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RESEARCH ARTICLE

Sucking lice and spiracular transpiration: turning a liability into a benefit and a necessity

Ian F. Burgess

ABSTRACT

Sucking lice feed on blood and therefore ingest more water than they require for maintaining hydration. This water must be excreted; however, unlike other blood-feeding insects, they do not produce urine but do become dehydrated within hours if unable to feed. Using human clothing lice and head lice, *Pediculus humanus* ssp., and high sensitivity balances, it was shown that recently fed lice lost mass consistently as water ingested with the blood meal was excreted via the respiratory system. If all spiracles were occluded using petroleum jelly, mass/water loss was inhibited. Blocking thoracic spiracles resulted in a slight reduction in the rate of mass loss compared with untreated lice, but blocking the abdominal spiracles resulted in an enhanced rate of mass loss. Lice immersed in water did not lose mass but maintained the same mass for several hours, after which they increased in mass as the tissues became turgid, indicating that the insects were able to block water ingress during the period of stability, but that after some time the mechanism failed allowing water to enter the lice by osmosis.

KEY WORDS: Anoplura, Excretion, Spiracles, Transpiration, Water management

INTRODUCTION

A necessary consequence of blood feeding by insects is ingestion of excess water beyond normal physiological requirements. Most hematophagous insects, such as mosquitoes, tsetse, triatomines and cimicids, have adopted strategies whereby they are able to excrete excess water rapidly as liquid faeces or urine either during or soon after engorgement but, at other times, maintain something close to water homeostasis (Wigglesworth, 1931; Bursell, 1960; Pereira et al., 1998; Benoit and Denlinger, 2010). In contrast, host-dependent ectoparasites, such as sucking lice and fleas, normally produce dry faeces. For these insects, defecation of previous blood meals mixed with the water derived from a recent blood meal could be potentially hazardous. Fluid droplets would foul host hair or skin, alerting it to the presence of the parasites, and excretory compounds have the potential to trigger irritant or immune sensitivity reactions, resulting in increased scratching and grooming.

Sucking lice of the suborder Anoplura all produce dry and 'powdery' faeces. Mumcuoglu et al. (1986) reported laboratory reared clothing lice (*Pediculus humanus humanus*) faeces contained just 2% water by mass. Although they do not produce urine and exhibit minimal faecal water loss, these lice are susceptible to death by dehydration; for example, human head lice, *P. h. capitis*, require around six blood meals daily (Maunder, 1983) and die in less than 24 h if removed from the host (Lang, 1975; Chunge et al., 1991). Groups of freshly fed lice can be seen to form condensation inside closed containers, indicating rapid production of water vapour either by cuticular or spiracular transpiration, and during culture of *Pthirus pubis* (Burgess et al., 1983), a starved second stage nymph was observed to form droplets of water from the thoracic spiracles during engorgement. In contrast, they are tolerant of immersion in water, with survival of some lice for over 12 h at ambient temperatures (Bacot and Talbot, 1919; Mumcuoglu et al., 2006; Candy et al., 2018).

All phthirapterans live within the pelage of their hosts and therefore in conditions close to or above the temperatures at which the cuticle lipids of most other insects undergo transition (Ramsay, 1935; Beament, 1961). This places lice in a temperature environment similar to that of xeric insects, but, because of their feeding and lifestyle behaviours, they are in other respects more similar to mesic insects. Therefore, how sucking lice manage their water overall, and the excess water from blood feeding, is of interest in relation to management of infestations as well as an understanding of their normal survival.

MATERIALS AND METHODS

Lice

Human lice from two sources were used in this study. Laboratory reared clothing/body lice, *P. h. humanus* Linnaeus 1758 (Culpepper, 1944), were fed one blood meal each day as they are able to withstand longer periods of starvation than head lice. Head lice, *P. h. capitis* De Geer 1767, were obtained either from children whose parent/guardian had requested help with treating an infestation or from schools that had invited us to screen children with parental consent for lice, under ethical approval from North Cambridgeshire Research Ethics Committee (REC reference no. 06/Q010640). The insects were used within 2–3 h of collection or maintained by regular (4–6 hourly) feeds on the back of a hand over a maximum of 24 h. Immediately before an experiment, each louse used was fed to repletion on the back of a hand, a process that took 5–10 min.

Blood meal mass

The quantity of ingested blood and the loss of water excreted from the blood meal were measured by changes in body mass of individual insects placed in aluminium foil weighing boats for the duration of an experiment. Masses were recorded before feeding, immediately after feeding and at intervals ranging from 1 to 60 min. Faeces produced during the experiment were weighed separately. For some experiments, batches of 20 clothing lice were weighed together in lightweight plastic weighing boats. Measurements were taken in a warm room temperature, 24±2°C, with an ambient humidity of 45±10% relative humidity (RH).

Medical Entomology Centre, Insect Research & Development Limited, Cambridge CB25 9AU, UK.

*Author for correspondence (ian@insectresearch.com)

I.F.B., 0000-0003-0747-3938

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Two types of balance were used for weighing. Single lice were weighed using a Sartorius 4503 MP6 micro-balance (maximum tare 4.1 g, precision=0.001 mg; Sartorius Weighing Technology GmbH, Goettingen, Germany). Batches of lice were weighed using an Ohaus GA200 semi-micro balance (tare 40 g, precision=0.1 mg, OHAUS Europe GmbH, Nänikon, Switzerland).

Rates of water loss after blood feeding for different groups of lice were compared using a one-way ANOVA, including a Tukey HSD, calculator for independent measures (https://www.socscistatistics.com/tests/anova/default2.aspx). Means are presented ±s.d.

RESULTS

The mean body mass of unfed adult female clothing lice, starved for 24 h, was 1.867±0.490 mg. The mean mass increase from feeding was 0.9767±0.4017 mg, an approximately 52.3% increase, taken as the blood meal mass and used as the baseline for subsequent mass changes. In most cases, mass loss was linear for approximately 60 min after feeding, after which the rate of change reduced in some lice, with overall a loss of approximately 30% by between 105 and 120 min after feeding (Fig. 1). Less mass loss was observed during periods of inactivity than when lice were crawling about, but in all cases the body mass returned to approximately the pre-feeding mass by the following day. If lice were not fed after the normal interval of 24 h they continued to lose mass, although at a slower rate.

Faeces were confirmed as a minor component of the total mass loss, increasing as a proportion of the loss from 3% after 1 h to 8% after 6 h, compared with a total loss of from 26% to 55% of the blood meal mass in groups of 20 lice weighed together over the same period (Fig. 2).

Head lice are smaller than clothing lice and take proportionately smaller blood meals more frequently. The mean body mass of adult females examined was 0.677±0.0523 mg and the mass of the blood meal was 0.112±0.013 mg, an approximately 16.5% increase, slightly less than reported by Speare et al. (2006) (0.705 mg body mass and 0.167 mg blood meal). Individual lice varied more in the rate of mass loss after feeding than clothing lice. Overall, the proportionate rate of loss for all insects was significantly greater in the sample of head lice (P=0.0059), so that by 180 min after feeding the mean mass loss was more than 75% of the ingested blood meal compared with approximately 40% in clothing lice (Fig. 3). Most head lice returned to their pre-feeding mass by approximately 4 h after feeding, compared with 17–24 h for clothing lice.

Each of the above measurements was conducted under conditions of RH that the lice were likely to encounter naturally, i.e. either room ambient of approximately 40–45% or incubator RH of 65±10%. In order to determine whether the water is lost by either passive means or by active means, experiments were conducted that were designed to identify which of these is the primary mechanism of excretion. If the water loss is passive, for example through cuticular evaporation, the rate should vary with changes in RH. To test this, I measured mass loss under widely differing RH conditions of 'saturated' air (as close to 100% RH as possible), 70% RH and 25% RH by placing clothing lice on a small weighing boat in a 150 mm diameter Petri dish containing either distilled water or saturated sodium chloride solution for ∼100% RH or 70% RH, respectively, or in a non-humidified incubator for 25% RH. For 'saturated' air, the Petri dish was enclosed in a larger plastic box with a clip-on top that was humidified to 95 ±1% RH. At each measurement, the lice were removed and lightly blotted to remove water droplets. It was expected that if humidity affected the rate of water loss, then the lice exposed to a saturated atmosphere would have some difficulty eliminating water, but the lice at the low RH of 25% would lose water rapidly through cuticular evaporation. However, if water is eliminated by an active excretory mechanism, it could be expected that the rate of water loss would not be affected by humidity. The result was that over more than 6 h there was only a slightly lower rate of mass loss of lice in the saturated atmosphere (Fig. 4), but no significant difference between any of the groups, indicating that water excretion was achieved principally through an active transport mechanism.

Following methods of Zachariassen (1991) and other workers, I investigated the possibility of active transpiration through the

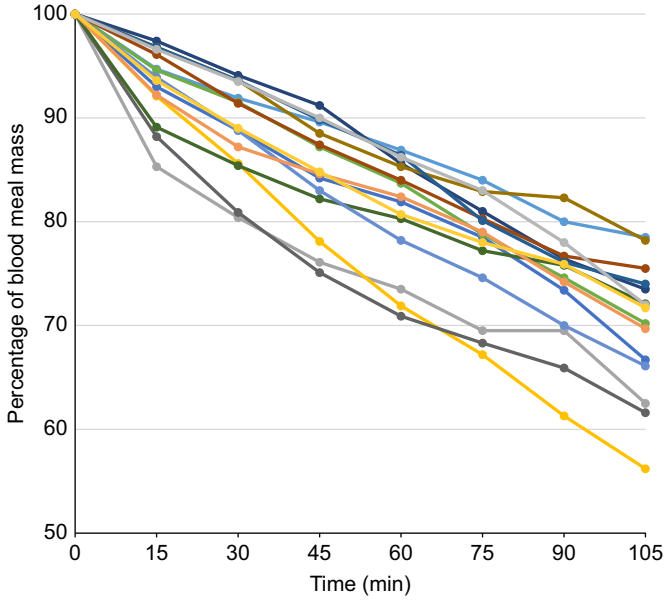

Fig. 1. Mass loss over time of 15 individual female clothing lice following a blood feed to repletion. The different colours represent individual lice.

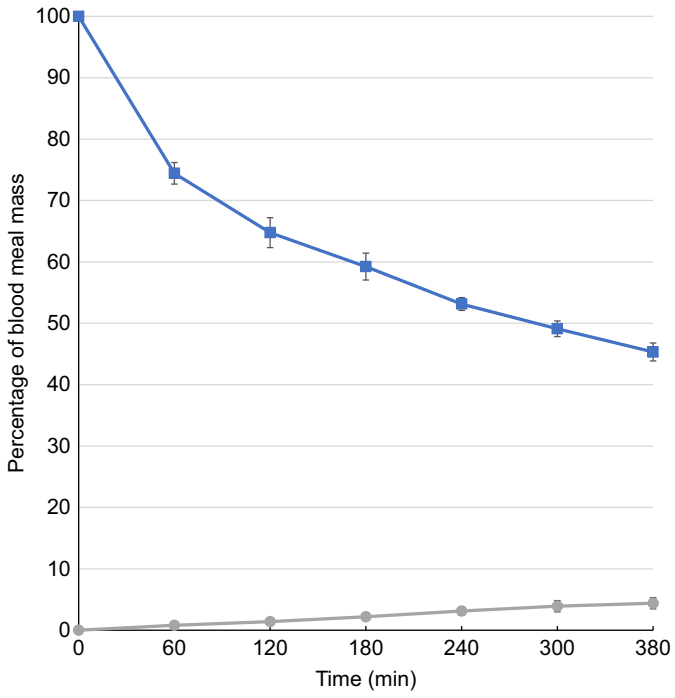

Fig. 2. Mean (±s.d.) mass loss over time of three groups of 20 clothing lice following a blood feed to repletion (blue squares) and concurrent mean (±s.d.) mass of faeces production (grey circles).

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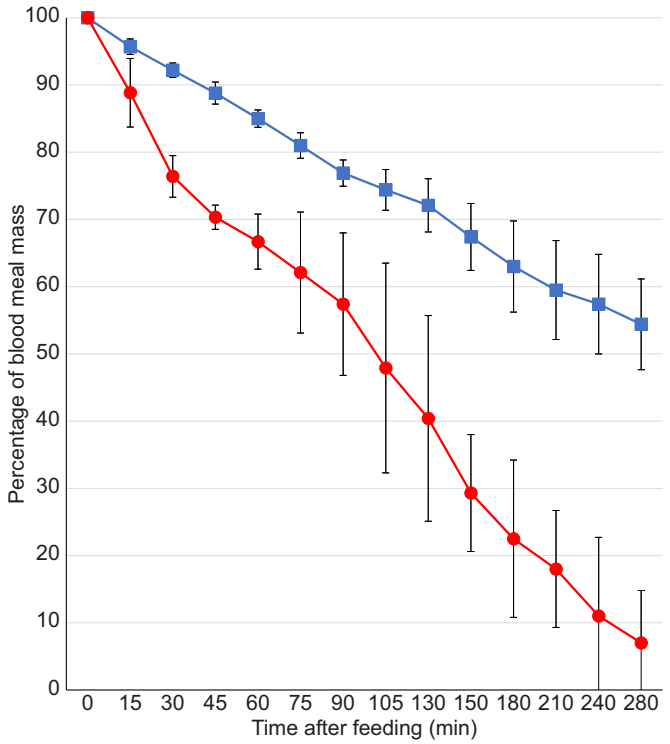

Fig. 3. Comparison of mean (±s.d.) mass loss over time of three groups of clothing lice (blue squares) and three similar groups of head lice (red circles).

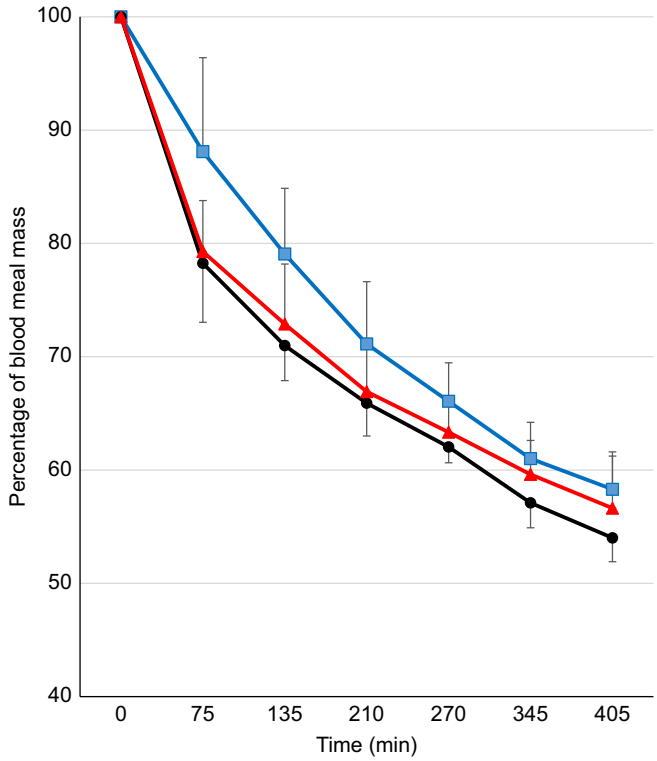

Fig. 4. Comparison of mean (±s.d.) mass loss over time from clothing lice maintained at different relative humidities (RH). 'Saturated' air (putatively 100% RH; blue squares), 70% RH (black circles) or 20% RH (red triangles) (some error bars omitted to avoid confusion).

respiratory system by physically blocking spiracles. Some occlusion media, such as colloidin and lacquers, proved unsuitable because they dried too rapidly and did not adhere to the sclerotised cuticle of the pleural plates surrounding the spiracles. Petroleum jelly applied across all spiracles (both the abdominal and thoracic spiracles) of clothing lice induced reduced activity in the insects and minimal mass change after an initial brief period of mass loss during application of the gel (Fig. 5). When it was applied only to the thoracic spiracles there was initially a slightly reduced rate of mass loss, but not significantly different from that of untreated lice (Fig. 5), which increased with time so that by 150 min the difference was significant (P=0.017). In contrast, if petroleum jelly was applied only to abdominal spiracles, the rate of mass loss after feeding was significantly increased (P=0.045) relative to untreated insects fed at the same time (Fig. 5).

It was physically more difficult to occlude the spiracles of head lice and this was less effective at reducing the rate of mass loss compared with untreated lice, so that after 17 h, approximately 50% of the mass of the blood meal had been lost, whereas untreated lice had lost mass equivalent to 160% of the initial mass of the blood meal. Head lice dehydrated beyond 18 h were mostly motile but non-viable, being unable to feed despite attempting to suck blood. They probed the skin, attached their stylets, and the cibarial and pharyngeal pumps could be seen through the transparent cuticle to be actively expanding and contracting. It was concluded that blood was not ingested because the lice were too dehydrated to produce the saliva required to prevent blood from clotting. In contrast, head lice with occluded spiracles were mostly non-motile after 18 h, despite not showing evidence of dehydration. Some lice had died, having ruptured their gut in the intervening period in a similar manner to lice treated therapeutically using dimeticone fluids (Burgess, 2009). Others appeared normal but showed minimal activity of the appendages and gut.

Immersion in water could test whether excretion is by an active mechanism, independent of aerobic conditions, because the spiracles would be occluded but without physical blockage. When immersed in water, all lice became immobile within 1–2 min and some fed insects initially showed some loss of mass. When lice were continuously immersed beyond that initial period, removed at intervals, blotted lightly and weighed, they showed no further mass loss but no mass gain between 60 and 360 min, after which some lice slowly increased in mass (Fig. 6). When immersed beyond 8–12 h, they all became turgid and immobile, but a majority immersed for less than 24 h recovered motility and were able to feed after dry incubation for 30–60 min at 30°C.

DISCUSSION

This study confirms earlier indications that Anoplura, as exemplified by human lice, lose mass rapidly after feeding and continue to lose mass in a steady manner on a more or less continuous basis. Apart from a small quantity of dry faecal matter, the entire mass loss appears to be due to active excretion of water via spiracular transpiration. Any occlusion or physical blockage of the spiracles, particularly those on the thorax, reduces or halts mass loss, as described previously when head lice were coated by non-volatile siloxanes (Burgess, 2009).

No other group of terrestrial insects has been shown to actively excrete water other than as urine, and in most cases respiratory transpiration is a potentially disadvantageous consequence of respiratory activity over which insects have limited control (Benoit and Denlinger, 2010). It appears that sucking lice have

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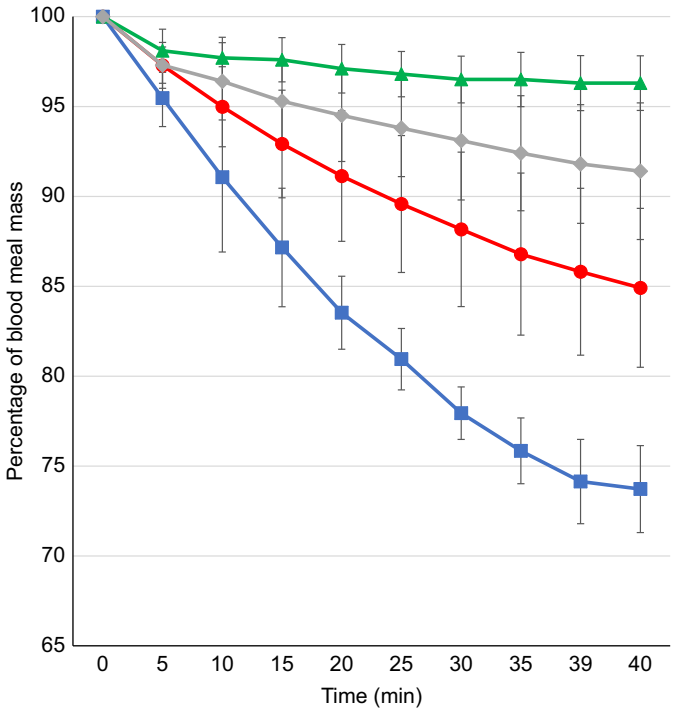

Fig. 5. Comparison of mass loss (mean±s.d.) over time of groups of clothing lice with spiracles coated with petroleum jelly. All spiracles coated (green triangles); thoracic spiracles only coated (grey diamonds); untreated with no spiracles coated (red circles); and abdominal spiracles only coated (blue squares).

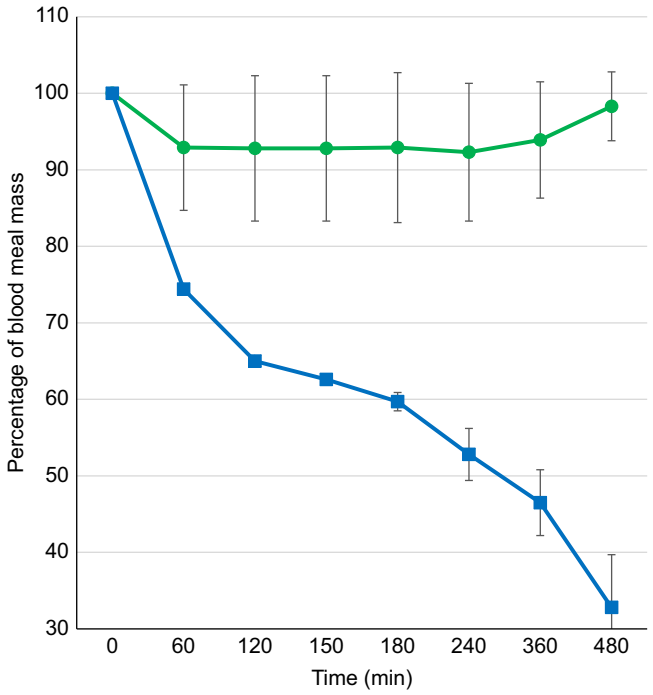

Fig. 6. Comparison of mass loss over time of clothing lice following a blood feed and lice from the same batch immersed in water. Group immersed in water (green circles) and group maintained in air (blue squares).

exploited this weakness to their advantage, in parallel with adopting a constant parasitic habit giving continuous access to surplus water in their diet. However, the active process of excretion of water by this route also has a disadvantage in that the insects appear incapable of adequately controlling or switching off the excretory process, so that, if removed from their host, they simply continue to lose water until they die from dehydration, a process that occurs more rapidly in lice that infest humans compared with other insects.

Respiratory water loss is a process that has raised various controversies, and few studies have adequately evaluated respiratory loss of water relative to cuticular transpiration. Only in tenebrionid and some other beetles living in xeric environments has respiratory transpiration been shown to significantly exceed water loss through the cuticle (Nicolson et al., 1984; Zachariassen et al., 1987). In at least one species, *Phrynocolus petrosus*, there was found to be minimal transpiration through the cuticle and the abdominal spiracles, so the majority of water loss was via pronotal spiracles (Zachariassen, 1991). The experiments occluding different spiracle groups on lice produced a similar result.

It has been accepted as a general underlying concept that one controlling factor for respiratory water loss in free-living terrestrial insects is management of spiracle opening and gaseous exchange through the mechanism of discontinuous gas exchange (Chown et al., 2006). In insects such as scarabeids living in relatively xeric conditions, this is relatively easily measured, and it has been shown that metabolic rate and gas exchange pattern are contributory factors to respiratory transpiration, and that respiratory excretion of water is a significant contributory factor in overall water loss (Chown and Davis, 2003). However, in the majority of species that have been evaluated for the impact of discontinuous gaseous exchange on water balance, it has also been possible to physically identify the open, flutter or closure states of spiracles (Lighton, 1996).

Unlike most insects living in a xeric environment, anopluran lice have an internal-valved atriate spiracle with a fixed aperture (Fig. 7). Opening of the respiratory tract is by means of a muscle running from the body wall to a chitinous rod that is inserted into the tracheal wall close to the base of the spiracle. Contraction of the muscle pulls the rod and straightens the upper extremity of the tracheal tract, where there is a narrow, flexible passage formed by the walls of the spiracular gland. This closure mechanism, described by Hase (1931) and Webb (1946), was believed to seal the tracheal trunk adjacent to the spiracle and was speculatively attributed to a mechanism for possible water conservation by Buxton (1947), yet, while on the host, there is no evidence that sucking lice have any requirement to conserve water in their bodies. The greater likelihood is that they experience a problem in dealing with excess water in a controlled manner.

In the relaxed state, the tracheal/spiracular aperture would be effectively closed because there is no pull on the trachea, reducing water loss in the unfed clothing louse; however, in the recently engorged louse, the tracheal tract appears to be pulled open on some regular basis during the initial period in order to actively excrete water, as shown in Figs 2 and 3. This characteristic sets the Anoplura apart from all other groups in the Psocodea, the Amblycera, Ischnocera and free-living ‘Psocoptera’, in that some members of each of these groups have been shown to have some capacity for active extraction of water from unsaturated atmospheres with an RH over 43% (Rudolph, 1982a,b, 1983). Water loss for those species only consistently occurred in dry air.

After feeding, the large midgut of a sucking louse normally exhibits energetic peristalsis over several hours, during which time it shrinks, presumably as water is extracted from the blood meal into the haemocoel. During that time also, only small quantities of older

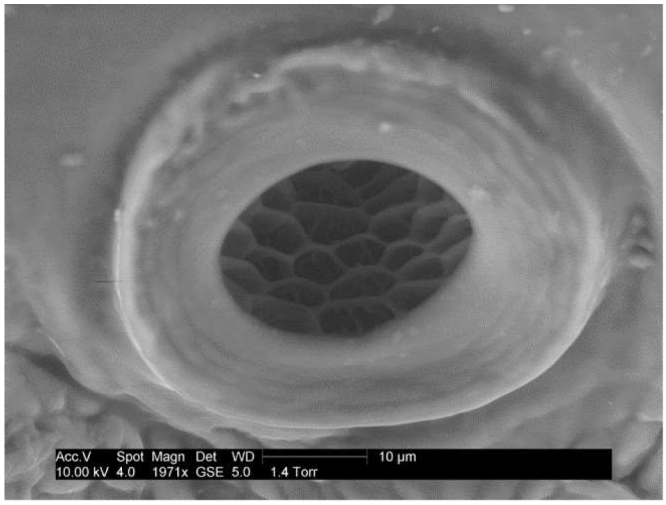

Fig. 7. Head louse thoracic spiracle. SEM of a thoracic spiracle to show the fixed opening and 'honeycomb' pattern of chambers within the atrium. Image from Burgess (2009).

digested material can be observed to be released into the hindgut for excretion as faeces. Water extracted from the ingested blood increases the turgor pressure inside the haemocoel, especially in laboratory reared clothing lice that take a large blood meal, so that any damage to the flexible cuticle of an engorged louse within 1–2 h of feeding results in fluid being physically expressed, sometimes pushing out adjacent pieces of internal organs at the same time. It is unclear whether this apparently high internal pressure of the haemocoel is a component of the mechanism for continuous excretion of water through respiratory transpiration, as indicated by Corbet (1988), or whether excretion is effected through some as-yet-unidentified active mechanism. However, if the spiracles are occluded to prevent water vapour excretion, the increased haemocoel pressure results in physical stress and gut rupture of the type observed in lice occluded using silicone-based head louse treatments (Burgess, 2009). There appears to be no facility for lice to expel water through the alimentary tract, either as liquid faeces, urine or via the retracted mouthparts, because droplets of water are never seen at either extremity.

Human lice immersed in water for long periods remain immobile until removed and dried, apparently respiring anaerobically (Bacot and Talbot, 1919; Candy et al., 2018). Because there is no apparent water uptake by lice during the first several hours of immersion (Fig. 6), this suggests some physical mechanism of water exclusion in the anaerobic environment. It may be assumed that in addition to the apparent natural closure of the duct leading to the trachea from the spiracle (Webb, 1946; Maunder, 1983), the waxy, hydrophobic surfaces of the complex structure of the spiracle atria would prevent water ingress (Fig. 6), and even after several hours in water, fluid has not been observed to enter the tracheae (Mumcuoglu et al., 2006; Candy et al., 2018). But during an extended period of immersion, water enters the body of the insect, which becomes turgid, distended and, ultimately, non-viable.

This investigation has shown that water taken in by a sucking louse as part of its blood meal is eliminated rapidly and that this process only occurs aerobically. Occlusion of different groups of spiracles demonstrated that the water is primarily excreted via the respiratory system but the mechanism for this was not identified. A possible site for this 'novel' excretory process that warrants further investigation is the thin-walled spiracular gland that forms the neck of the trachea close to the spiracle, identified by Webb (1946) as having a 'secretory' function.

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Competing interests
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