

Mate-guarding in the genus *Creutzeria* (Astigmata: Histiostomatidae), an aquatic mite genus inhabiting the fluid-filled pitchers of *Nepenthes* plants (Nepentheaceae)

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Abstract

A number of genera of Histiostomatidae inhabit phytotelmata, being found fully submerged and filtering organic matter from the fluid. One such phytotelm habitat is the fluid-filled pitchers of species of *Nepenthes*, a carnivorous plant genus widely distributed in the Old World tropics. As in most histiostomatid species, males of *Nepenthes*-inhabiting mites seek out tritonymphal females and guard them from other males, a behavior termed “precopulatory mate-guarding”. Upon ecdysis of the tritonymph to adult, mating takes place. Successful mate-guarding prevents other males from gaining access to a virgin female and helps ensure the guarding male’s paternity of any resultant offspring.

While most *Nepenthes*-inhabiting genera are ambulatory, walking about on decomposing arthropods in the pitchers, species of the genus *Creutzeria* are natatory and propel themselves through the fluid using legs greatly modified for this purpose. Males of purely ambulatory species typically climb onto the dorsum of a tritonymphal female and firmly clasp her idiosoma with their legs. Some species do not move about on their own accord, but rely on the female to carry them. In others, two pairs of legs are used for clutching the female; the other two pairs are very long, thereby allowing the male to walk about while holding the securely clasped female off the substrate. Swimming is a more laborious task, especially since the male must propel not only himself through the fluid but also the female. The mate-guarding behavior of *Creutzeria* spp. was studied by direct observation under a stereomicroscope and from videos filmed through a stereomicroscope. In addition, phase contrast and scanning electron microscopy were used to examine the morphological adaptations associated with mate-guarding and mating. Legs I of males are greatly modified for clasping a tritonymphal female during mate-guarding and legs III and IV for swimming. Legs II are a morphological compromise, being used for swimming as well as for grasping the substrate for resting when legs I are occupied with a female.

Key words: *Creutzeria*, *Zwickia*, *Nepenthes*, precopulatory mate-guarding, mating behavior

Introduction

A male’s fitness is typically measured by the number of offspring sired. In this regard, an individual’s reproductive success can be advanced by inseminating the partners of other males as well as by preventing his own sexual partners from being inseminated by rival males (Parker 1970). The latter is generally accomplished by means of precopulatory and/or postcopulatory mate-guarding (Alcock 1994; Choe & Crespi 1997).

Precopulatory mate-guarding is commonly observed in the astigmatic family Histiostomatidae, species of which are typically found in moist or wet habitats. Some taxa are fully aquatic and include species that inhabit phytotelmata. One such phytotelm habitat is the fluid-filled pitchers of species of *Nepenthes*, a carnivorous plant genus of over 82 species that ranges in the Old World tropics from Madagascar and the Seychelles in the west, through Southeast Asia, to New Caledonia in the east (Clark 2001). Several histiostomatid genera, some undescribed, are obligatory inhabitants of

Nepenthes pitchers and adapted to withstand digestive enzymes produced by the plant. As in many other histiostomatid species, males seek prequiescent and quiescent tritonymphal females and actively guard them from other males (personal observation). Mating takes place soon after the tritonymphal female molts to an adult. Successful mate-guarding prevents other males from gaining access to a virgin female and thereby helps to insure the guarding male's paternity of resultant offspring.

The Histiostomatidae is a very large and diverse family, and its diversity is reflected in both behavioral and morphological adaptations associated with mate-guarding. Most species inhabiting *Nepenthes* pitchers are ambulatory and found walking about on decomposing arthropods or on the sides of the pitcher. Natatory behavior has, however, been observed in the genera *Zwickia* (Fashing 2004) and *Creutzeria* (Fashing *et al.* 1996). Swimming is a more laborious task than walking and requires extensive modification of the legs (Fashing 2004; Fashing *et al.* 1996). This in turn imposes restrictions in male adaptations for mate-guarding. Mate-guarding and its associated morphological adaptations have been described for the genus *Zwickia*, a taxon that is both ambulatory and natatory (Fashing 2004). The following account describes precopulatory mate-guarding in the genus *Creutzeria*, a taxon that is solely natatory (Fashing *et al.* 1996).

Methods and materials

The genus *Creutzeria* was established by Oudemans (1932) to accommodate *C. tobaica* collected from *N. tobaica* Dans. in Java. Since then, only one additional species has been described; *C. seychellesnsis* Nesbitt from *N. pervillei* Blume in the Seychelles (Nesbitt 1979). Neither of the two described species were available for this study. The genus is, however, widespread and contains a number of undescribed species including those used in this study. Based on my observations and collections, the genus can be split into two species groups based on size: a western group with idiosomal lengths in the 500–650 μm range that inhabit *Nepenthes* pitchers in Madagascar and the Seychelles, and an eastern group with idiosomal lengths in the 200–350 μm range that inhabit *Nepenthes* pitchers in southern Asia and northern Australia. Both groups have similar morphological adaptations for swimming and for mate-guarding by males, and were collectively used to describe mating behavior.

Species of *Creutzeria* observed in this study were collected from the following localities and *Nepenthes* species: Cape York Peninsula, Australia (*N. mirabilis* (Lour.) Druce); Brunei (*N. albomarginata* T. Lobb ex Lindl., *N. ampullaria* Jack, *N. bicalcarata* Hook. f., *N. gracilis* Korth); Dauphin, Madagascar (*N. madagascariensis* Poiret); and southern Thailand (*N. gracilis*).

Since specimens from Madagascar were provided by colleagues and preserved in alcohol, they could be used only for morphological studies. Those from Australia, Brunei and Thailand were transported back to the laboratory in *Nepenthes* pitchers, the pitcher contents placed in petri dishes, and mate-guarding behavior observed using a stereomicroscope. In the case of the Australian *Creutzeria*, video recordings of mite behavior were made for later study.

To study the morphological adaptations associated with mate-guarding, specimens from all localities were cleared in Nesbitt's solution, mounted on microscope slides in Hoyers medium, and examined using phase contrast and interference microscopy. Setal nomenclature for the legs follows Grandjean (1939). Measurements were taken on idiosomal, leg and tarsal lengths of males, females and tritonymphs of *Creutzeria* **sp. nov.** from Madagascar. In addition, specimens from all localities were prepared for scanning electron microscopy (SEM) by dehydrating in ethyl alcohol, drying in a Samdri-PVT-3B critical point dryer (Tousimis), affixing to stubs with double-sided sticky tape, and

coating with gold palladium in a Hummer Sputter System (Anatech). SEM microscopy was performed on an AMR-1810.

Results and discussion

Most histiostomatid genera inhabiting *Nepenthes* pitchers are solely ambulatory and can be observed walking about on the decomposing arthropods or on the sides of the pitcher. When a male encounters an unguarded tritonymphal female, he climbs onto her dorsum and utilizes his legs to firmly clasp her idiosoma. In this position he can prevent other males from gaining access to the female and thereby reserve her as his own. In some species, males are not able to move about when guarding a potential mate and passively rides on the female's dorsum as she moves about on the substrate. In other ambulatory species, the male uses two pairs of legs to grasp the female and the other two pairs, which are very long, for walking about while holding the securely clasped female off the substrate.

Species in the genus *Creutzeria* are solely natatory and propel themselves through the fluid using legs extremely modified for that purpose (see Fashing *et al.* 1996 for details). Swimming is a laborious task, especially for a male when mate-guarding since the male must not only propel himself through the fluid but also the clasped female with her added weight and drag. Males therefore have additional modifications for both mate-guarding and swimming.

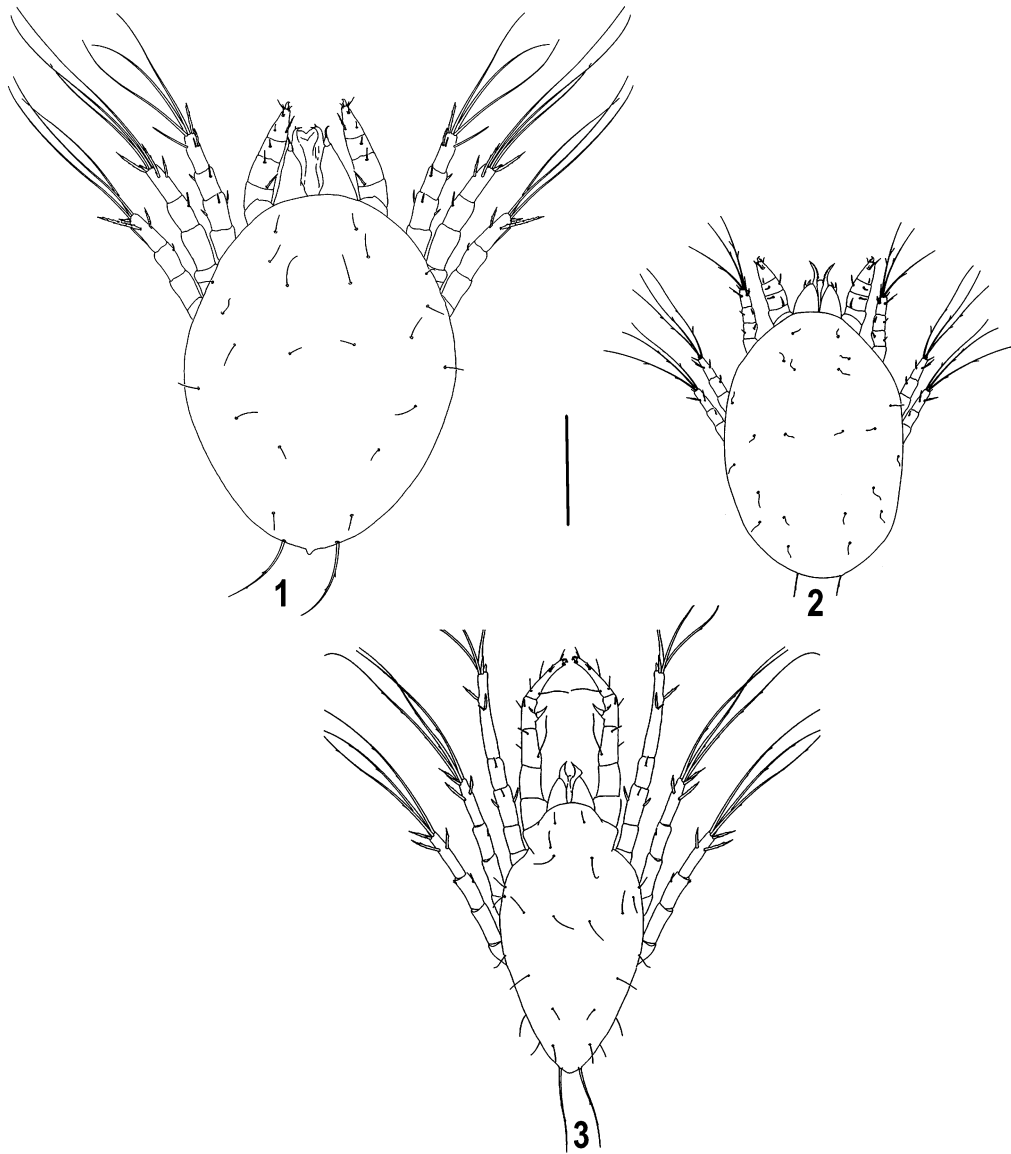
Legs III and IV are used solely for swimming by adult males as well as by adult females and immature instars and are therefore morphologically similar. The two terminal tarsal setae and claws are extremely long to aid in swimming (Figs 1–3). Legs II of females and immature instars are also used solely for swimming and are therefore morphologically similar to legs III–IV, whereas legs I are modified for grasping the substrate when resting. Legs I and II of males, however, are quite different from those of females (Figs 1–6).

Legs I of males are adapted for mate-guarding and, in comparison to legs I of females and immature instars (Figs 1, 2, 5), they are extensively modified for this purpose (Figs 3, 6, 7). They are more robust and well over twice as long as those of females and immature instars (Table 1). Elongate legs I are essential since, when in amplexus with a female, the male is mounted dorsally and clasps the female by surrounding her idiosoma with legs I (Figs 8–10). The tarsi of legs I are elongated, flattened ventrally and arched dorsally (Figs 6, 7, 11), and the tibiae are also elongated and flattened in relation to the tibiae of females and other instars (Fig. 7). Tibial setae *hT* are enlarged, somewhat flattened laterally, and notched at the base (Figs. 6, 7). Directly in front of seta *hT*, the tibia has a concave depression; combined, seta *hT* and the tibial depression form a deep groove (Figs 6, 7). Upon encountering a female tritonymph, a male slides the “tibial grooves” of his elongate front legs over the trochanters of the tritonymph's second legs, thereby surrounding them (Fig. 11). The flattened tarsi clasp the venter of the tritonymph's idiosoma (Fig. 11).

Legs II of males are a morphological compromise since they are not only used for swimming, but also for grasping the substrate for resting when legs I are occupied with a female. They are quite robust and long: 73% of idiosomal length, vs. 38% in females, 29% in tritonymphs, 35% in protonymphs and 39% in larvae. Their length is necessary to allow a male to reach beyond a clasped female and grasp the substrate. Unlike those of females and immature instars, the claws of legs II in males are not setiform nor are they similar in length to the two long, terminal setae. Instead, they are much shorter and somewhat claw-like for grasping the substrate as well as somewhat more elongate and flattened to aid in swimming (Figs 3, 4). With the female securely held in place with his first pair of legs, a male can actively swim about using legs II–IV while mate-guarding.

As an adaptation for swimming while carrying a female, males also have significantly longer legs than the corresponding legs of immature instars and adult females (Figs 1–3), even when

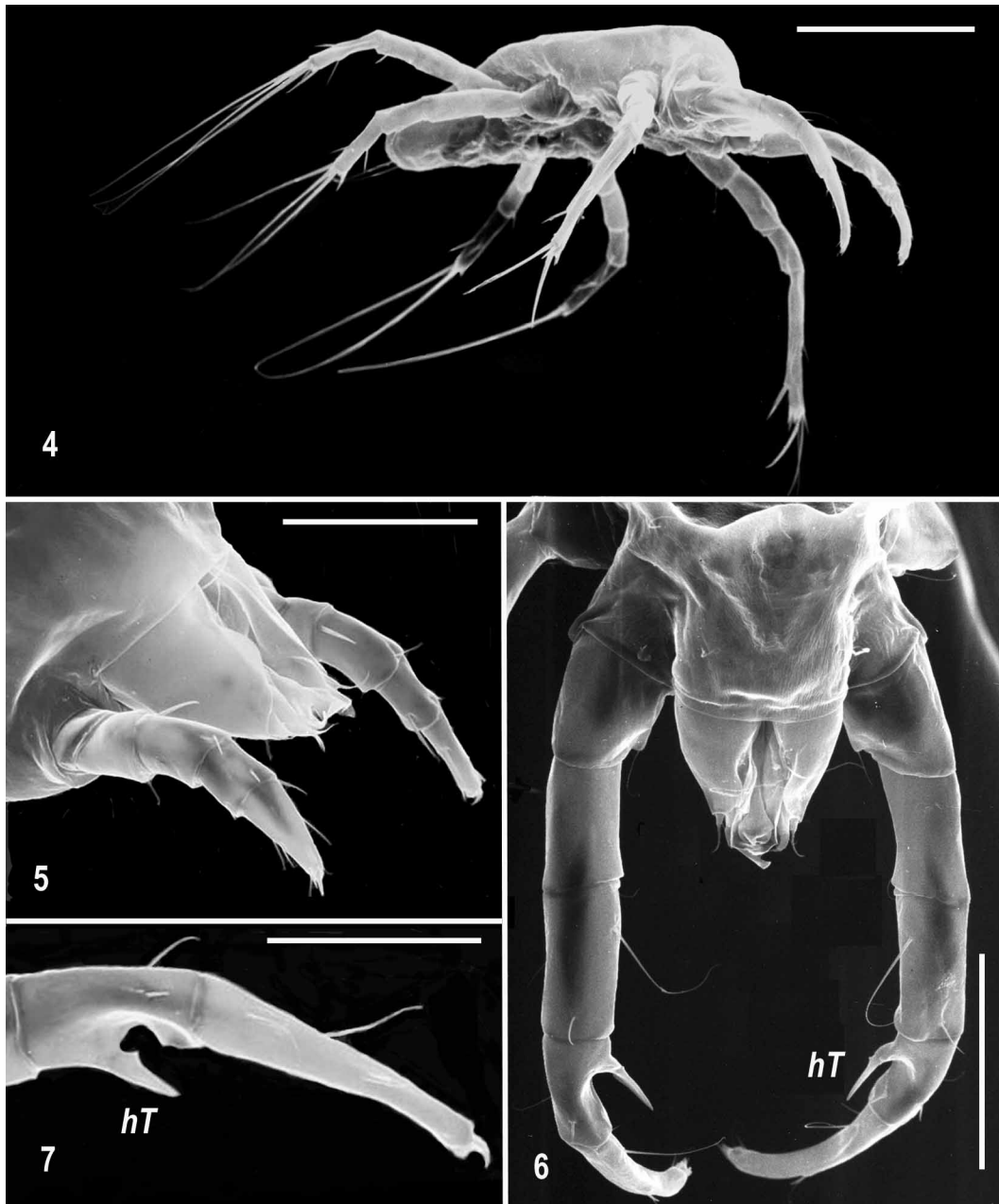
adjusted to a percentage of idiosomal length (Table 1). The longer leg length increases the length of the stroke, thereby making them more efficient for swimming. In addition, the idiosoma of a male is slender and stream-lined (Figs 3, 4) when compared to the oval idiosoma of a female or tritonymph (Figs 1, 2, 8, 9), thereby producing less drag when swimming.



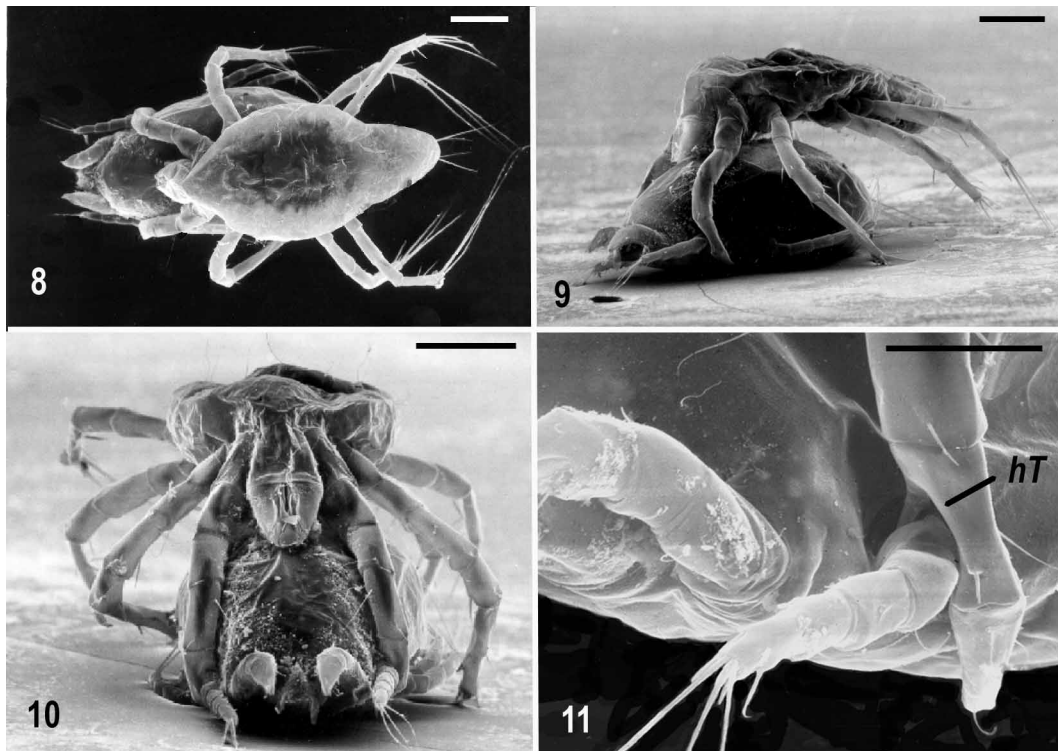
FIGURES 1–3. Drawings of *Creutzeria* sp. nov. (Madagascar). Dorsal illustration of the female (1), tritonymph (2), and male (3). Scale bar = 200 μ m.

The only other histiostomatid genus known to be natatory is *Zwickia*, and species in that genus are ambulatory as well as natatory (Fashing 2004). Their natatory behavior has also led to exceptional modifications in males for both swimming and mate-guarding (Fashing 2004). In this regard, they share several behavioral and general morphological features with *Creutzeria* spp. males. They too have longer legs than the corresponding legs of adult females and tritonymphs, especially legs I and II. In addition, legs I function primarily in mate-guarding, are extremely modified for this

purpose, and are used to clasp a female's idiosoma when mate-guarding (Figs 12–14). In *Zwickia* spp., however, it is the tarsi of legs I, not the tibiae, that are modified for clasping. In this regard, tarsi I are longer relative to those in other instars, and ventral setae *ra* and *s* are stout and broadly flattened laterally. These two setae, combined with the concave nature of the region between them, form a deep groove (Fig. 14). Upon encountering a female tritonymph, a male *Zwickia* spp. mounts dorsally and slides the “tarsal grooves” of his front legs over the proximal regions of the femora of the



FIGURES 4–7. SEM photographs of *Creutzeria* sp. nov. (Madagascar). 4. Frontal/lateral view of male; 5. Frontal/lateral view of tritonymph gnathosoma and legs I; 6. Dorsal view of male gnathosoma and legs I; 7. Dorsal/lateral view of male tibia and tarsus. *hT* = tibial seta. Scale bar = 200 μ m (Fig. 4), 100 μ m (Figs 5, 6), 50 μ m (Fig. 7).



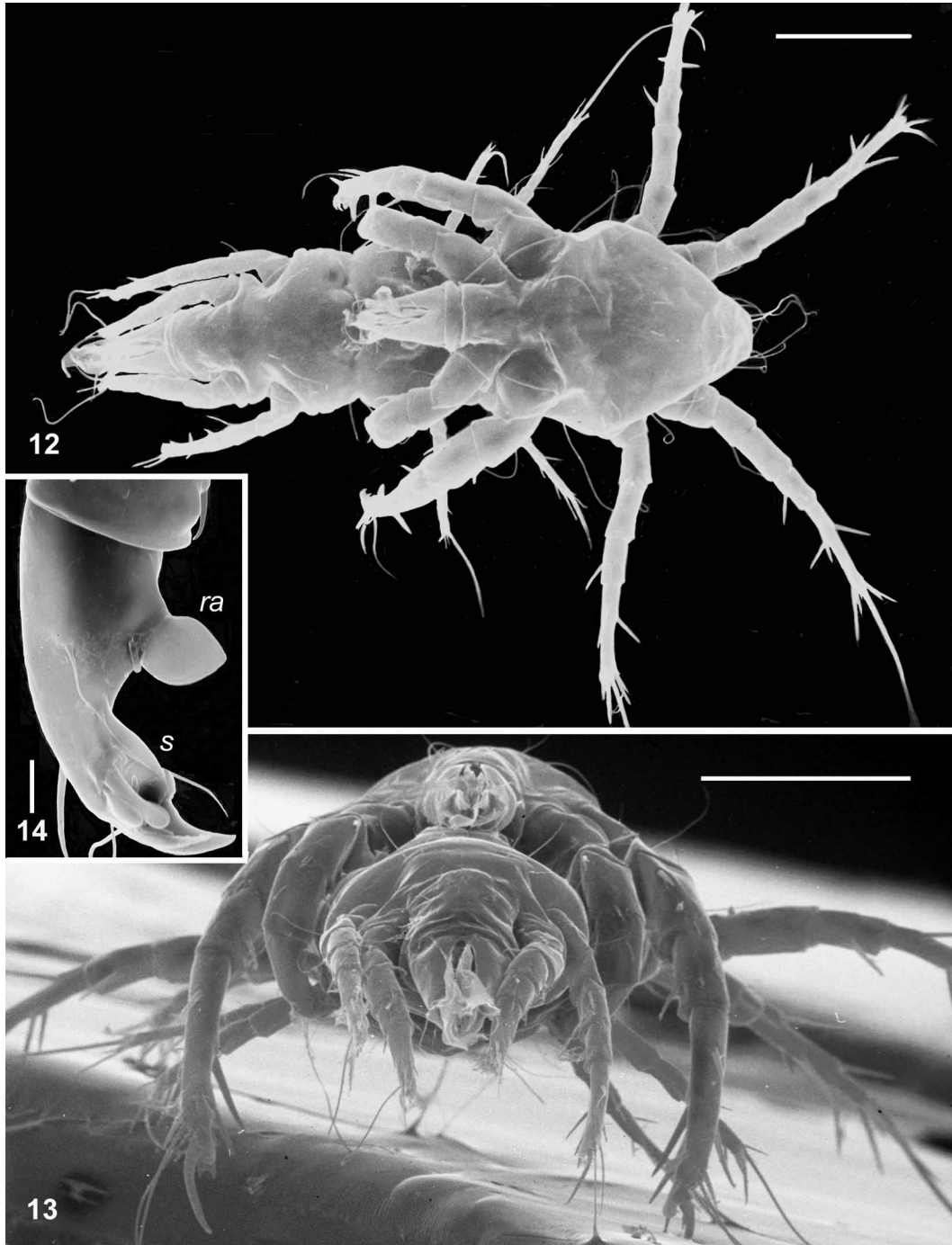
FIGURES 8–11. SEM photographs of *Creutzeria* sp. nov. (Madagascar) male and tritonymph in amplexus. 8. Dorsal view; 9. Lateral view; 10. Frontal view; 11. Lateral view of male leg I with tibia “clasping” trochanter II of tritonymph. Scale bar = 100 µm (Figs 8–10), 50 µm (Fig. 11).

TABLE 1. Measurements of males, females and immature instars of *Creutzeria* sp. nov. from Madagascar. Means and their standard errors (µm) for idiosomal lengths and leg lengths (upper) and for leg lengths expressed as a percentage of idiosomal length (lower). Sample size equals 9 for tritonymphs, 10 for others.

	Idiosoma	Leg I	Leg II	Leg III	Leg IV
Male	518 ± 8.24	344 ± 3.11 67 ± 0.92	377 ± 5.23 73 ± 1.07	309 ± 3.33 60 ± 0.88	294 ± 2.77 57 ± 0.79
Female	628 ± 20.81	196 ± 3.95 32 ± 1.20	236 ± 4.82 38 ± 1.29	260 ± 4.08 42 ± 1.55	255 ± 4.59 41 ± 1.65
Tritonymph	479 ± 11.12	128 ± 1.95 27 ± 0.78	140 ± .92 29 ± 0.82	166 ± 2.57 35 ± 1.12	163 ± 2.40 34 ± 1.01
Protonymph	383 ± 13.12	132 ± 4.01 35 ± 0.85	133 ± 2.11 35 ± 0.88	150 ± 3.10 39 ± 0.80	135 ± 3.41 35 ± 0.63
Larva	264 ± 4.82	99 ± 2.05 38 ± 1.17	102 ± 1.16 39 ± 0.57	111 ± 1.31 42 ± 0.62	—————

female’s third legs (Figs 12, 13), not the second legs as in *Creutzeria* spp (Figs 8–11). In *Creutzeria* spp. the flattened tarsi of legs I clasp the female’s venter to hold her securely in place, but in *Zwickia* spp. the muscular front legs tightly clasp the sides of the female’s idiosoma (Figs 12, 13). Unlike *Creutzeria* spp., *Zwickia* spp. tritonymphs are modified to facilitate mate-guarding; a waist-like constriction between the propodosma and hysterosoma (Fig. 12) allows the male to more easily clasp

and hold her in place (Fashing 2004). While mate-guarding, a male *Zwickia* can readily move about on solid substrate using legs II, or swim through fluid substrate using legs III and IV (Fashing 2004).



FIGURES 12–13. SEM photographs of *Zwickia* sp. nov. (Brunei Darussalam) modified from Fashing 2004. 12. Dorsal view of male and tritonymph in amplexus; 13. Frontal view of male and tritonymph in amplexus; 14. Ventral view of male tarsus I. *ra*, *s* = tarsal setae. Scale bar = 100 μ m (Figs 12, 13), 10 μ m (Fig. 14).

It is obvious that as long as a male is mate-guarding, other males do not have access to a female for copulation. Although mate-guarding behavior helps to insure the guarding male's paternity of the resultant offspring, it is not without a cost. It is advantageous to a male's fitness to mate with as many females as possible, and time spent guarding females results in loss of time available for mating with additional females (Alcock 1994). The extreme morphological modifications associated with mate-guarding found in male *Creutzeria* spp., *Zwickia* spp., and many other histiostomatid genera, indicates the benefits must far outweigh the cost.

Much remains to be learned about mating behavior in the genus *Creutzeria* as well as other histiostomatid genera. Dickinson (1997) postulated that pre-copulatory mate guarding should be found in species where receptive females are in short supply. Short supply does not necessarily mean a lack of females, but can result from a short period of female sexual receptivity that occurs immediately after the molt. In such a scenario it would be advantageous for a male to guard a quiescent immature female in order to be present when she molts. While it is not known how long *Creutzeria* spp. females are receptive after the tritonymphal molt, it is known that most males in amplexus are clasping tritonymphal females rather than adults. In addition, males were never observed clasping gravid females. These observations imply that males release females relatively soon after they molt to the adult stage, and, if so, it is possible that this is due to a short period of receptivity. Alternatively, it could mean that females mate only once and males therefore need not remain with them after mating.

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