# FINE STRUCTURE OF THE GENITAL PAPILLAE OF *HORMOSIANOETUS MALLOTAE* (FASHING) (ASTIGMATA: HISTIOSTOMATIDAE), AN OBLIGATORY INHABITANT OF WATER-FILLED TREEHOLES

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**ABSTRACT** - Scanning and transmission electron microscopy (SEM and TEM) reveal that the genital papillae (= ring organs) of *Hormosianoetus mallotae* (Fashing) consist of a central disk of modified cuticle surrounded by a thickened ring of normal cuticle. Beneath the disk is an electron-lucent chamber separating it from modified cells below that contain numerous mitochondria in close association with plasma membrane plications, a fine-structural characteristic of cells with an active transport function. *Hormosianoetus mallotae* is an obligate inhabitant of water-filled treeholes and a completely aquatic species. Evidence points toward osmoregulation and/or ion regulation as the probable function of genital papillae in freshwater mites, and, based on fine structure, this is their probable function in *Hormosianoetus mallotae* as well. To date, the genital papillae of only one other species of histiostomatid, *Histiostoma feroniarum* (Dufour), have been investigated. Although both species share many fine-structural characteristics, they also differ in a number. *Histiostoma feroniarum* is found in a wide variety of habitats (e.g., stable manure, decaying vegetation, rotting mushrooms, fungal beds, under tree bark), and is usually only partially submerged and wading in the semi-aquatic environment. Differences in fine-structural characteristics of the genital papillae between *Hormosianoetus mallotae* and *Histiostoma feroniarum* are thought to be associated with habitat differences.

**Key Words** - Acari, Histiostomatidae, *Histiostoma, Hormosianoetus,* osmoregulation, astigmatic mites, genital papillae, Claparède organs, axillary organs, USA.

## INTRODUCTION

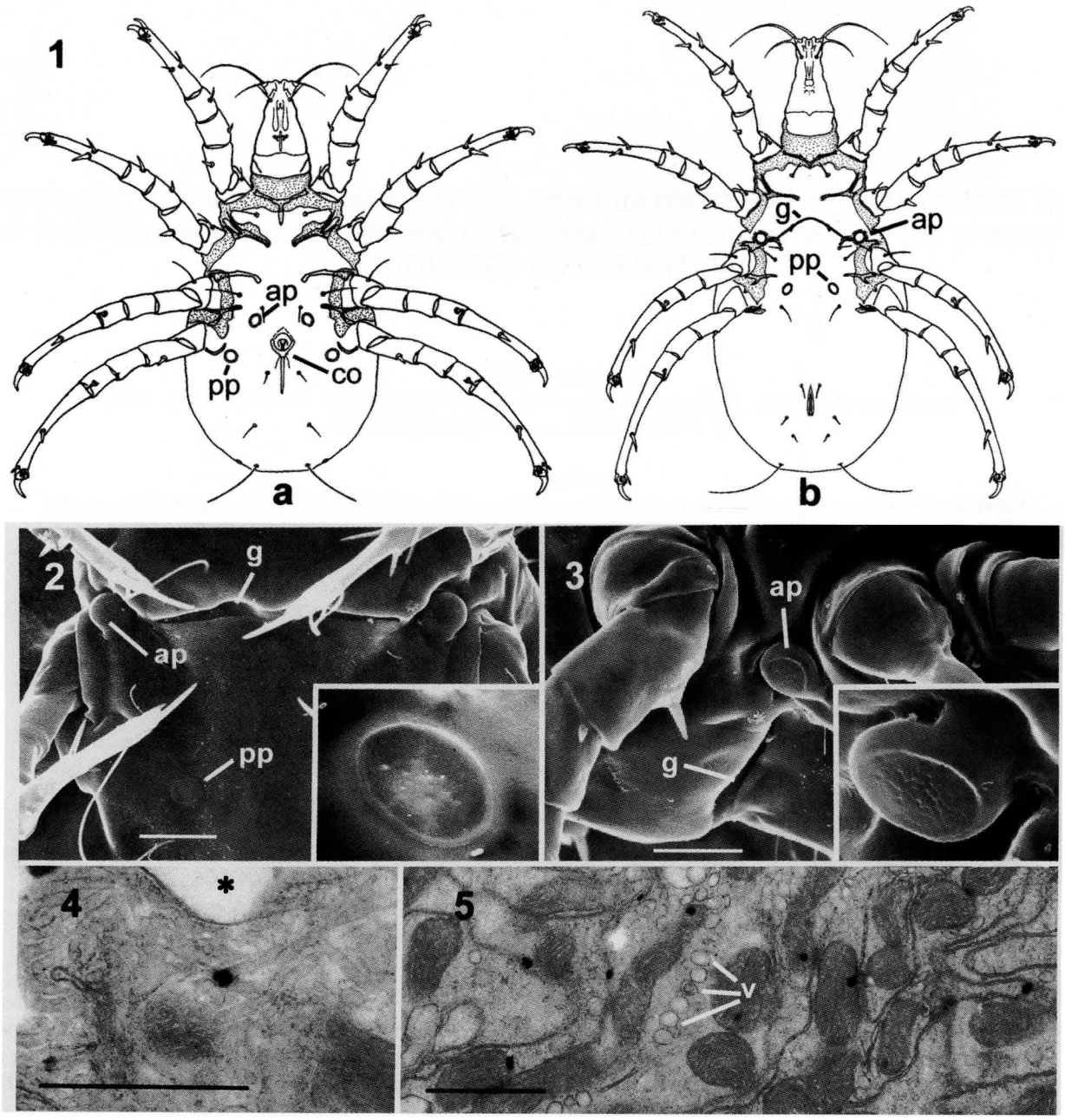
A characteristic of the mite order Astigmata and other Acariformes is the presence of genital papillae, structures usually finger-shaped and located within the progenital chamber. The papillae can be everted like the fingers of a glove when the genital region is protruded by means of hemolymph pressure. Adults, tritonymphs and deutonymphs possess two pair of genital papillae, and protonymphs possess one pair. Larvae and prelarvae lack a "genital opening" and therefore lack genital papillae. However, they usually possess homonomous structures called Claparède organs located externally between the bases of legs I and II.

With the exception of the deutonymphal instar, members of the family Histiostomatidae are uniquely characterized among the Astigmata by genital papillae that are external (superficial) rings of sclerotized cuticle more or less flat against the normal cuticle and not associated with the genital opening. Larval Claparède organs are similar in appearance to genital papillae but differ in location. The extensive differences in external appearance and location of histiostomatid genital papillae and Claparède organs even led Whitaliński *et al.* (2002) to coin the term "ring organs" to distinguish them from normal astigmatid genital papillae.

The genital papillae of the Histiostomatidae have been the subject of fine-structural investigations for only one species, *Histiostoma feroniarum* (Dufour). The present study reports on the fine structure of the genital papillae of a second species, *Hormosianoetus mallotae* (Fashing), an inhabitant of water-filled treeholes (Fashing, 1973).

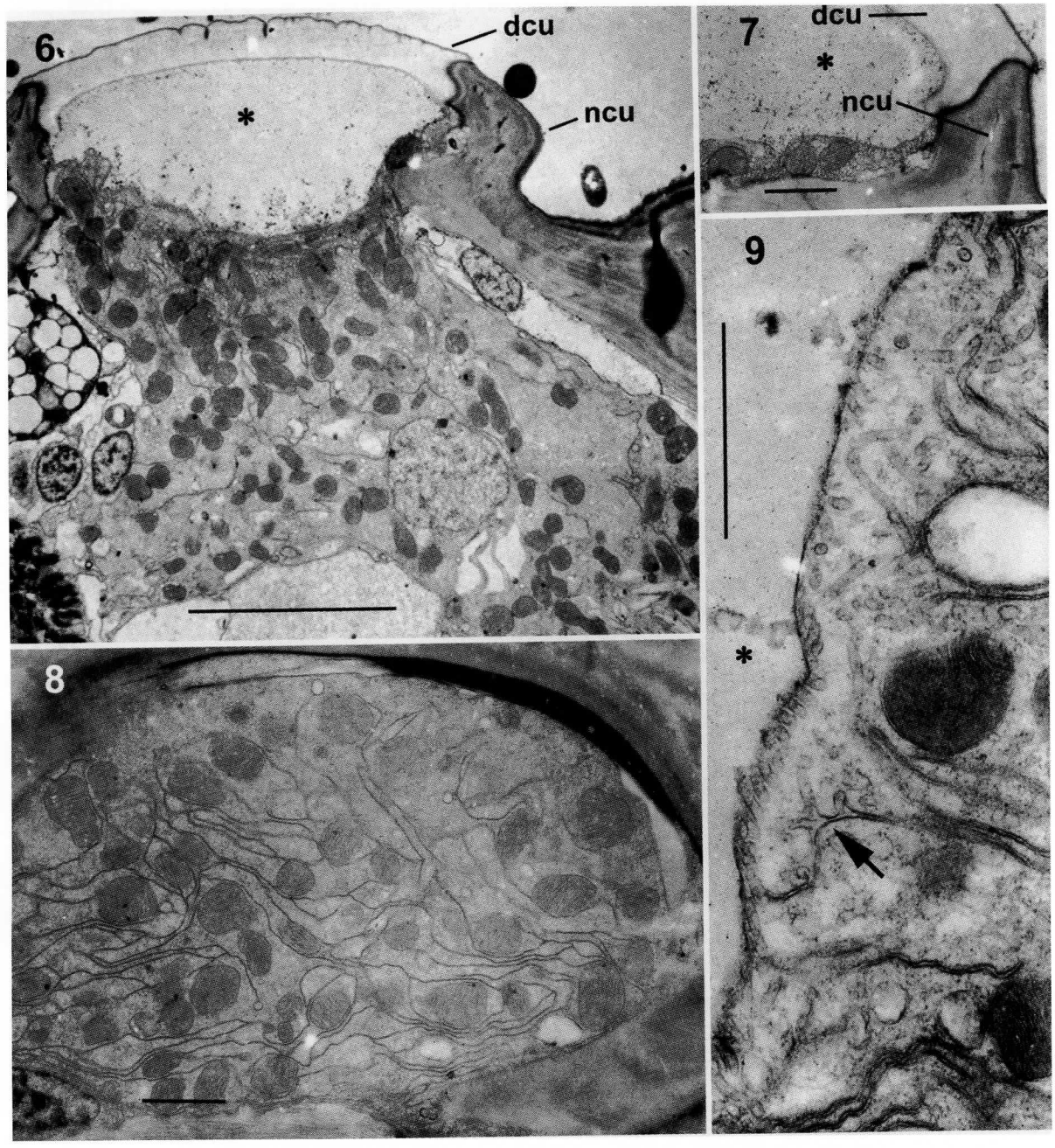
## METHODS AND MATERIALS

Samples consisting of water, detritus and associated arthropods, including *Hormosianoetus mallotae,* were collected in April from water-filled treeholes in eastern Virginia, USA, and maintained in finger bowls in the lab-




Figs. 1-5. *Hormosianoetus mallotae* (Fashing) - 1. Distribution of genital papillae in male (a) and female (b); 2. SEM, female ventral view; insert = enlarged view of posterior papilla; 3. SEM female, lateral view; insert = enlarged view of anterior papilla; 4. TEM, extensive proliferation of membranous vesicular tubules near surface in apical region of cell; 5. TEM, vesicles in apical region of cell: ap, anterior papilla; co, copulatory organ; g, oviporus; pp, posterior papilla; v, vesicle; asterisk, electron-lucent chamber (Scale bars: Figs. 2, 3, = 30 μm; Figs. 4, 5 = 1 μm).

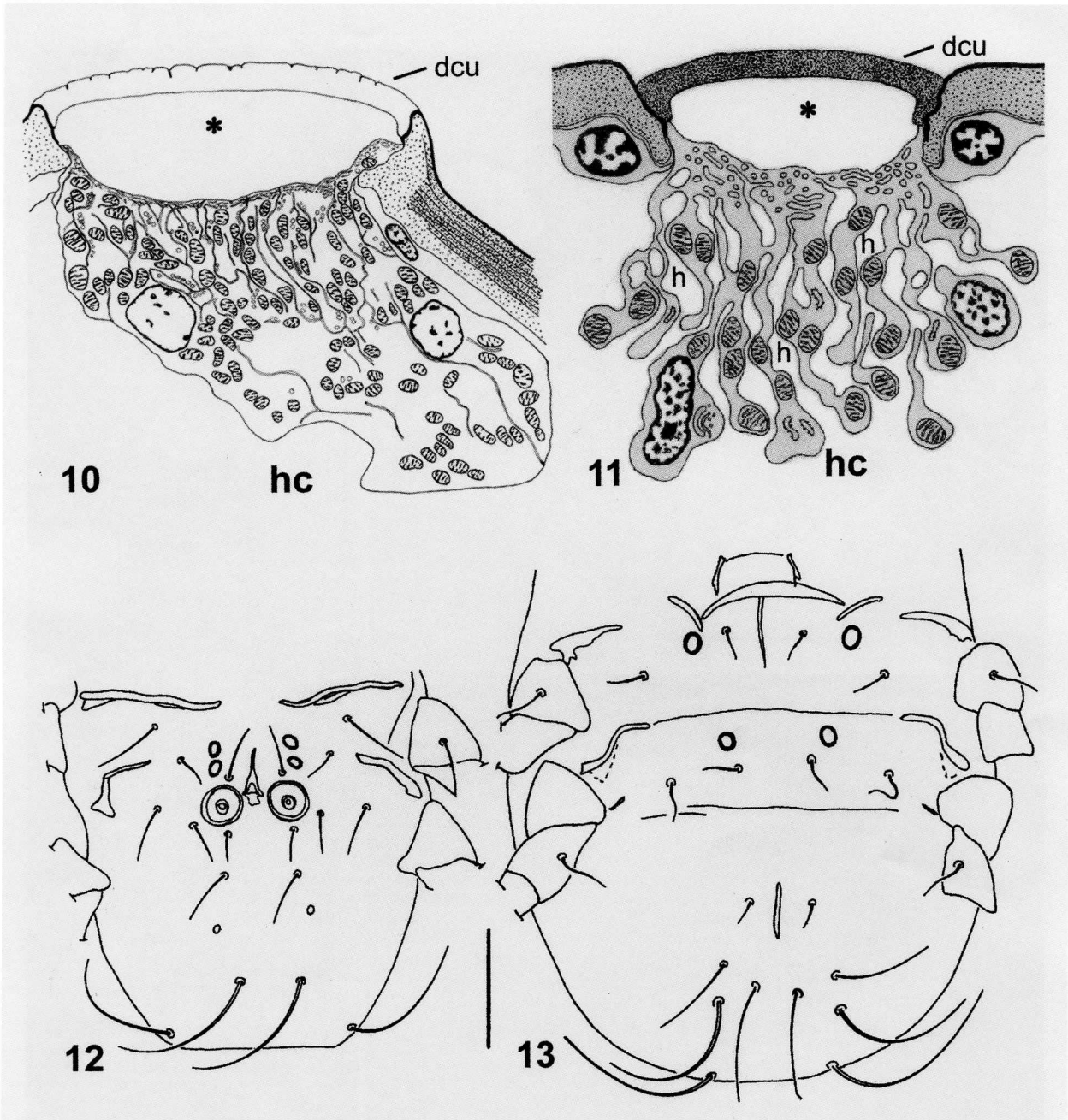
oratory. Adult male and female specimens obtained from these cultures were used for investigation. For transmission electron microscopy (TEM), the idiosomal integument of living mites was first ruptured with a minuten nadel to facilitate fixation, and the specimens then placed in a fixative of 3.5% gluteraldehyde, 2.5% paraformaldehyde, and 2% acrolein in cacodylate buffer (pH 7.4) for 12 hr at 4°C. After several brief cacodylate buffer rinses, they were post-fixed for 1½ hr at 4°C followed by an additional 1½ hr in 1% $OsO_4$ in cacodylate buffer. Specimens were then briefly rinsed in 50% acetone and soaked overnight in 2% uranyl acetate 70% acetone solution at




Figs. 6-9. *Hormosianoetus mallotae* (Fashing), TEM of female anterior papilla - 6. axial section; 7. border between the modified cuticle of the disk and the surrounding normal cuticle, sectioned axially; 8. cross section slightly below apical region of cell; 9. formation of membranous vesicular tubules by plasma membrane (e.g., arrow) in apical region of cell: dcu, disc cuticle; ncu, normal cuticle; asterisk, electron-lucent chamber (Scale bars: Fig. 6 = 5 μm; Figs. 7, 8, 9 = 1 μm).

4°C. Dehydration was completed in acetone, and Spurr's medium used for infiltration and embedding. Thin sections were stained in lead citrate, and TEM performed on a Zeiss EM 109.

For observation under the scanning electron microscope (SEM), live specimens were vigorously rinsed in several baths of distilled water in an attempt to cleanse them of debris. They were then briefly submerged in distilled water near the boiling point in order to force protraction of appendages. Specimens were then dehydrated in ethyl alcohol, dried using the critical point procedure, individually affixed to stubs using double-sided sticky tape, and coated with gold palladium in a sputter coater. Microscopy was performed on an AMR 1200.




Figs. 10-11. Schematic diagram of genital papillae in axial section - 10. *Hormosianoetus mallotae* (Fashing), 11. *Histiostoma feroniarum* (Dufour) (after Whitaliński *et al.,* 2002): dcu, disk cuticle; h, hemolymph in canals penetrating the cell; asterisk, electron-lucent chamber; hc, hemocoel.

Figs. 12 (Male) -13 (Female). Ventral view of the hysterosoma - *Austranoetus kerguelenensis* Fain illustrating very small genital papillae (after Fain, 1972) (Scale bar = 100 μm).

## RESULTS

As in all histiostomatid mites, the genital papillae of *Hormosianoetus mallotae* differ in location between males and females (Fig. 1). In females the anterior papillae are located between coxae II and III, adjacent to the transverse genital opening, and the posterior pair are closer together and located on coxal fields IV (Figs. 1b, 2, 3). Some male histiostomatid mites have both pairs in a compact group located just anterior to the genital opening (Fig. 12). However, males of *Hormosianoetus mallotae*, like the majority of histiostomatid mites, have the anterior pair on coxal fields IV above the copulatory organ, and

the posterior pair more separated, parallel to the copulatory organ, and just posterior to coxal fields IV (Fig. 1a).

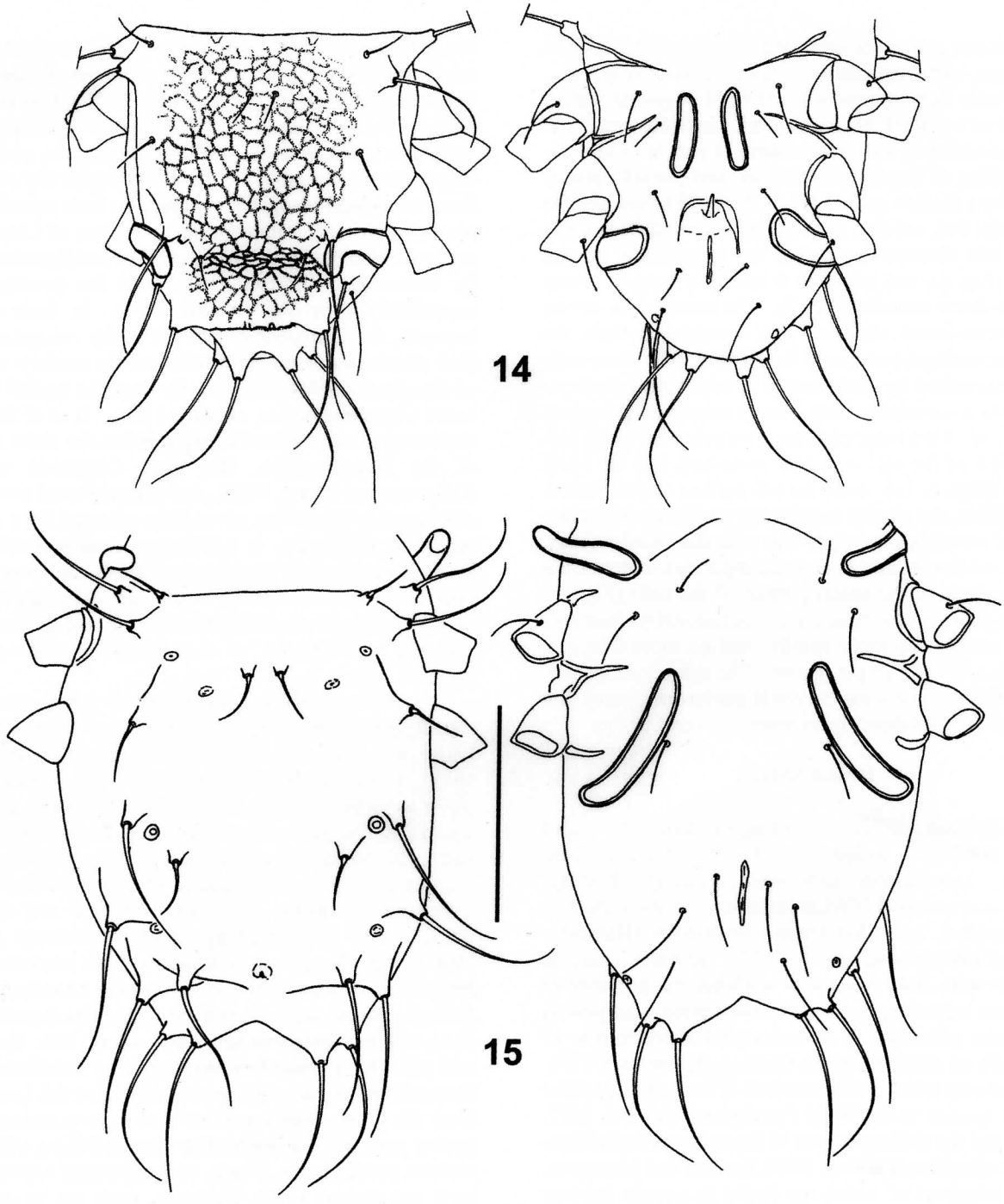
Under light microscopy and SEM, a genital papilla appears as a central disk containing numerous small pits and surrounded by a thickened band of cuticle. The anterior papillae of females are elevated and partially underscored by a shallow groove (Figs. 2, 3). TEM reveals that the central disk consists of a thin layer of modified cuticle that is less electron-dense than the surrounding normal cuticle (Fig. 6), and joined to it by a thin band of a very electron-dense material (Fig. 7). The central disk covers an electron-lucent chamber that separates it from the large, specialized, polar cells below (Fig. 6). These cells are characterized by the presence of numerous mitochondria in close association with plasma membrane plications (Figs. 6, 8). Membrane plications are primarily in the apical region of the cell with few extending into the basal portion (Figs. 6, 10). Near the cell surface directly below the chamber, the plasma membranes proliferate into a network of numerous membranous vesicular tubules (Figs. 4, 9). Vesicles, sometimes containing a dark material, are also observed in the apical portion of the cells (Fig. 5). Smooth endoplasmic reticulum is occasionally observed. Nuclei are located more basally, and no more than two were observed in a single section. The number of specialized cells in a genital papilla could not be determined, but it is probable that there are no more than three or four.

## DISCUSSION

Published studies concerning the fine structure of genital papillae of astigmatic mites exist for only three species: *Naiadacarus arboricola* Fashing (Fashing, 1988), *Acarus siro* L. (Whitaliński *et al.*, 1990) (both in the Acaridae), and *Histiostoma feroniarum* (Histiostomatidae) (Whitaliński *et al.*, 2002). All three share in common with *Hormosianoetus mallotae* the presence of numerous mitochondria in close association with plasma membrane plications, a fine-structural characteristic of cells with an active transport function (Komnick, 1977). Such features are also characteristic of the genital papillae of mite species in the Order Prostigmata (Alberti, 1977, 1979 ) and the axillary organs of the astigmatic mite family Algophagidae (Fashing, 1984; Fashing and Marcuson, 1996), members of which are found in aquatic habitats ranging from semiaquatic (wading in fluid) to fully aquatic (submerged) (Fashing and Wiseman, 1980; Fashing *et al.*, 2000; OConnor, 1982). Axillary organs are located laterally on the idiosoma between legs I and II, and are found in all instars with the exception of the deutonymph (Fashing, 1984). Since the presence of axillary organs is coupled with vestigial genital papillae, it is postulated that they have taken over the function of the genital papillae (Fashing, 1984; Fashing and Marcuson, 1996). The location of the axillary organs between legs I and II, the fact that algophagid mites lack Claparède organs (larval homonoms of genital papillae), and the similarity in fine structure between axillary organs and Claparède organs, led Alberti and Coons (1999) and Whitaliński *et al.* (2002) to postulate that axillary organs are probably structures homonomous to Claparède organs that deviate from the typical ontogenetic pattern by their retention in postlarval instars. Although rare, retention of Claparède organs has been reported to occur in the prostigmatic family Halacaridae (Bartsch, 1974) and the prostigmatic superfamily Tydeoidea (André, 1991). In these cases, however, the Claparède organs are easily recognized by their normal shape and size, whereas the axillary organs of algophagid mites differ greatly from the typical astigmatic Claparède organs of related taxa. It is of interest that larval instars of the Carpoglyphidae, the sister group of the Algophagidae, also lack Claparède organs (OConnor and Moser, 1985), and that postlarval retention of Claparède organs has never been recorded for a member of the Astigmata. It is therefore more probable that the axillary organs of the Algophagidae are independently evolved structures. Further studies are definitely necessary to conclusively determine whether the axillary organs are homonomous, or analogous, to Claparède organs.

The morphological attributes of active transport cells found in genital papillae and axillary organs are also found in the chloride cells of aquatic insects (Komnick, 1977). Komnick (1977) has amassed a great deal of evidence to support the hypothesis that the chloride cells of aquatic insects play an important role in osmoregulation and/or ion regulation, and an analogous function has been postulated for the genital papillae of halacarids and freshwater mites (Alberti, 1977, 1979; Alberti and Coons, 1999; Evans, 1992; Fashing, 1984; Whitaliński *et al.*, 1990, 2002). Being a fully aquatic species inhabiting the freshwater of water-filled treeholes, the hemolymph of *Hormosianoetus mallotae* is hypertonic to the aquatic medium with the result that salts (as ions) are lost. Specialized cells that recover lost salts would be beneficial, and the modified genital papillae probably serve this function. Over the years, other functions have been postulated for genital papillae of actinotrichid mites including adhesive suckers, sense organs, glands, and respiratory organs (Alberti and Coons, 1999). However, there are no experimental bases for these interpretations and, to reiterate Whitaliński *et al.* (2002), they should be rejected.

The genital papillae of *Hormosianoetus mallotae* and *Histiostoma feroniarum* have many morphological features in common, but they also differ in several respects. The cuticular disk of *Histiostoma feroniarum* is electron-dense and has a smooth surface (Fig. 11) (Whitaliński *et al.*, 2002), whereas it is not electron-dense and has a pitted surface in *Hormosianoetus mallotae* (Figs. 2, 3, 6, 10). In both species the disc cuticle and sur-




Figs. 14 (Male) -15 (Female). Dorsal and ventral views of the hysterosoma - An undescribed species of histiostomatid mite from the fluid-filled flower bracts of *Heliconia imbricata* (Kuntze) Baker illustrating enlarged and elongated genital papillae (Scale bar = 100 μm).

rounding unmodified cuticle are joined by a thin band of electron-dense outer epicuticular material, a connection found to be relatively weak in *Histiostoma feroniarum* and thereby resulting in the loss of the disk during specimen process (Whitaliński *et al.*, 2002). Disk loss during specimen preparation was never observed in *Hormosianoetus mallotae*. The most extreme difference between the two species is found in the specialized cells located in the base of the papillae. In *Histiostoma feroniarum*, the infoldings of plasma membranes have spaces between

them resulting in wide and long channels (canals) that deeply penetrate the cell body to the apical cytoplasm (Fig. 11). In the apical cytoplasm they continue as tubular canaliculi located close, and parallel to, the apical cell membrane. The wide canels and tubular canaliculi are therefore compartments of extracellular space continuous with the hemocoel and therefore filled with hemolymph (Fig. 11) (Whitaliński *et al.*, 2002). Such interdigitating canals provide maximal exposure of the cell surface to the hemolymph. In contrast, the infoldings of plasma membrane in the cells of *Hormosianoetus mallotae* genital papillae appear to originate at the apex of the cell and are more concentrated in that region (Fig. 10). The membranes are tightly appressed to one another and most do not penetrate further than the center of the cell. There are therefore no extracellular spaces creating canals of hemolymph that indent the cells as found in *Histiostoma feroniarum*. In addition, plasma membrane plications appear to be more extensive in *Hormosianoetus mallotae*, and mitochondria more numerous. In fact the fine-structural characteristics of the specialized cells of *Hormosianoetus mallotae* are more similar to those found in the genital papillae of *N. arboricola* (Acaridae) (Fashing, 1988) than to those found in the genital papillae of *Histiostoma feroniarum*. It is interesting to note that *N. arboricola* is also a fully aquatic species and shares the water-filled treehole habitat with *Hormosianoetus mallotae* (Fashing, 1994). *Histiostoma feroniarum*, on the other hand, is found in a wide variety of habitats including wet grain, stable manure, sewage bacteria beds, decaying vegetable matter, decaying mushrooms, fungal beds, oak slime flux, and under tree bark (Hughes and Jackson, 1958; Hughes, 1976), where it is usually only partially submerged and wading in the semiaquatic environment. It is therefore probable that the differences observed between *Hormosianoetus mallotae* and *Histiostoma feroniarum* are related to habitat differences, the former being fully aquatic and the latter being only semiaquatic.

Although trophic instars of all histiostomatids possess mouthparts highly specialized for filter-feeding and therefore require a film of water in which to feed, species in this large and diverse family can be found in a wide variety of habitats and are often habitat specific. Among these are the subcortical galleries of insects, the nests of animals (e.g., bees, ants, birds and mammals), vertebrate dung, decomposing vertebrate carcasses, and decomposing vegetable matter (Hughes and Jackson, 1958; OConnor, 1982), even that of halophilic plants (Fain, 1976). Some species have been recorded as parasitic in the cocoons of annelids (Oliver, 1962) and others as commensals in the ears of elephants (Fain, 1970) and African buffalo (Fain and Zumpt, 1974). Yet other species are fully aquatic, with some specific to various plants that form phytotelmata such as treeholes, bromeliads, bamboo internodes, *Heliconia* flower bracts, and the leaves of carnivorous pitcher plants (Fashing, 1973, 2002; Fashing and OConnor, 1984; Fashing *et al.*, 1996; Wurst and Kovac, 2003). One also finds diversity in the size and shape of the genital papillae in the various histiostomatid species, and this is most probably associated with the osmoregulatory demands imposed by the habitat. For example, the genital papillae of species such as *Austranoetus kerguelenensis* Fain that are halophilic, favoring intertidal habitats, are typically quite small, ringlike structures (Fain, 1972) (Figs. 12, 13). At the other extreme are species with greatly enlarged and elongated genital papillae as represented by an undescribed species that inhabits the fluid-filled floral bracts of *Heliconia imbricata* (Kuntze) Baker. Males of this species have anterior genital papillae that extend over coxal fields three and four, whereas the posterior genital papillae originate ventrally posterior to coxal fields four, wrap around the lateral side of the idiosoma, and project posteriorly as turrets between dorsal setae $d_2$ and $e_1$ (Fig. 14). In females it is the anterior genital papillae that originate ventrally, wrap around the lateral side of the idiosoma, and project dorsally as turrets. The posterior genital papillae extend ventrally over, and well beyond, coxal fields four, with their posterior margins extending like short turrets from the idiosoma (Fig. 15). Studies on a number of species from a diversity of habitats are therefore necessary to fully understand the fine-structure of the genital papillae of members of the family Histiostomatidae, as well as to gain additional insight into their specific function(s) in different habitats.

The genital papillae of both *Hormosianoetus mallotae* and *Histiostoma feroniarum* have many ultra - structural features in common with those of other astigmatic mites, thereby reinforcing the hypothesis that the ring organs are in fact modified genital papillae. Although Whitaliński *et al.* (2002) coined the term "ring organs" for the genital papillae of histiostomatids due to their unique structure and location, the use of this term is not recommended since it can lead to the erroneous assumption that they are functionally something different than the so-called "genital papillae" of other taxonomic groups.

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