*

Characteristics	<i>H. antarcticus</i>		<i>R. gerlachei</i>		<i>A. antarcticus</i>	
	Cormorant	Humble	Cormorant	Humble	Cormorant	Humble
Water content						
Initial mass (μg)	89.6 \pm 4.3	91.2 \pm 3.8	191.2 \pm 6.2	193.1 \pm 7.2	191.5 \pm 5.6 ^a	193.4 \pm 6.2 ^a
Dry mass (μg)	28.3 \pm 3.4	28.3 \pm 4.9	64.4 \pm 4.0	64.4 \pm 6.5	64.0 \pm 4.9	63.9 \pm 5.3
Water mass (μg)	61.3 \pm 2.5	62.9 \pm 2.1	126.8 \pm 4.3	128.7 \pm 5.9	127.5 \pm 3.7	129.5 \pm 2.8
Body water (%)	68.4 \pm 4.1	68.9 \pm 3.3	66.3 \pm 3.4	66.6 \pm 4.6	66.6 \pm 2.9	67.1 \pm 2.3
Water loss						
Loss rate (%/h) at 5°C						
Individual	5.43 \pm 0.21	5.72 \pm 0.41	6.32 \pm 0.42	6.22 \pm 0.42	0.34 \pm 0.04	0.36 \pm 0.03
Group of 5	5.04 \pm 0.33	5.12 \pm 0.22	6.05 \pm 0.26	5.91 \pm 0.53	0.24 \pm 0.05	0.29 \pm 0.03
Group of 10	4.32 \pm 0.45	4.43 \pm 0.42	5.62 \pm 0.31	5.53 \pm 0.39	0.22 \pm 0.02	0.22 \pm 0.03
Group of 20	4.04 \pm 0.81	4.32 \pm 0.52	5.34 \pm 0.23	5.14 \pm 0.33	0.23 \pm 0.01	0.21 \pm 0.04
Loss tolerance (%)	34.5 \pm 2.1	34.6 \pm 2.3	35.3 \pm 1.2	35.2 \pm 1.6	33.14 \pm 2.1	34.53 \pm 1.9
Activation energy (J/mol)	73.4 \pm 3.2	72.9 \pm 3.5	58.1 \pm 4.1	58.4 \pm 4.3	153.3 \pm 3.2 ^b	152.1 \pm 2.9 ^b
					32.4 \pm 4.4 ^c	31.9 \pm 3.2 ^c
CTT (°C)	ND	ND	ND	ND	25.5 \pm 2.1 ^b	26.1 \pm 2.4 ^b
Suivivorship at 0.00a _v (hours)	6.5 \pm 0.3	6.2 \pm 0.5	5.2 \pm 0.6	5.7 \pm 0.7	99.6 \pm 3.1 ^b	94.6 \pm 7.9 ^b
Water gain						
Free water drinking	+	+	+	+	+	+
CEH (RH)	95–98	95–98	100	100	100	100

Values are the mean \pm SE of 30 individuals each. *CTT* Critical transition temperature; *CEH* critical equilibrium humidity; *ND* not detected; + drinks free water

^a Values similar to those reported by Block (1977)

^b High

^c Low

right and crawl, once they had lost approximately 35% of their body water (Table 1; ANOVA; $P > 0.05$, $df = 3,87$, $F = 6.22$). The mites remained in this debilitated condition despite efforts to restore regular activity by placing them in close proximity to droplets of free water or by exposing them to water saturated air (100% RH). Specimens from both island populations responded similarly (ANOVA; $P < 0.05$, $df = 6,174$, $F = 1.41$). Thus, the proportion of body water loss that can be tolerated by these mites is independent of their body size.

The net transpiration (water loss) rate for isolated individuals of *A. antarcticus* was nearly 20 \times lower than the rates observed for *H. antarcticus* and *R. gerlachei* (Table 1; Fig. 1; ANOVA; $P < 0.05$, $df = 3,87$, $F = 5.10$). Again no differences were noted in populations from the two islands (ANOVA; $P > 0.05$, $df = 6,174$, $F = 1.17$). These net transpiration rates are consistent with the periods of survival noted in dry air (0% RH) and the corresponding dehydration tolerance limits (Table 1). A similar relationship between dehydration tolerance and survival in dry air to net transpiration rate was observed at 25°C (ranked statistically;

ANOVA; $P < 0.05$): *R. gerlachei* ($29.5 \pm 0.4\%/h$) $>$ *H. antarcticus* ($36.5 \pm 0.2\%/h$) \gg *A. antarcticus* ($0.8 \pm 0.4\%/h$). The water loss rate for *A. antarcticus* was similar to that found by Worland and Block (1986, 2003). No reduction in net transpiration rate occurred for *H. antarcticus* or *R. gerlachei* when held in groups of 5, but a significant group effect (lower net transpiration rate) was noted for *A. antarcticus* at this group size (30% increase in water conservation, Table 1; ANOVA; $P < 0.05$, $df = 2,58$, $F = 4.97$). The net transpiration rate continued to drop at group sizes of 10 and 20, and the largest group size tested, 20, showed a significant reduction in net transpiration rate for all three mite species (Table 1), but the group effect was most pronounced for *A. antarcticus*. We conclude that water loss can be regulated behaviorally by formation of clusters and despite similarities in body size, *A. antarcticus* retains water more effectively than *R. gerlachei*, which is least effective in retaining water. The smallest, *H. antarcticus*, falls between both extremes, suggesting that surface area to volume properties alone do not contribute to this difference in net transpiration rate.

In *A. antarcticus*, activation energy (E_a) for water loss changed dramatically as temperature increased, producing a distinct CTT at 25°C (Table 1; Fig. 2). The change in E_a displayed a fivefold difference between low (<CTT) and high (>CTT) temperature ranges (ANOVA; $P < 0.05$, $df = 2,58$, $F = 5.03$), representing a biphasic relationship of a two-component curve. In contrast, the E_a for *H. antarcticus* or *R. gerlachei* did not change but instead exhibited a continuous slope characteristic of an uninterrupted Boltzmann temperature function ($r^2 > 0.93$ for *H. antarcticus* and $r^2 > 0.91$ for *R. gerlachei*; ANOVA; $P < 0.001$), indicating equivalent depletion of water mass with a rise in temperature. Mites from both island populations had similar E_a values (Table 1; $P > 0.05$, $df = 6,174$, $F = 1.11$). Net transpiration rate and E_a varied inversely: the E_a for *R. gerlachei* was lower than in *H. antarcticus*, and *R. gerlachei* lost water more rapidly (Table 1; ANOVA; $P < 0.05$, $df = 6,174$, $F = 2.97$). This difference was due to the fact that *H. antarcticus* experienced larger proportional increases in the amount of water loss from one temperature to the next (hence, yielding a steeper slope used to derive E_a) than *R. gerlachei*. Such large proportional jumps in water loss over successive temperatures only began for *A. antarcticus* at 25°C and did not occur at lower temperatures, thus creating a biphasic relationship leading to the CTT, whereas for *H. antarcticus* and *R. gerlachei* the jumps were consistently and proportionately large over the entire 0–50°C temperature range; i.e., no CTT was detected. This suggests that a cuticular modification present in *A. antarcticus*, but lacking in *H. antarcticus* and *R. gerlachei*, suppresses the progressive increases in water loss over a range of low temperatures.

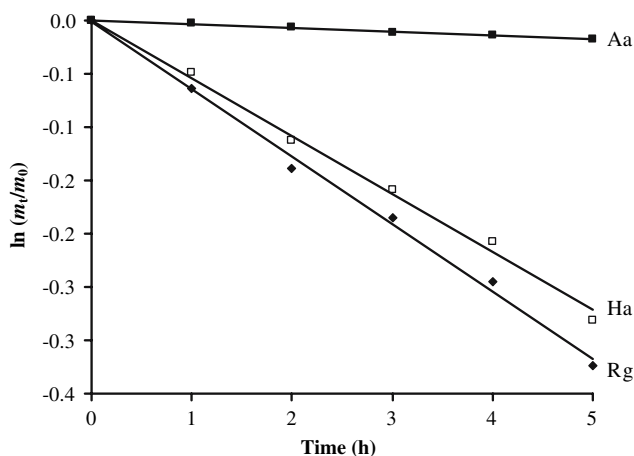


Fig. 1 Proportion of water mass lost at 0% RH (4°C) over a 5 h period in living female adults of *Alaskozetes antarcticus* (Aa), *Hydrogamasellus antarcticus* (Ha) and *Rhagidia gerlachei* (Rg). The slope of the regression is the net transpiration rate (integumental plus respiratory water loss). Each point represents the mean of 30 individuals; SE bars lie within confines of symbols used on the graph

Water gain

When mites were introduced into the bioassay arena they displayed vigorous searching behavior, crawling rapidly, pausing on occasion, and then continuing to crawl. *A. antarcticus* was attracted to droplets of water, and especially to moist filter paper disks that prompted expression of drinking posture (stopping, raising their posterior end, making water contact with their mouthparts); blue dye could be seen filling the interior of the gut. The result was a clustering effect around droplets or directly on the moist paper. Dissection caused release of blue dye from the gut under 100× light microscopy (Table 1), thus confirming the ingestion of water. In contrast, encounters with droplets or moist filter paper by *H. antarcticus* and *R. gerlachei* were passive, occurring when the mites crawled throughout the bioassay arena; no deliberate movements were made toward the water sources. Upon contact, however, they stopped crawling, sometimes becoming trapped within the droplets, and pharyngeal pumping and action of the mouthparts (light microscopy 100×) suggested that fluid was imbibed. Blue dye was detected in the gut upon dissection of mites that were thoroughly rinsed with clean water (Table 1). This evidence indicates that all three mites can obtain water by drinking directly from droplets or from damp substrates.

At 98% RH and 4°C, only *H. antarcticus* was capable of maintaining water mass over a 2 day period; all other relative humidities that were tested below saturation resulted in declines in water mass for the three mites (Fig. 3). A similar trend was observed at 25°C, although *H. antarcticus* could not counter water loss at this high temperature.

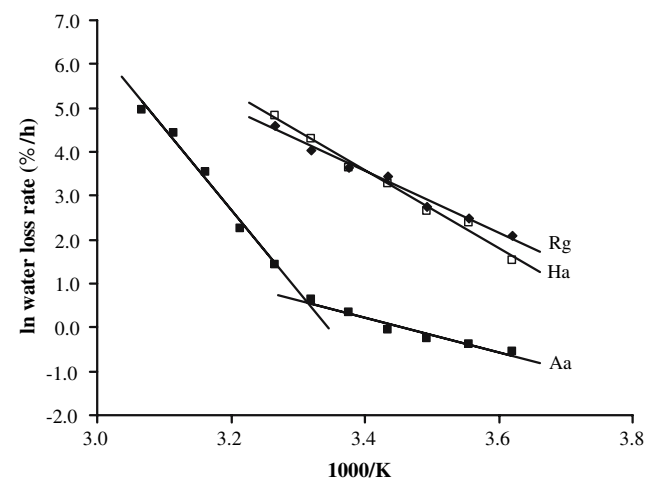


Fig. 2 Net transpiration rate as a function of temperature in living female adult mites of *A. antarcticus* (Aa), *H. antarcticus* (Ha) and *R. gerlachei* (Rg). The slope of the regression = $-E_a/R_{\text{gas}}$, where E_a is the activation energy for water loss and R is the gas constant. Each point is the mean of 30 mites; SE bars lie within graph symbols

As anticipated, the decline in water mass was less pronounced at higher relative humidities (all three species combined, $R \geq 0.88$; ANOVA; $P < 0.001$), indicative of wholly passive processes and gains by sorption. At 98% RH, the change in water mass was positive for *H. antarcticus* and was maintained in dynamic equilibrium for a period of several days (hence gain = loss), implying the existence of an active mechanism because gain occurred against the gradient created by activity of the mite's body water ($a_w = 0.99$) and that of the surrounding test air ($a_v = \%RH/100$), i.e. $0.99a_w > 0.98a_v$, which would otherwise favor water loss by simple diffusion (Wharton 1985). At 100% RH, water gain was noted for all three mites. The fact that net losses were measured at 98% RH for *A. antarcticus* and *R. gerlachei* (Fig. 3) argues against operation of an active mechanism. Thus, the CEH for *H. antarcticus* lies somewhere between 95–98% RH and is dependent on low temperature, while the CEH $\geq 99\%$ for *A. antarcticus* and *R. gerlachei* (Fig. 3). We conclude that *R. gerlachei* and *A. antarcticus* cannot use water vapor as a primary source of water.

Cuticular hydrocarbons

A. antarcticus was coated with 2–3 \times more cuticular hydrocarbon than the other two mite species (Fig. 4; ANOVA; $P < 0.05$, $df = 3,6$, $F = 6.97$). The slightly lower amount of cuticular hydrocarbon on *R. gerlachei* than on *H. antarcticus* was not significant (ANOVA; $P > 0.05$, $df = 2,4$, $F = 3.97$). Quantities of cuticular hydrocarbons did not differ between populations (ANOVA; $P > 0.05$, $df = 6,12$, $F = 2.98$), indicating that this is a species-specific characteristic not unlike the other water balance characteristics observed in the two island populations (Table 1). The most relevant correlation is that *R. gerlachei* had the highest net transpiration rate and the least amount of cuticular hydrocarbons, *A. antarcticus* had the lowest net transpiration rate and the most cuticular hydrocarbons, and *H. antarcticus* was intermediate for both characteristics (Fig. 4; Table 1). As anticipated, increased amounts of cuticular hydrocarbons increased the ability of the mites to retain water, a feature that is consistent with the water-proofing function of this class of lipids.

Discussion

Absence of a CEH ($\geq 99\%$ RH) is one of the prominent water balance characteristics for *R. gerlachei* and *A. antarcticus*. This indicates that they do not have the capacity to absorb water vapor from the air and must obtain water as a liquid (Arlan and Ekstrand 1975). *R. gerlachei* is an

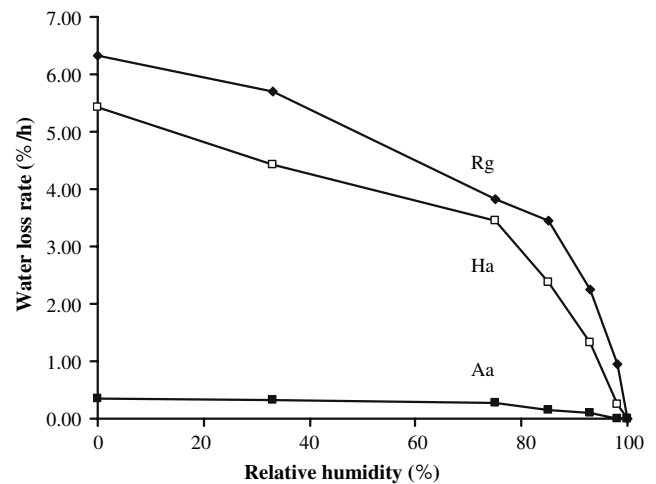


Fig. 3 Effect of relative humidity (% RH) at 4°C on percentage change in water mass in living female adults of *A. antarcticus* (Aa), *H. antarcticus* (Ha) and *R. gerlachei* (Rg). Each point is the mean of 30 individuals; SE bars lie within graph symbols

aggressive and voracious predator on other mites and springtails, suggesting that it obtains most of its water from its prey. This is also the case for another predatory mite, *Balaustium* sp. near *putnami*, from temperate regions that obtains a majority of its fluids through predation (Yoder et al. 2006). Like *Balaustium* sp. near *putnami*, *R. gerlachei* drank free water; it was not attracted to water sources but instead encountered droplets passively. By contrast, *A. antarcticus* compensates for a lack of water vapor absorption by its extreme desiccation resistance and water retention properties, as observed in a desert-inhabiting

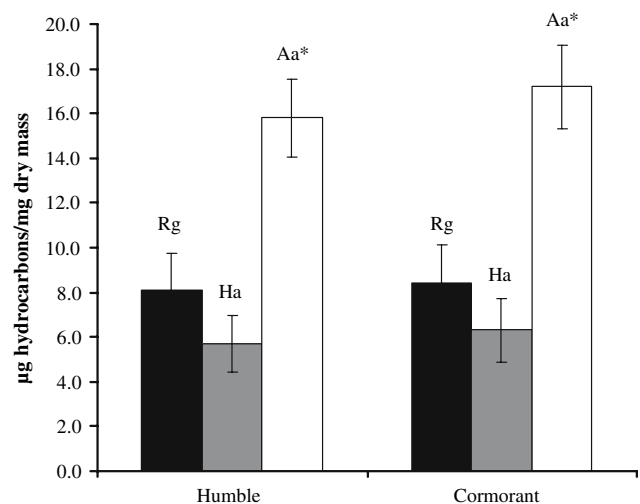


Fig. 4 Quantity of cuticular hydrocarbon extracted from the surface of *A. antarcticus* (Aa), *H. antarcticus* (Ha) and *R. gerlachei* (Rg) collected on either Humble or Cormorant Islands. Vertical error bars are \pm SE of the mean for three groups. * denotes significant difference between species

mite, *Hemisarcoptes cooremani* (Yoder and Houck 2001), that similarly shares a heavily sclerotized cuticular armor; the evolution of this thick cuticle is likely to enhance water retention, thus negating the need for a water vapor uptake processes. Thus, the lack of water vapor absorption reflects two different strategies: heavy reliance on prey as the sole source of water for *R. gerlachei* and a reduced requirement for water in *A. antarcticus* because they are maximally suited for water conservation. High CEHs have been noted in hydrophilic soil arthropods that utilize the accumulation of sugar and polyols to uptake water vapor through their cuticle (Bayley and Holmstrup 1999). But, it is unlikely that *H. antarcticus* uses sugar and polyol accumulation to absorb water vapor. Rather, *H. antarcticus* more likely uses an oral uptake mechanism that is common to many species of mites (Hadley 1994). Additionally, it is uncommon for Oribatid and Prostigmata mites to use water vapor to replenish their internal water stores (Yoder and Klompen 2001). In contrast, *H. antarcticus* drinks free water but it is also capable of absorbing water from the atmosphere, although it can do so only at high relative humidities (95–98% RH). With such a high CEH, the ability of *H. antarcticus* to absorb water vapor is limited and occurs only at relative humidities close to saturation. This limited capacity for water vapor absorption is in contrast to that observed in several other mites, e.g. Astigmata mites (CEHs are in the 70% RHs) and for *Gromphadorholaelaps schaeferi* (Yoder and Barcelona 1995), *Echinolaelaps echidnina* (Wharton and Kanungo 1962), and *Phytoseiulus persimilis* (Gaede 1992), which have CEHs in the range of 83–93% RH. *H. antarcticus* thus has three options: it can apparently use water vapor as a primary source of water at high relative humidities, it can drink free water, and utilize water from its prey to balance its high rates of body water loss. Use of atmospheric water vapor appears to be a mechanism common to most mesostigmatid mites (Yoder and Klompen 2001), including this polar species, *H. antarcticus*.

The group effect noted in *H. antarcticus* and *R. gerlachei* is likely a laboratory artifact because we have not observed clusters of these mites in the field, and they did not readily aggregate in the laboratory during storage. Clustering is a common reaction in acarines as they undergo dehydration as a means to counter water stress (Glass et al. 1997). Although a clustering-stress response is feasible, especially considering the fact that *H. antarcticus* and *R. gerlachei* dehydrate rapidly and are prone to desiccation, we suspect this does not normally occur in the field. Net transpiration rates of *H. antarcticus* and *R. gerlachei* are extremely high, 20–30× greater than the 1–4%/h rate that is typical for mites of a similar body size, e.g. *Julolaelaps celestiae* (Yoder and Klompen 2001), *Galendromus occidentalis* (Yoder 1998 as *Typhlodromus*

occidentalis), *Dematophagoides farinae* (Arlian and Wharton 1974) and *Glycyphagus domesticus* (Seethaler et al. 1979). The high net transpiration rates of *H. antarcticus* and *R. gerlachei* imply that they are hydrophilic, as interpreted by Hadley (1994). This is consistent with the moisture-rich habitats where we generally find these mites in the field. The high net transpiration rates for *H. antarcticus* and *R. gerlachei* have two implications: ecologically, this necessitates that the mites remain in moisture-rich habitats where their prey abounds, and behaviorally the mites must rely upon predation to balance body water losses. Low temperature facilitates this strategy by suppressing transpiration and thus preventing the mites from quickly reaching their limits of dehydration.

The presence of a CTT (25°C) is the notable water balance characteristic that distinguishes *A. antarcticus* from the other two species. This is likely linked to their unique, heavily sclerotized cuticular armor that conveys resistance to desiccation to *A. antarcticus*, and is not present in the other two mites. *H. antarcticus* and *R. gerlachei*, both of which have soft cuticles and high net transpiration rates, lack CTTs. A similar cuticular modification is evident in a desert-inhabiting species, *Hemisarcoptes cooremani*, that also has a thick cuticle and the presence of a CTT (Yoder and Houck 2001). *H. cooremani* also has $E_{a,s}$ in the same low and high temperature ranges as we observed in *A. antarcticus*, suggesting that the desiccation-resistance properties of their cuticles are similar. Desiccation hardness in *A. antarcticus* is further enhanced by a cuticular hydrocarbon layer that is twice as great as observed in *H. antarcticus* and *R. gerlachei*. This lowers the net transpiration rate by decreasing integumental water loss (Hadley 1994), differentially increasing survival by several days in *A. antarcticus*, as compared to *H. antarcticus* and *R. gerlachei*, both of which have low levels of cuticular hydrocarbons. *A. antarcticus* is a clustering species, thus the water conserving group effect we noted is likely to operate in the field. To increase its internal water content, this mite relies solely on obtaining water as a liquid (i.e. water directly or in conjunction with its food) or surface condensation associated with saturated air (100% RH) as noted in this study, and also by Block (1981), Cannon (1986), and Worland and Block (1986). For *A. antarcticus*, the ability to retain water (net transpiration rate) is clearly more important than its ability to gain water, implying that it is xerophilic with regard to water balance (Hadley 1994) and modified for water conservation, a strategy that is particularly useful during prolonged periods when food and water may be scarce.

The 25°C CTT for *A. antarcticus* implies that at 25°C there is a change in the cuticle, a transition previously linked to cuticular hydrocarbon modifications (Gibbs 2002). Not all mites have a CTT (Arlian and Veselica

1979), but those that do typically have CTTs in the 25–35°C range, as shown in *Varroa destructor* (Yoder et al. 1999 as *Varroa jacobsoni*) and *Glycyphagus domesticus* (Seethaler et al. 1979). The CTT provides only basic ecological information that can apply to thermal tolerance or the prediction of habitat suitability, and must be interpreted with caution (Yoder et al. 2005a). However, there is no doubt that the CTT represents a change in the cuticle (Rourke and Gibbs 1999; Yoder et al. 2005a, b) that is accompanied by a change in E_a (Gibbs 2002). A key point is that the phase change occurring at the CTT is reversible; E_a 's and the resultant change at the CTT are consistent whether the E_a 's are measured by starting at a low and progressing to higher temperatures (ramp up) or the reverse (ramp down) (Hadley 1994). The cuticular lipids are capable of re-organizing as temperature declines (Rourke and Gibbs 1999). Once temperatures go below the CTT, the lipids re-organize, producing a compact impermeable boundary that elicits a change in E_a . This creates the CTT, the consequence of which is E_a suppression at lower temperatures. Thus, *A. antarcticus* becomes more water tight as temperature declines, a feature not observed in either *H. antarcticus* or *R. gerlachei*, species that lack a CTT. In *H. antarcticus* and *R. gerlachei*, net transpiration rates remain high because they lack the capacity for a temperature-dependent lipid re-organization sufficient to elicit a decline in the E_a as temperature drops. The prevailing ground temperature during the austral summer in the vicinity of Palmer Station is 10–30°C, but rarely rises above 25°C. Thus, during periods when *A. antarcticus* is most active, this mite is rarely exposed to temperatures above the CTT. This allows *Antarcticus antarcticus* to maximize water retention properties without sacrificing activity or heavy reliance on the presence of free water.

Certain water balance characteristics of these three mite species were alike. Percentage body water content averaged 67%, and dehydration tolerance limits were generally in the same 20–30% range observed for most arthropods (Hadley 1994). With no differences between the water balance requirements of mites from different islands, sub-speciation is unlikely. In the majority of mites that have been studied, mostly from the temperate zone, percentage body water content is slightly higher, in the 73–75% range, thus suggesting that these Antarctic mites may require less body water, a common desiccation hardness feature. Lower percentage body water content usually correlates with a higher dry mass (= fat; Hadley 1994). For *A. antarcticus*, an additional feature contributing to their low percentage body water content is their thick cuticle, which is well known to be associated with low body water content in beetles (Benoit et al. 2005; Hadley 1994). Like other arthropods, the Antarctic mites must retain at least 2/3 of their body water to survive.

High amounts of storage fat are common features of cold-hardy arthropods, and this is likely integral to survival of these mites in Antarctica (Hayward et al. 2001, 2003). In summary, the diverse strategies for maintaining water balance employed by these three mite species implies that no single mechanism is used exclusively by Antarctic mites for this purpose. Additionally, all the mechanisms used by the Antarctic species are present in species from lower latitudes, indicating that the Antarctic mites utilize generalized, not novel polar-specific, methods to prevent dehydration.

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