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Lazy hitchhikers? Preliminary evidence for within-habitat phoresy in pygmephoroid mites (Acari, Scutacaridae)

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Abstract

Mites of the family Scutacaridae have been found in termite nests and also phoretic on termites. In contrast to what has been reported until now for scutacarid mites, the respective species did not possess claws on leg I for attachment on their host, but clasped to the termites' coxae probably by using their large, pad-like empodia on legs II and III. This mode of attachment is a totally new and unique discovery for Scutacaridae. The fact that the mites were present on non-winged termites, which are no suitable dispersal units, points to a yet undiscussed function of phoresy: apart from serving as long-distance transport between habitats, it may also be an energy-saving way of movement within a habitat (in this example, the termite nest).

Keywords association | phoretic transport | termites | phoronts | *Imparipes* | *Reticulitermes*

1. Introduction

Mites (Acari) are minute arachnids with body sizes that usually range below 1 mm. As their abilities for active dispersal are very limited because of their small size and the absence of wings, many mite taxa disperse to new habitats passively via air or water currents (anemochory and hydrochory) or commonly attach themselves to other, larger and more mobile organisms (phoresy) (e.g. Schatz 1991, Krantz & Walter 2009). Several animal taxa can serve as phoresy hosts, and most of all social insects play a very important role (e.g. Szymkowiak et al. 2007). In many cases, the relationship between mites and their insect hosts is not only restricted to phoretic transport, but the mites also live as inquilines in the nests of their hosts where they find favorable conditions for reproduction and might have a positive influence on their hosts by playing a sanitary role (Biani et al. 2009).

Members of the mite family Scutacaridae (Heterostigmatina, Pygmephoroidea) inhabit different types of soil and, based on biological studies on few species, are considered to be fungivorous. Approximately half of the about 800 known species were found in association with an animal, either as phoronts, as inquilines, or both. Most scutacarid species can be found associated to ants, to beetles of the family Carabidae and to small ground-dwelling mammals, but other insects like bees or termites, birds and even arachnids are also used as hosts (Baumann 2018). While some other mite taxa have special developmental stages as dispersal units (e.g. hypopi of Astigmata), in Scutacaridae the adult females perform phoresy. For attaching to their hosts, they are known to use large claws on tibiotarsus I (e.g. Karafiat 1959, Khaustov 2008, Baumann 2018). In some species of the genera *Archidispus* (6 species), *Lamnacarus* (1) and *Scutacarus* (6), female dimorphism in connection

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with phoresy has been demonstrated by various authors (see Baumann 2018): there are phoretic morphs with large claws and non-phoretic morphs with small or absent claws on leg I. The non-phoretic morphs, which are also smaller and weakly sclerotized, are considered to be 'energy saving' forms mainly responsible for rapid reproduction (Ebermann 1991). Finally, several presumably exclusively soil inhabiting scutacarid species only consist of females without claws on leg I.

During collections in Spain, a new scutacarid species was encountered in association with termites. Most individuals occurred free-living inside the termite nests, but some termites also bore phoretic scutacarids, which encouraged the closer study of the phoretic association. By keeping termites and mites in glass tubes in the laboratory, it was possible to observe their interactions. The study revealed a method of attachment new for scutacarid mites, and moreover a possible new function of phoresy apart from the typical long-distance, between-habitat dispersal.

2. Material and Methods

Five parts of dead wood of *Pinus halepensis* (Pinaceae) of approx. 30 cm length infested with termites and surrounding soil were collected on the 18.02.2017 near El Saler, Valencia, Spain (39°20'24.6"N, 0°18'43.0"W) and transported to the Laboratory of Acarology of the Instituto Agroforestal Mediterráneo, Universitat Politècnica de València (UPV) for inspection under a Nikon stereo microscope. Termites were determined as *Reticulitermes banyulensis* Clément, 1978 based on the information available in Clément et al. (2001). Scutacarid mites were encountered in the tunnels constructed by termites inside the wood when it was cracked open. Groups of about 40 termites consisting of immatures, soldiers, workers and few young alates were put in three glass tubes (diameter 3 cm, height 10 cm) filled with soil and parts of wood from the collection site. As it was not possible to proof whether the termites were infested with phoretic mites, approximately 30 scutacarids collected from the tunnels were added to each tube. The tubes were kept in darkness at room temperature, and only in one of the tubes a relatively stable colony could be maintained. In this very basic setup, the termites thrived for 3 months until the state of the small colony worsened within few days and all individuals died. During this time, a manipulation of the termites inside the tube was not feasible, but the interplay between termites and mites was observed through the glass on a daily base. Subsequent efforts to establish further termite colonies under more controlled conditions failed and because of this, no quantitative study could be performed. Only a limited number of termites was available for simple behavioral experiments and the observations presented in this work can thus only be considered as preliminary information.

For the behavioral experiments, termites (soldiers and workers, 5 individuals per dish) were put into two plastic dishes with moist plaster of Paris. The dishes were also supplied with small particles of wood so that the termites could hide beneath them. Fifteen mites were added in each vessel and the termites were checked for phoretic mites after 24 hours in the first and after 7 days in the second trial.

Phoretic scutacarids from these experiments as well as free moving specimens from the tunnels were collected with brushes and microscopic slides were prepared using Swan's embedding medium (Swan 1936) for their identification. Additional specimens were studied by Environmental Scanning Electron Microscope (ESEM) in low vacuum mode and by conventional SEM at the Graz Centre for Electron Microscopy (ZFE) & Institute of Electron Microscopy and Nanoanalysis (FELMI).

3. Results

The morphological investigation showed that the mite species strongly resembles *Imparipes adleri* Delfinado and Baker, 1976, which has been described from the USA in association with the termite *Reticulitermes virginicus* (Banks, 1907), but it displays characters indicating that is a new species that is yet to be described. In the further text, it will thus be referred to as *Imparipes* nr *adleri*. No morphological differences between the phoretic and the free moving specimens were present.

In the behavioral experiments, in the first trial (after 24 hours) one termite was infested with two phoretic scutacarids. In the second trial (after 7 days) only two termites were still alive, both of them with attached scutacarids. On one termite there were five and on the other termite seven mites. The scutacarids always were located between the termites' coxae, embracing the coxa with their legs, the gnathosoma facing towards the termites' body (Fig. 1A). When the termites were touched with tweezers or put into water, the mites reacted strongly to this external interruption and quickly left their host. The morphological investigation of scutacarids collected from their termite hosts showed that they did not possess claws on leg I (Fig. 1B). On the other hand, claws and empodia were well developed on legs II and III (Fig. 1B, C, D).

The daily examination of the small termite colony in the glass tube showed that at the beginning of the observations only about 5 % of the termites were infested with phoretic scutacarids and the number of attached mites was low (1-2 individuals per termite). After three months, when the colonies were in a subjectively evaluated 'bad state' characterized by increasing numbers of dying termites, infestation rate and number of scutacarids per termite were very high (95% of all termites regardless of the caste infested, up to 10 scutacarids per individual). No scutacarids could be encountered on alates. As the termites inside the tube could not be manipulated, the given percentages are estimates.

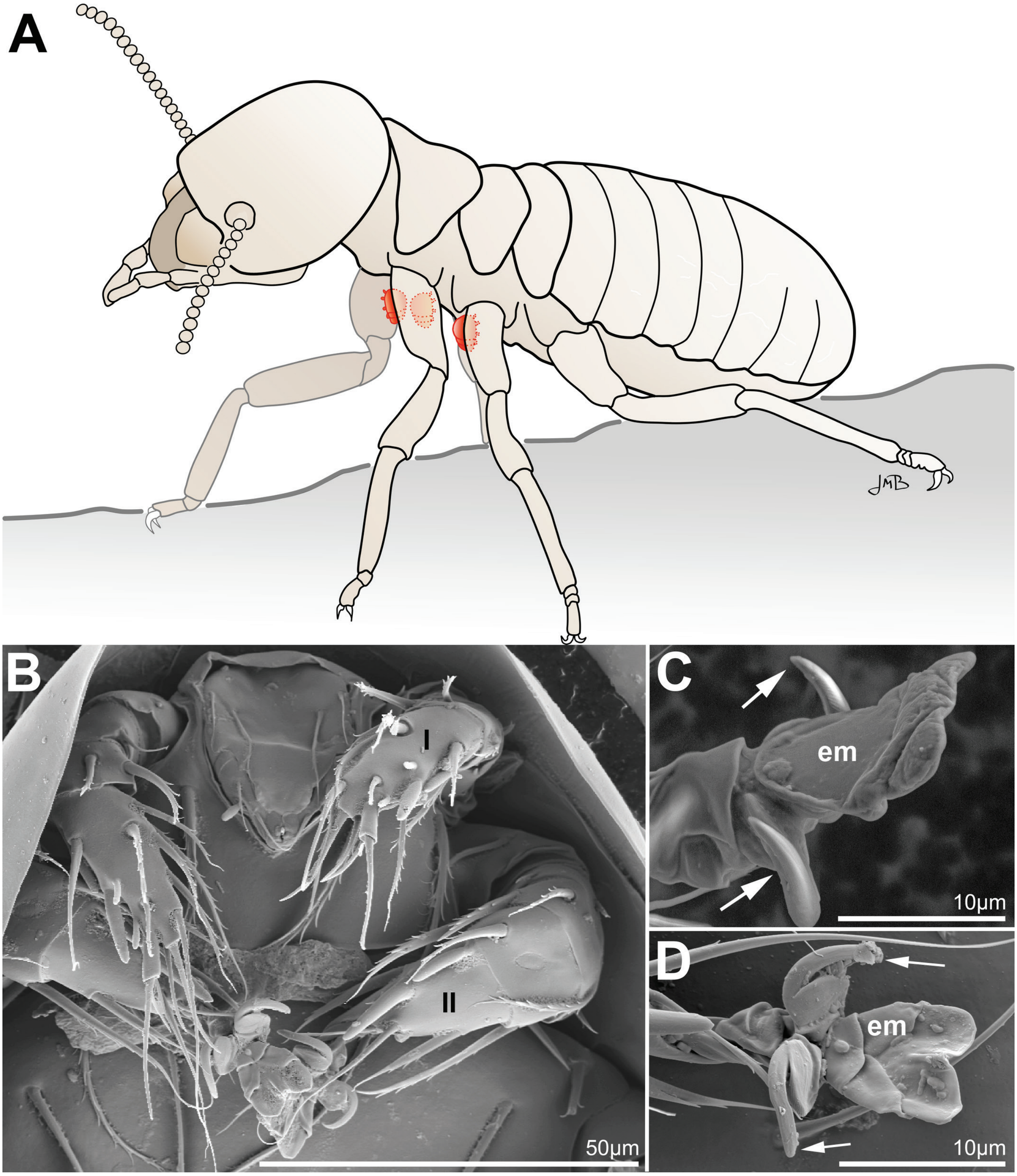

Figure 1. (**A**) Position of *Imparipes* nr *adleri* (in red) on a termite host, between the termite's coxae; (**B**) SEM image of the anterior ventral part of *I.* nr *adleri*, showing the gnathosoma, clawless legs I, and legs II with 2 small claws and large empodium; (**C**) close-up (ESEM image in low vacuum mode) of praetarsus of leg II, ventral side; (**D**) close-up (ESEM image in low vacuum mode) of praetarsus of leg II, dorsal side. I = leg I, II = leg II, em = empodium. Arrows point to claws.

In one occasion, short appetence behavior was observed: a female located near a termite positioned itself on its legs III and IV and raised legs I and II in the air when the termite started to move. In the end, the termite was not mounted, but the observed behavior was very similar to the appetence behavior prior to phoresy that has been described for several scutacarid species (Baumann 2018). Another female was observed seeking its final phoresy location between the termite's coxae. The respective mite reached the abdomen of the termite through the surrounding substrate without evident previous appetence behavior and moved freely over the insect's body until it reached the coxae, where it remained motionless.

The termites never removed the scutacarids, neither when autogrooming themselves nor when allogrooming nestmates. However, sometimes mites avoided the grooming termites by simply walking away to the dorsal site of thorax or abdomen of the termite, only to return to their original position shortly after.

4. Discussion

4.1 Attachment on host

The observed form of phoresy is an entirely new discovery for the mite family Scutacaridae as until now, only females with claws on leg I have indeed been observed phoretic on their hosts. There are some rare reports of phoretic scutacarids without claw on leg I (Baumann 2018); however, the respective species were extracted together with their presumed hosts through Berlese funnels and thus phoresy was proposed although the attachment *per se* had not been observed. In the closely related family Microdispidae, several species without claw on leg I have already been reported as phoretic (e.g. Hajiqanbar & Sobhi 2018).

Apparently, females of *I.* nr *adleri* attach to the termite coxae mainly with the help of their large empodia in form of a pulvillus on legs II and III. The termite coxae are only sparsely hairy and thus offer sufficient smooth surface for attachment by these adhesive pads. Moreover, the width of the mites (measured between articulations of their legs: around 120 µm) corresponds with the width of the termite coxae, which additionally facilitates an attachment by clasping. Although the observed empodia are considerably large, there are several descriptions of other scutacarid species (with as well as without claws on leg I) with empodia of comparable sizes. It is not known yet whether the respective species also use these structures for attaching on hosts.

The attachment location between the coxae is a favorable area that protects the mites from environmental influences. There are also other scutacarid species that can be found on the coxae of their hosts, for example on ants or bark beetles (Baumann 2018). In general, phoretic mites use not to occur distributed randomly on their host, but to gather in 'protected places' like said coxae or under the elytra of beetles (Eickwort 1994).

4.2 Function of phoresy

Generally, phoresy is defined as a means of dispersal in order to reach new habitats that the phoronts themselves could not reach due to their limited mobility (e.g. Krantz & Walter 2009, Camerik 2010). Active dispersal/migration of the host and accordingly also phoresy of the phoront are practically always considered as temporally clearly delimited actions which cover large distances and should follow the most direct route to the best habitat (e.g. Binns 1982; Krantz & Walter 2009). Subterranean termites like the genus *Reticulitermes* possess winged reproductive individuals which can disperse and found new colonies. Due to the common understanding of phoresy, these individuals should thus be the preferred host caste. In the present case, no phoretic scutacarids could be found on alates, but this might be due to the simple study set-up and the little number of alates available. The attachment of phoretic scutacarids to unwinged termites may not be an adaptive behavior at first glance as these individuals only have limited mobility. However, they also disperse since the termites' nests themselves are not locally bound. In fact, whole colonies slowly progress with amoeba-like foraging movements, and new colonies can emerge through budding (Thorne et al. 1999). As the reported form of phoresy is quite unstable (the mites actively leave the termites when they are slightly disturbed), it could represent a hitherto undiscussed function of this behavior by serving as 'short-distance transport' within the termite nest.

Although females of *I.* nr *adleri* can be found throughout the termites' nest, they only reproduce and lay eggs near termite corpses that have been buried with particles of soil by their nestmates (pers. obs.). When the corpses decay, fungal hyphae start to grow, and these fungi serve as food for the scutacarids. By mounting termites, the scutacarids can probably easily reach sites with buried corpses within the nest. The same way, they can follow the termites on their foraging movements, which use to happen underground (Thorne et al. 1999). Presumably, scutacarids could also move through the termite nest by their own locomotion, but phoresy seems to be an attractive, energy-saving alternative strategy. As they are moving in a safe environment where accidental dismounting of the host has no severe consequences, no selective pressure towards firm attachment should be present. The absence or reduction of claws on leg I could thus be regarded as another way to save energy (compare with Ebermann 1991).

The observations presented in this paper still need to be supported by quantitative data gained through replicated experiments. By doing so, it will be possible to answer questions that are open to date (e.g.: After which time period do the mites attach to their host? What is the influence of environmental factors on the phoretic behavior? Can the mites also be found attached to alates?). Anyhow, our preliminary observations already allow two important conclusions:

First, in contradiction to current knowledge, all scutacarid mites species might actually be able to perform phoresy, even those without claws or with only thin and tiny claws on leg I [for example, *Scutacarus nudus*, which is associated with ants (Baumann 2018)]. This new knowledge may help to explain wide geographical distributions of presumed non-phoretic scutacarids.

Second, when defining the term ‘phoresy’, we should consider the idea that it may not only be an indispensable form of long-distance transport used by minute organisms in order to overcome hostile regions and to reach new suitable habitats. Additionally, it could also be an energy-saving solution of these organisms for moving around in a safe environment within their habitat. Such a safe habitat can be found in termite nests, like described in this paper, but nests of ants, which are known to harbor a variety of associated mites (e.g. Campbell et al. 2013), would be another example.

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