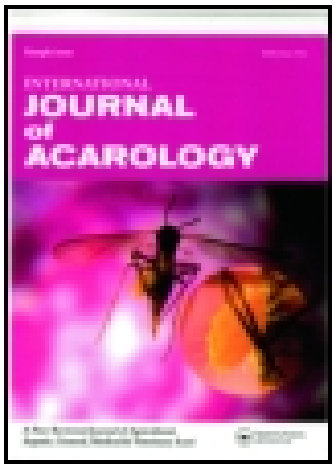

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First report of mite parasitization in frog-biting midges (Corethrella species)

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SHORT COMMUNICATION

First report of mite parasitization in frog-biting midges (*Corethrella* species)

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Water mites (Acari: Hydrachnidiae) were collected from males of frog-biting midges (Corethrellidae) in Gamboa, Panama. All *Corethrella squamifemora* males collected were parasitized with water mites. The mites were attached to the soft tissues of the thoracic region of the midges. No mites were found on female frog-biting midges of the same species. This study is the first report of mite parasitization in frog-biting midges.

Keywords: frog-biting midges; Corethrellidae; Hydrachnidae; mite parasitism

Larvae of water mites (Acari: Hydrachnidiae) are common parasites of emergent aquatic insects including Chironomidae and Culicidae (Smith and Oliver 1976; Smith 1988; Karunaratne and Amerasinghe 1992; Rolff et al. 2000; Martin and Stur 2005). Most aquatic larvae of water mites are first attached during the last aquatic stage of their host insect and are transferred to the adult during emergence of the host (Sabatino et al. 2003). Although mite-parasitizing mosquitoes have long being studied (Mullen 1975; Milne et al. 2009), there are no reports of this phenomenon in the closely related family of frog-biting midges (Corethrellidae). In this study, we report the occurrence of water mites in frog-biting midges of the genus *Corethrella* Coquillet and discuss sexual differences in mite prevalence.

Frog-biting midges belong to the monogeneric family Corethrellidae (Order Diptera), sister taxon to Culicidae (Mosquitoes) and Chaoboridae (Phanton midges). The family Corethrellidae currently has 105 described species distributed in tropical and subtropical areas (Borkent 2008; Robert and Borkent 2014). Female frog-biting midges are haematophagous and feed on anuran blood, presumably to obtain the protein required for egg production (McKeever 1977). In contrast to other blood-feeding insects that rely on chemical cues to locate their hosts (Clements 1999; Lehane 2005), female frog-biting midges cue in on the mating calls produced by frogs and toads (McKeever 1977; Bernal et al. 2006; Borkent 2008; Bernal and de Silva 2015). Female frog-biting midges are commonly observed biting calling frogs and can be collected in large numbers using sound-producing traps. In contrast, the behaviour of adult male Corethrellids has not been observed in the wild and males are rarely collected (Borkent 2008). Indeed, males are unknown for most species of frog-biting midges (Borkent 2008). To further our understanding of male frog-biting midges, we set up ultraviolet (UV) LED CDC traps (Bioquip, Inc., Rancho Dominguez, CA, USA) in areas surrounding the facilities of the Smithsonian Tropical Research Institute in Gamboa, Panama (9°07.0′N, 79°41.9′ W). UV traps have proven effective in the capture of males of several species of these midges (Borkent 2008). We deployed UV traps during the rainy season (July–August 2013) at a variety of distances from frog-breeding areas (0–30 m) where female frog-biting midges are abundant. We opened the traps at sunset and closed them after 1.5–2 hrs. Once the traps were closed for the night, the collection containers were brought to the laboratory where the insects were euthanized in the freezer and later examined under the light microscope.

All frog-biting midges captured were carefully examined for the presence of external parasites. Mites found were mounted with Hoyer's medium following Singer (1967). Identification of the specimens was performed using the key provided by Walter et al. (2009). Given that only mite larvae were found and the current status of Neotropical water mite taxonomy (Cook 1980; Walter et al. 2009), a more refined identification was not possible.

We collected 22 males of *Corethrella* species, which included *C. quadrivitatta* Shannon and Del Ponte (1 male), *C. squamifemora* Borkent (3 males), *C. peruviana* Lane (9 males) and *Corethrella* undescribed species (9 males). Frog-biting midges were identified following the key by Borkent (2008). Three of these male midges (*C. squamifemora*) were parasitized with water mites (Superfamily Arrenuroidea, Mideopsidae; Figure 1a). Parasitized male midges carried between one and three mites attached to the ventral areas of the thorax (Figure 1b–d). The mites were attached at soft, membranous inter-segmental areas of the thorax. In one male, three midges were attached at the protorax, in the ventral area of the neck. These observations are consistent with previous studies that report high mite occurrence in the thoracic region of mosquitoes (*Arrenurus* species; Karunaratne and Amerasinghe 1992) and chironomids

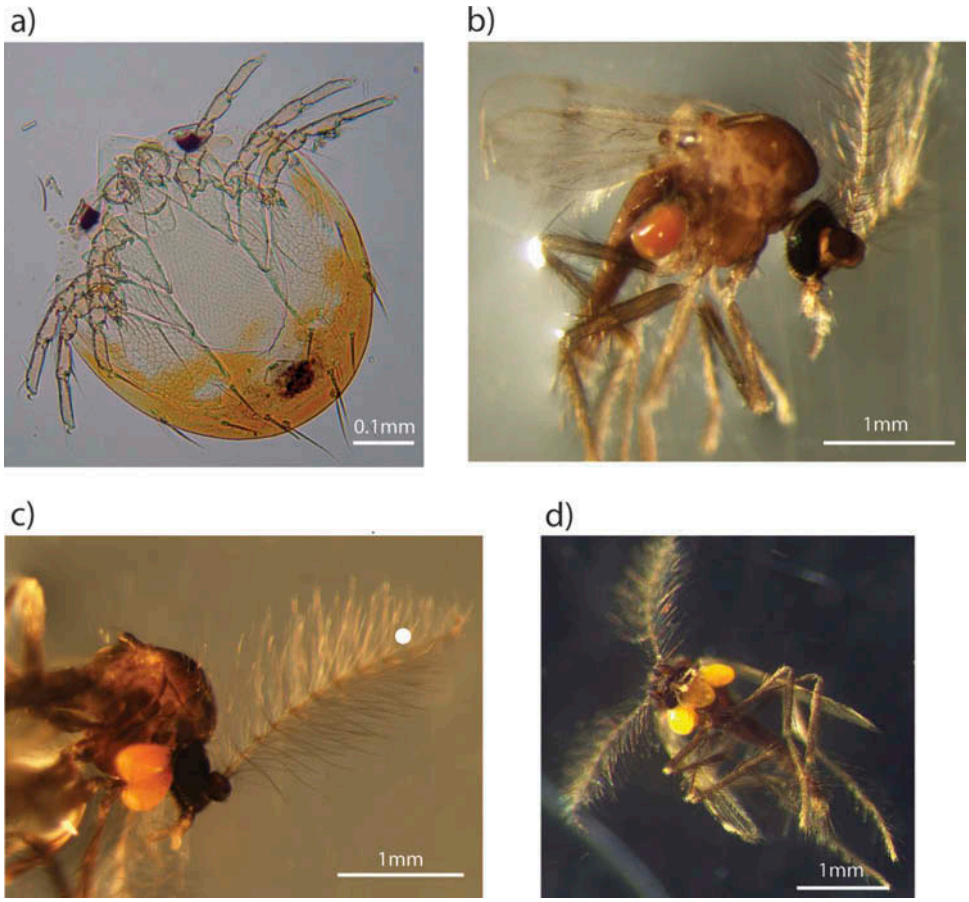

Figure 1. Water mite parasitism in male frog-biting midges *Corethrella squamifemora*. Photos of a water mite (Acari: Hydrachnidia) originally found attached to midge (a), and males with mite(s) attached at the meta-thorax (b) or prothorax (c and d) are shown.

(*Sperchon* species and *Aturus* species; Martin 2005; Martin and Stur 2005). Despite the low sample size, it is interesting to note that mite infection was restricted to one species of frog-biting midges and all male *C. squamifemora* collected carried mites.

Over the last decade we have collected and carefully examined thousands of female frog-biting midges from the same study site without ever finding mite parasites (Bernal et al. 2006; de Silva et al. 2014; Bernal and de Silva 2015). In fact, sound traps opened simultaneously with the UV traps captured hundreds of females, with no cases of mites parasitizing them. In contrast, we found mites in 3 out of 22 males captured (13.6% of all species, 100% of *C. squamifemora*). Our results thus suggest higher mite parasitization rates in male than in female frog-biting midges. Sexual biases in water mite parasitism in insects seem to be a widespread phenomenon (Smith and Cook 1991; Lajeunesse et al. 2004; Fairn et al. 2008). Such apparent preference for a specific sex is probably due to differences in host behaviour between the sexes resulting in one sex having greater exposure to larval mites (Mullen 1977) or due to protandry (males of potential hosts emerging before females) (Smith and McIver 1984a). While female-biased mite parasitism by *Arrenurus* species occurs in some mosquitoes and damselflies (Smith 1983; Karunaratne and Amerasinghe 1992; Robb and Forbes 2006), higher prevalence in males is known in dragonflies (Lajeunesse et al. 2004). The high infestation of water mites in male dragonflies seems to be explained by the higher amount of time males spent in proximity to water compared to females, which increases the chances that water mites can infest them. (Lajeunesse et al. 2004). It is unclear, however, why prevalence is higher in females in mosquitoes or damselflies. Our results do not seem to be explained by males spending more time in contact with water either. In frog-biting midges, only females return to the water for oviposition. It thus seems that for a water mite, being on a male host limits its opportunities to return to aquatic habitats to complete its life cycle. It has been suggested that parasitic water mites could move from a male to a female during mating to avoid such disadvantage if infesting a host from the wrong sex (McLachlan 1999), but the complete absence of water mites in female frog-biting midges suggests the hypothesis that mite presence in males resulted from isolated errors is inappropriate in this system.

Mite parasitism can have adverse effects on survival, fecundity, body condition and flight activity of their host (Åbro 1982, 1984; Smith 1988; Forbes 1991, 1993;

Rolff et al. 2000). Mite-induced reduction in egg production in mosquitoes and damselflies of various species parasitized by *Arrenurus* spp. has been widely recognized (Lanciani and Boyt 1977; Smith and McIver 1984b; Forbes 1991; Fairn et al. 2008). Larval water mites usually create a deep incision into the thorax of their host causing cellular damage to their flight muscles (Gillies and Wilkes 1972; Smith 1983; Reinhardt 1996). This damage often affects infected mosquitoes or damselflies reducing their flight capability (reviewed by Smith 1988). There were no apparent negative signs of carrying the mites in the male Corethrellids infected. These midges actively flew to the UV traps suggesting normal flying ability. Studies that further investigate this host-parasite interaction are necessary to understand the effects of water mites on frog-biting midges.

Although mite parasites of mosquitoes have attracted considerable attention given their potential role in disease control (Mullen 1975; Smith 1988), mites on closely related families are poorly understood. To our knowledge, this study is the first report of the parasitic relationship between frog-biting midges and water mites and highlights potential sexual differences in mite prevalence that deserve further investigation.

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