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Correlations in morphology between the sexes in feather mites (Acari: Astigmata): precopulatory guarding and reproductive morphologies

by

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To my parents, Don and Marilyn, for their endless love, support and encouragement throughout all of my adventures.

And for Brandon, my beacon on the horizon.

Abstract

Sexual dimorphism is prominent across animals. In addition to differences in size and colouration, the sexes may also differ in non-genitalic contact traits whereby the grasping morphologies of males are matched by either cooperative or resistant corresponding structures in females. Resistance traits may also be genitalic and are indicative of sexually antagonistic coevolution whereby the sexes adapt and counter-adapt traits to maximize their own fitness. For both hypotheses of cooperative and antagonistic coevolution, theory predicts a correlation in the dimensions of male and female structures. I aim to determine whether correlated morphologies between the sexes in the Proctophyllodidae and Trouessartiidae are cooperative or antagonistic. Furthermore, I evaluate whether directional trends exist in the evolution of genitalic size in the genus *Trouessartia*. My studies indicate that feather mites are exceptions to many trends, and due to their diversity are excellent organisms for further study in regards to sexual selection.

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Table of Contents

List of Tables

List of Figures

List of Appendices

Chapter 1. Sexual differences in feather mites (Acari: Astigmata)

1.1 Intersexual differences in morphology

Morphology, strictly defined as the study of form (Ball, 1977) is a term often used in place of "structure" or "form" in describing the physical appearance of organisms. Morphological differences between the sexes are widespread throughout animals. These differences between the sexes can be as obvious as the bright colouration exhibited by males in some taxa (e.g., red colouration in male cardinals vs light brown colouration in females; Wolfenbarger, 1999) or as subtle as modifications in the number and distribution of antennal sensilla in noctuid moths (Jefferson *et al*., 1970). These intersexual differences, though fantastically diverse, are mostly explained by sexual selection, which has long been acknowledged as a driver in the evolution of morphologies and behaviours associated with reproductive success (Andersson, 1994). Sexual selection arises due to the differential investment of the sexes in gamete production (anisogamy; Bateman, 1948). Anisogamy together with other aspects of higher parental investments by one sex, typically females, results in a competition between individuals of the opposite sex, typically males, for mates (Trivers, 1972; Parker *et al*., 1979). This competition promotes the evolution of morphologies or behaviours that increase the possessor's likelihood of mating with more or better partners. When these traits are truthful indicators of fitness, females may actively select mates with characteristics that will increase the viability of their offspring (Kodric-Brown & Brown, 1984).

1.2 Correlated evolution of morphology

Sexual selection is often linked to the elaboration of primary and secondary sexual characters (Andersson, 1994). Though sexual selection is recognized as a factor in the differentiation of non-genitalic morphologies (West-Eberhard, 1983; Coleman *et al*., 2004; Emlen *et al*., 2005), the rapid divergence of male genitalic form in internally fertilizing animals is also proposed to be strongly influenced by sexual selection by female choice (Eberhard, 1985; Hosken &

Stockley, 2004). This theory predicts that females will favour the paternity of males with a desired trait over other males lacking this trait (Eberhard, 1996). Male morphologies may be preferred if they "fit" with the female's genitalia in a way superior to other males, or if they more effectively stimulate the female (Eberhard, 2010). Traditional female choice models predict that female morphologies and behaviours will be 'cooperative' with desirable male traits (Eberhard, 2010) and therefore may exhibit some degree of correlation.

In contrast, females can employ behaviours or morphologies to counteract the negative impacts of reproduction inflicted by some males. Depending on the species, costs to the female include damage to the genitalic tract via male genitalia (Crudgington & Siva-Jothy, 2000), reduced longevity (Stutt & Siva-Jothy, 2001), and various reductions in reproductive success (Alexander *et al*., 1997) Although sexually antagonistic coevolution (SAC) has been questioned by Eberhard (2004, 2010), several studies have reported correlated antagonistic traits associated with male and female genitalia (Koene & Schulenburg, 2005; Brennan *et al*., 2007; Rönn *et al.,* 2007; Tatarnic & Cassis, 2010; Kamimura, 2012) and with non-genitalic contact structures (Arnqvist & Rowe, 2002; Bergsten & Miller, 2007). In these studies, the sexes adapt and counter-adapt traits to promote their own fitness gains, over their partner's, in an evolutionary arms race (Parker *et al*., 1979; Arnqvist & Rowe, 2005; Rönn *et al.,* 2007).

1.3 Feather mites as models

Mites (Arachnida: Acari) are among the most diverse animal groups, second in richness only to insects. With over 55,000 described species (Walter & Proctor, 1999) and an estimated 500,000 - 1,000,000 species worldwide (Krantz & Walter, 2009); they show an enormous range of sexual behaviour and morphology, and are ideal for testing questions of correlated evolution and sexual selection. Mites also occupy diverse habitats and may be symbiotic on plants or animals, or free living in the soil, water, or foliage (Krantz, 2009). Birds alone are host to over 2,500 species of mites (Proctor & Owens, 2000) the vast majority of which belong to the suborder Astigmata (Proctor, 2003). Mites

occupy all surfaces of their avian host including the skin, feather down, feather vane and inside the quill (Proctor, 2003).

Precopulatory guarding often occurs when females have a brief fertilization window or when the female has little ability to store sperm (Parker, 1974; Grafen & Ridley, 1983). Male Astigmata of many species use non-genitalic structures to hold onto and guard tritonymphal females prior to their eclosion as adult females (Witaliński *et al*., 1992; Bochkov & OConnor 2005). In some taxa, males posses enlarged legs III or IV to grasp nymphal females (OConnor, 2009), while males of other taxa attach to females using a pair of ventral adanal suckers. In some members of the feather mite Proctophyllodidae, these suckers fit over top of a pair of docking papillae on the dorsal region of the tritonymphal female, which are suggested to enhance coupling (Witaliński *et al*., 1992). In Chapter 2, I measure the dimensions of male adanal suckers and female docking papillae in several genera within the Proctophyllodidae, to decipher whether correlations in these structures reflect cooperative design.

Genitalic form is also highly variable across feather mites. In most feather mites, sperm transfer is achieved when the male inserts his aedeagus into the female's copulatory opening located at the tip of a sclerotized internal spermaduct (Proctor, 2003). To do this, the male attaches to the female's dorsum via his adanal suckers and orients himself in the opposite direction to the female (Walter & Proctor, 1999). Though the female spermaduct is typically internal (Proctor, 2003), in several taxa of feather mites and other Astigmata it extends externally to various lengths as a copulatory tube (Gaud & Atyeo, 1996a). The evolution of external copulatory tubes in the Astigmata and the intersexual coevolution of genitalic traits are proposed by some to have arisen through SAC (Klimov & Sidorchuk, 2011). Similar to cooperative traits, structures that have arisen through conflict are also predicted to be correlative. In the feather mite genus *Trouessartia*, the female spermaduct extends externally past the supranal concavity and can reach up to 20% of the female's body length in size (pers. obs.). Though Santana (1976) posited that the *Trouessartia* male receives the

female's spermaduct in his copulatory opening in an apparent sex role reversal, little is known about the correlated morphologies of the genitalia.

As the main focus of my thesis (Chapter 3), I set out to determine whether male and female genitalia in the Trouessartiidae (with a strong emphasis on *Trouessartia* spp.) correlate in size, and whether there is evidence for directional selection acting on female external spermaduct length. By using the avian host phylogeny as a proxy for the mite phylogeny, I looked for patterns in the occurrence of external spermaducts to deduce whether this trait has likely coevolved with male genitalic size, which might suggest either cooperative or antagonistic coevolution. While observing these mites, I found that females also possessed other striking morphological differences from males. The first difference was the degree of elaborate dorsal ornamentation on the hysteronotal shield, composed of small groove-like lacunae. The second was the range in size of the *h1* setae from hair-like microsetae to spade-like macrosetae, while the male *h1* setae were invariably hair-like. Both morphological features are located in the approximate region of the placement of the male's adanal suckers during attachment. Therefore, in addition to my analyses of intersexual correlations in genitalia, I also took measurements of the females' dorsal ornamentation and *h1* setae to discover whether these structures were correlated with dimensions of the male adanal suckers. In addition, I determine whether ornamentation and *h1* setae variability are potentially used to disrupt male attachment –similar to the phenomenon documented in dytiscid beetles (Bergsten & Miller, 2007).

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Chapter 2. Like a glove: do the dimensions of male adanal suckers and tritonymphal female docking papillae correlate in the Proctophyllodidae (Astigmata: Analgoidea)?

2.1 Abstract

Precopulatory guarding of tritonymphal females by adult males is common in feather mites (Acari: Astigmata). Within the Proctophyllodidae (Astigmata: Analgoidea), some genera possess morphological adaptations in both sexes, which are suggested to enhance male attachment. One such adaptation in tritonymphal females is the development of a pair of fleshy lobe-like docking papillae, while males possess a pair of ventral adanal suckers that are proposed to fit over top of these projections. To determine whether these morphologies are cooperative in nature, we measured the dimensions of the male adanal suckers and tritonymphal female docking papillae in three genera of mites: *Neodectes* spp., *Proterothrix* spp. (Proctophyllodidae: Pterodectinae), and *Proctophyllodes* spp. (Proctophyllodidae: Proctophyllodinae). We looked for correlations in these measurements as an indication of selective cooperation. Our results did not reveal any such correlations between these morphologies in tritonymphal females and adult males. We propose several reasons for why we may not have detected morphological correlations related to the biology of feather mites, as well as future steps to expand on this research in future.

2.2 Introduction

The male mating strategies of precopulatory and postcopulatory mate guarding are common tactics employed by males in sperm competition (Parker, 1970). Although both strategies aim to maximize male fertilization success, these behaviours are predicted under different reproductive conditions. Precopulatory mate guarding is expected when females mate only once, when females mate for a limited time (a brief fertilization window), or when there is little ability of the female to store sperm; these qualities encourage males to stake a claim to unreceptive females before they become receptive (Parker, 1974; Grafen &

Ridley, 1983). In contrast, postcopulatory mate guarding is predicted when females mate repeatedly and are receptive to additional males following an initial copulation (Parker, 1974). In the case of precopulatory mate guarding, the fitness gained by guarding an unreceptive female exceeds that which is gained in a continued search for mates (Parker, 1974). This form of mate guarding is argued by Simmons (2001) to function more as a means of monopolizing females until they are receptive than as a tactic to avoid sperm-competition.

While mate guarding has been widely reported throughout the animal kingdom (beetles: Alcock, 1991; birds: Birkhead, 1979; Hammers *et al*., 2009; cephalopods: Huffard *et al*., 2008; lizards: Cuadrado, 1998; mammals: Schubert *et al*., 2009); precopulatory guarding is especially common among invertebrates (Deinert *et al.,* 1994; Bel-Venner & Venner, 2006; Arnqvist *et al*., 2007; Parker & Vahed, 2010; Takeshita & Henmi, 2010). Several studies in arthropods document that the onset of precopulatory mate guarding occurs when females are still juveniles (Evstigneeva, 1993; Fiers, 1998; Holdsworth & Morse, 2000; Zhu & Tanaka, 2002; Oku, 2009; Estrada *et al*., 2010; Jones *et al*., 2010). In some taxa this guarding is cued by the emission of pheromones by the immature female that announce her stage in the moult cycle to potential mates (Dunham, 1978; Thompson & Manning, 1981). Some immature female mites (Arachnida: Acari) also emit pheromones termed "arrestants" that stimulate guarding behaviour in consepecific adult males (Sonenshine, 1985).

In mites, precopulatory guarding has been found in many taxa where males guard the penultimate female stage, which depending on taxon can be the deutonymph (Helle, 1967; Potter *et al*., 1976; Yasui, 1988; Oku, 2009) or the tritonymph (Witaliński *et al*., 1992; Bochkov & OConnor 2005). Some male spider mites (Tetranychidae: *Neonidulus*) have enlarged legs I to guard nymphal females (D.E. Walter, Royal Alberta Museum, pers. obs.). Likewise, many male Astigmata possess enlarged legs III and/or legs IV to aid in guarding nymphal females (Krantz & Walter, 2009), while others use a pair of ventral adanal suckers to attach to immature and/or mature females (Witaliński *et al*., 1992). In most male Astigmata, these suckers are composed of a thick exocuticle that

forms slightly concave sucker plates that are covered by a flexible layer encompassing the sucker periphery, which facilitates attachment of the sexes through suction (Witaliński, 1990).

In the feather mite genus *Proctophyllodes* (Astigmata: Proctophyllodidae), the tritonymphal females possess a pair of dorsal, soft protuberances which are hypothesized to fit into the male adanal suckers (Atyeo & Braasch, 1966), which are particularly elongated in this genus. The protuberances of *Proctophyllodes* spp. and *Psoroptes* spp. (Astigmata: Psoroptidae) have been described in detail by Witaliński *et al*. (1992) whose findings suggest that the dimensions of the docking papillae and the adanal suckers correspond in length, diameter and axis-to-axis distance. A comparable mechanism for attachment has been illustrated in the beaver fur-mite *Schizocarpus mingaudi* Trouessart (Astigmata: Chirodiscidae) whereby the larval cuticle is drawn into a conical depression in the male's soft cuticle (Fain *et al.*, 1984). Some fur mites have an additional attachment site between the male's adanal suckers and discs on the immature female mite; in this instance, the discs are considerably larger than the adanal suckers, which makes their insertion into the suckers highly unlikely (Fain *et al.*, 1984).

Morphological traits associated with copulation and intromission are often correlated between the sexes (Eberhard, 2004). These correlated characters can arise through antagonistic coevolution wherein the sexes engage in an evolutionary arms race to gain control of reproduction (Rowe & Arnqvist, 2002; Bergsten & Miller, 2007; Tatarnic & Cassis, 2010); or these traits may be "selectively cooperative" arising through sexual selection by female choice (Eberhard, 1985). In this study, we measured the dimensions of the male adanal suckers and the tritonymphal female docking papillae in representatives of three genera of Proctophyllodidae (*Neodectes* spp., *Proctophyllodes* spp., and *Proterothrix* spp.). These traits are supposedly beneficial to both sexes, as efficient coupling may increase the fertilization success of both sexes, and potentially reduce damage to the female's integument by localizing attachment

to a particular area. As such, we hypothesized that these traits would be strongly correlated to improve attachment of the adult male to the tritonymphal female.

2.3 Materials and Methods

2.3.1 Specimen collection

We collected three genera of mites in the family Proctophyllodidae, including *Neodectes* spp., *Proterothrix* spp. (Proctophyllodidae: Pterodectinae), and *Proctophyllodes* spp. (Proctophyllodidae: Proctophyllodinae). Mites were collected from 31 avian hosts captured in Australia, Canada, China, the Philippines and Spain (Table 2-1). Mites were retrieved using a variety of methods depending on the host's place of capture. Birds collected in China and the Philippines were mist-netted and mites were removed by eye from the dead host. In Spain, feathers were plucked from live birds and soaked in 70% ethanol to rehydrate specimens (see Galván *et al*., 2008). In Australia, preserved bird specimens were sampled from either the Western Australian Museum or the Queensland Museum by one of the authors (HP). For these birds, mites were removed in two ways: if hosts were prepared as dry skins, the skins were ruffled over a sheet of white paper and the mites were picked out with fine forceps and placed into 80% ethanol; if birds were preserved in ethanol, mites were sucked up from the bottom of the container using a syringe. Birds collected in Canada were stored individually after capture at -20°C until processing by HP or KB. Frozen birds were thawed and then washed in a container with ~15 mL of 95% ethanol, ~10 mL of Palmolive® dish detergent and sufficient water to submerge the bird. We massaged each bird thoroughly within solution to remove mites from the body, wing feathers, and retrices. We rinsed the bird body and poured the washing solution through a Fisher-Scientific 53-µm mesh filter. Mites were washed from the sieve with 80% ethanol and stored in 75 mL screw-cap containers. For birds caught in Canada and Spain, the ethanol solution was examined at 20-40X magnification using a Leica MZ16 dissecting microscope.

We mostly selected pairs of mites that were in precopula (i.e., male and tritonymphal female) for slide mounting. Mites were cleared in lactic acid

overnight and mounted in PVA mounting medium (BioQuip Products; Rancho Dominguez, CA, USA) on glass slides. Slides were cured on a warming tray at 45°C for a minimum of four days and were then examined at 400X on a Leica DMLB compound microscope (Leica Microsystems Inc., Richmond Hill, ON, Canada) using differential interference contrast (DIC). Mites were identified to genus in all cases and species when possible using relevant taxonomic literature (Atyeo & Braasch, 1966; Gaud & Atyeo, 1996). Most proctophyllodids from hosts outside of Europe and North America are undescribed, and are noted simply as 'sp.'. Exemplars of each taxon are deposited in the E.H. Strickland Entomological Museum at the University of Alberta.

2.3.2 Correlation measurements and analysis

To analyze whether the male adanal suckers and tritonymphal female docking papillae correlated morphologically, we took digital images at 200 and 400X of adult males and tritonymphal females from each bird using Image Capture (Apple Computer, Cupertino, CA, USA) and a Canon PowerShot S40. Images were uploaded to ImageJ (National Institutes of Health, Bethesda, Maryland) and measured for male and tritonymph body size as well as for dimensions of the male adanal suckers and tritonymphal docking papillae. For both sexes we measured the length of the idiosoma from the anterior margin of the prodorsum to the posterior of the body excluding the opisthosomal lamellae in males (see Figure 2-1a,b). For tritonymphs, we took three measurements of the docking papillae: the width, the longest (medial) length and the shortest (lateral) length (Figure 2-1a). For males, we measured the width at the adanal sucker tip and at the base as well as the depth of the sucker (Figure 2-1c,d). Spearman's correlation coefficient analyses were performed in SPSS version 20 (SPSS Inc., Chicago, IL, USA) to determine whether the dimensions of the male adanal suckers and female tritonymphal docking papillae were correlated. For an estimate of measurement error, we measured one tritonymphal female and one adult male *Neodectes* sp. ten times to determine the degree of variation in measurements as indicated by the coefficient of variation (Zar, 2010).

2.3.3 Proctophyllodes troncatus *measurements*

In the literature, 'docking papillae' have been associated with the female tritonymph of *Proctophyllodes* spp. (Atyeo & Braasch, 1966); however, whether or not females are the only sex to possess these papillae has not been tested. To clarify whether docking papillae were restricted to female tritonymphs and not to any other nymphal stage or sex, we measured the length of the idiosoma in adult male, adult female and nymphal *Proctophyllodes troncatus* Robin captured from house sparrows (*Passer domesticus* (Linnaeus)) in Romania (Figure 2-1). We measured a total of 20 adult male and female *P. troncatus*, as well as 196 nymphal *P. troncatus*. Ideally we would have been able use the number of genital papillae to differentiate between protonymphs (which have one pair) and tritonymphs (which have two pairs), but the slightly degraded nature of the specimens rendered the nymphal genital papillae essentially invisible even under DIC lighting. For all nymphs we determined whether docking papillae were present. We grouped these mites into categories for adult males, adult females, nymphs with docking papillae, and nymphs without docking papillae and analyzed the distribution in a histogram produced in SPSS. We hypothesized that adult females would be larger than adult males, that adult males would be larger than nymphs and that, if nymphs with docking papillae were tritonymphal females, that these nymphs should be on average larger than nymphs without docking papillae (predicted to be nymphal males or protonymphal females).

2.4 Results

2.4.1 Correlation analyses

Of the 49 pairs of mites analyzed, 17 were omitted as the individuals were still firmly in precopula and the docking papillae were not observable. In the remaining pairs, body size was positively correlated between adult males and tritonymphal females ($r_s = 0.66$, n = 32, $P < 0.01$) (Figure 2-2). Among the tritonymphal females measured, four *Proterothrix* spp., one *Neodectes* spp. and eight *Proctophyllodes* spp. individuals did not have docking papillae. For correlation analyses we omitted specimens without docking papillae (n = 13) to

remove the influence of zero counts. We also omitted males where we were unable to measure adanal sucker length (n = 2 of 19 pairs) or basal area of the sucker (n = 1 of 19 pairs). There were no correlations between the dimensions of tritonymphal docking papillae and male adanal suckers (Table 2-2). The medial and lateral lengths of the female docking papillae did not correlate with male adanal sucker length ($r_s > 0.24$, n = 17, $P > 0.13$; see Figure 2-3c,d); nor was width of the docking papillae correlated with the distal ($r_s = 0.05$, n = 19, $P = 0.85$) or basal ($r_s = 0.43$, n = 18, $P = 0.07$) widths of the adanal suckers (Figure 2-3a,b).

Measurement error (mean ± standard deviation, coefficient of variation) as indicated by variation in our repeated measurements was minimal. For adult males: body length (315µm ± 1.64, CV = 0.005), adanal sucker area (107.98µm^2 ± 8.28, CV = 0.08), distal sucker width (10.76µm ± 0.32, CV = 0.03), basal sucker width (9.98µm ± 0.75, CV = 0.07) and sucker depth (2.93µm ± 0.12, CV = 0.04). For tritonymphal females: body length (309.17µm ± 0.81, CV = 0.003), lateral docking papillae length (2.95µm ± 0.21, CV = 0.07), medial docking papillae length (7.58µm ± 0.39, CV = 0.05) and docking papillae width (8.99µm ± 0.25, CV = 0.03)

2.4.2 Proctophyllodes troncatus *measurements*

Distributions of body length for *P. troncatus* adult males, adult females, and nymphs are displayed in Figure 2-4. Adult males ranged in size from 250 – 285 µm in length and were significantly smaller than adult females in length which were 330-380 µm ($t_{38} = 22.6$, $P < 0.001$). For nymphs, there were two distinct size clusters; nymphs with docking papillae (n = 81) ranged in size from 240-375 µm in length and were significantly larger than nymphs without docking papillae (n = 115) which were 175 – 255 µm in length ($t_{194} = 20.6$, $P < 0.001$).

2.5 Discussion

Although copulation occurs between adult astigmatan feather mites, it is common for males to couple with tritonymphal females prior to mating (Atyeo

& Braasch, 1966; Witaliński *et al*., 1992). In our study of *Neodectes* spp., *Proterothrix* spp. (Proctophyllodidae: Pterodectinae) and *Proctophyllodes* spp. (Proctophyllodidae: Proctophyllodinae) we observed dorsal docking papillae in tritonymphs of all genera similar to those described by Witaliński *et al*. (1992) in *Proctophyllodes stylifer* (Buchholz), *Proctophyllodes picae* (Koch), *Psoroptes cuniculi* (Delafond), and *Psoroptes natalensis* Hirst. Surprisingly not all tritonymphal females within a genus possessed docking papillae. This may be due to either intrageneric variability in the presence of docking papillae, or a result of males accidentally grabbing a nymph of the wrong sex or stage, a phenomenon which has been reported in male tarsonemids (Garga *et al*., 1997).

It was surprising that we did not find a correlation between the dimensions of the female docking papillae and the male adanal suckers. In arthropods, several studies have documented correlations between the sexes in reproductive characters both internally (e.g. the genitalia of *Apamea* moths (Mikkola, 1992)), and externally (e.g. the grooves and pits on female katydids which facilitate grasping by male clasping structures (Rentz, 1972)). In their study of *Proctophyllodes* and *Psoroptes* spp. mites, Witaliński *et al*. (1992) report size similarities between the docking papillae and male adanal suckers; however, these results were strongly categorical, whereby lengths and diameters of these structures were compared as ranges, instead of specific measurements. We did not find any quantitative correlation between these structures in our study. Given that the mode of precopulatory attachment is likely the sucking force between the adanal suckers and the docking papillae (Witaliński *et al*., 1992), one would expect that closely correlated dimensions would be integral to promoting a cohesive coupling. The absence of correlation between tritonymphs and adult males in dimensions of the docking papillae and adanal suckers may indicate that size is not the only characteristic that matters.

It is possible that we did not measure the size of the papillae when they are fixed within the adanal suckers. Witaliński *et al*. (1992) suggest that the docking papillae swell during attachment due to increased haemolymph pressure induced from suction of the male suckers. During coupling, the male is

hypothesized to press his adanal suckers against the female cuticle, at which point the sucker plate is pulled inwards, sealing the docking papillae within the male suckers (Witaliński *et al*., 1992). A similar suction-based mechanism of maintaining contact between adult males and immatures is hypothesized in *Schizocarpus* spp. fur mites (Fain *et al.,* 1984). If this is so, then it is possible that the papillae of independent tritonymphs are not representative of their size when in copula. However, we would also expect that the width of the papillae would be consistently smaller than that of the adanal suckers, which we did not find. Another consideration is the role of glandular secretions in the attachment of males to females. In some uropodid mites, the phoretic deutonymph uses glandular secretions from the pedicellar gland to attach to their carrier (Bajerlein & Witaliński, 2012). In *Pterodectes* spp. (Proctophyllodidae: Pterodectinae) glands associated with the adanal suckers are hypothesized to aid in male attachment (Popp, 1967), while sticky secretions exuded from the male suckers may also enhance fixation during coupling in fur mites (Fain *et al.,* 1984). If glandular secretions are used to improve adhesion between adult males and tritonymphal females in feather mites, then it is likely that the strict correlation between dimensions of the adanal suckers and docking papillae is unnecessary.

Another influence in why we did not detect a correlation between male and tritonymphal morphologies may be that we assumed that all tritonymphs with docking papillae were female. Although both Atyeo and Braasch (1966) and Witaliński *et al.* (1992) refer to tritonymphs with dorsal papillae as female, they may not be exclusive to female tritonymphs. We tried to account for this alternative by measuring body length of nymphal *P. troncatus* and grouping them by presence or absence of docking papillae. Our results show that docking papillae are present in larger nymphs and absent in smaller nymphs, suggesting that these larger nymphs with papillae are female (the larger adult sex) while the smaller nymphs without papillae are male (the smaller adult sex). It is also possible that when slide-mounting specimens, morphologies may have been distorted due to the orientation of the body and the flattening of the specimen on the slide, thus affecting our measurements. Though the results of this study are

largely preliminary, they do provide a basis for future studies of the mechanisms promoting precopulatory guarding in feather mites. To expand on this research, it will be beneficial to consider intraspecific as well as interspecific variations in traits to resolve the role of the docking papillae in coupling and precopulatory guarding in feather mites. Microscopic observation of the process of precopulatory coupling in live proctophyllodid mites may also provide clues about changes in dimensions of the docking papillae and/or adanal suckers before and after the male affixes to the tritonymph.

2.6 Acknowledgements

We would like to thank Peter Pap for providing *Proctophyllodes troncatus* specimens, Sarah Bush for sending mites from China and the Philippines, and Ismael Galván for mites from Spain. Thanks go as well to B. Doty for his assistance with this manuscript. This research was funded through a Natural Sciences and Engineering Research Council of Canada Discovery Grant to HP.

Table 2-1. Taxonomic authorities and locality data for avian hosts of *Neodectes* spp., *Proctophyllodes* spp., and *Proterothrix* spp. (Analgoidea: Proctophyllodidae). The presence or absence of docking papillae in tritonymphs is indicated for each taxon.

Mite Taxonomy and Authority	Host Genus	Host Specific Epithet	Authority	Locality	Docking Papillae Present
Neodectes spp. Park & Atyeo	*Acanthisitta*	*chloris*	(Sparman, 1787)	No data	Yes
	Pachycephala	*philippinensis*	(Walden, 1872)	Aurora Memorial Park, Philippines	Yes
	Sitta	*frontalis*	Swainson, 1820	Philippines	Yes
Proctophyllodes spp. Robin	*Acanthis*	*hornemanni*	(Hoiboil, 1843)	Smith, AB, Canada	No
	Bombycilla	*cedrorum*	Vieillot, 1808	Athabasca, AB, Canada	No
	Carpodacus	*purpureus*	(Gmelin, 1789)	Athabasca, AB, Canada	Yes
	Catharus	*ustulatus*	(Nuttall, 1840)	Edmonton, AB, Canada	Yes
	Certhia	*americana*	Bonaparte, 1838	Edmonton, AB, Canada	Yes
	Cincloramphus	*cruralis*	(Vigors & Horsfield, 1827)	Outcamp Creek, Australia	Yes
	Cyornis	*herioti*	Ramsay, 1886	Mt. Cagua, Philippines	Yes
	Melospiza	*melodia*	(A. Wilson, 1810)	AB, Canada	Yes
	Pica	*hudsonia*	(Sabine, 1823)	Edmonton, AB, Canada	Yes
	Sitta	*canadensis*	Linnaeus, 1766	Spruce Grove, AB, Canada	Yes
	Turdus	*migratorius*	Linnaeus, 1766	Edmonton, AB, Canada	Yes
P. microcaulus Gaud	*Eremophila*	*alpestris*	(Linnaeus, 1758)	Manyberries, AB, Canada	Yes
P. megaphyllus Trouessart	*Plectrophenax*	*nivalis*	(Linnaeus, 1758)	Barrhead, AB, Canada	Yes
P. musicus Vitzthum	*Turdus*	*migratorius*	Linnaeus, 1766	Edmonton, AB, Canada	Yes

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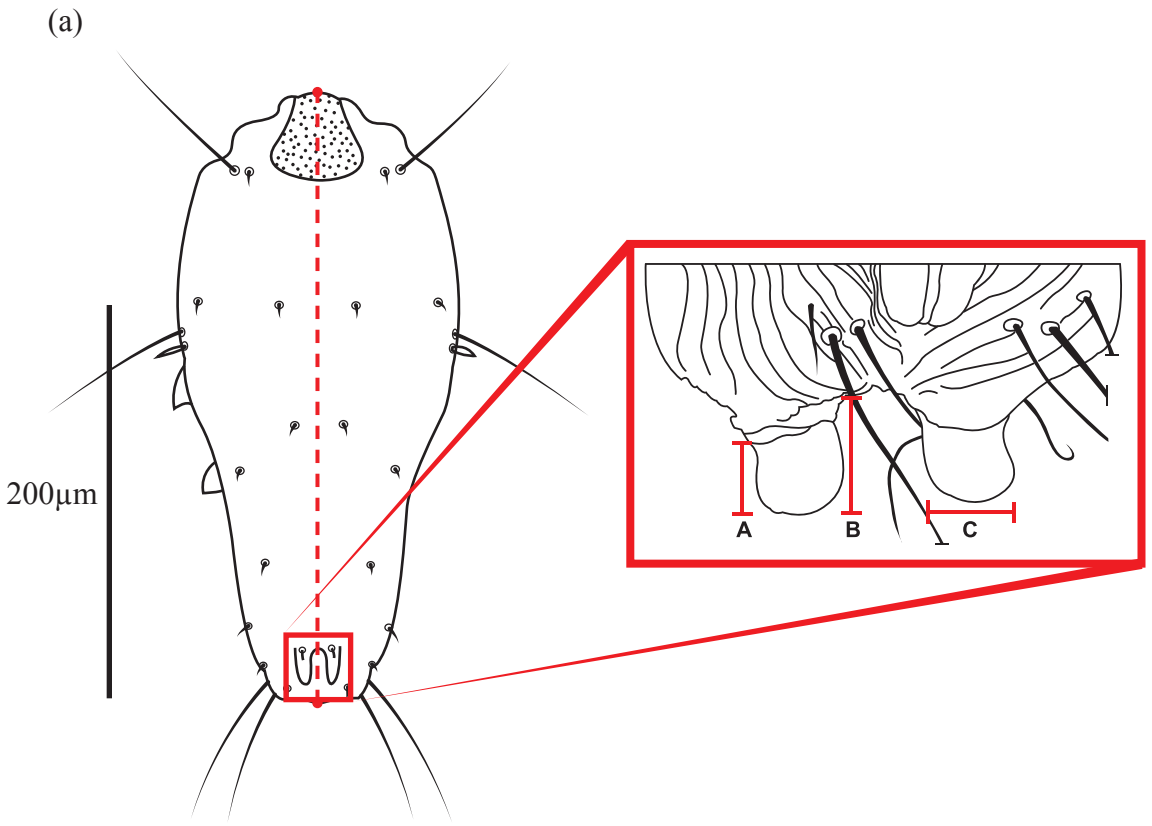
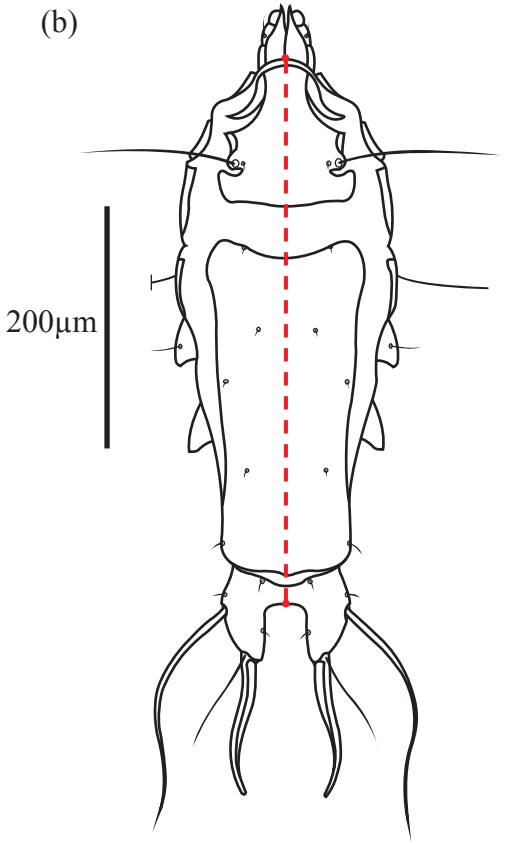
Mite Taxonomy and Authority	Host Genus	Host Specific Epithet	Authority	Locality	Docking Papillae Present
P. pennifer (Trouessart & Neumann)	*Cinclidium*	*leucurum*	(Hodgson, 1845)	Jing Xin, China	No
P. schoenicli Atyeo & Braasch	*Emberiza*	*schoeniclus*	(Linnaeus, 1758)	Spain	Yes
P. sylviae Gaud	*Sylvia*	*atricapilla*	(Linnaeus, 1758)	Spain	Yes
P. glandarius (Koch)	*Bombycilla*	*garrulus*	(Linnaeus, 1758)	AB, Canada	No
P. vesca Atyeo & Braasch	*Myadestes*	*townsendi*	(Audubon, 1838)	Camp Creek, AB, Canada	No
Proterothrix spp. Gaud	*Rhipidura*	*cyaniceps*	(Cassin, 1855)	Aurora Memorial Park, Philippines	Yes
	Orthonyx	*temminckii*	Ranzani, 1822	Wilson's Peak, Killarney, Australia	Yes
	Cracticus	*quoyi*	(Lesson, 1827)	Gunn Point, Australia	No
Keys to *Proterothrix* spp. Gaud	*Conopophila*	*rufogularis*	(Gould, 1843)	Derby-West Kimberley, Australia	Yes
	Cyornis	*herioti*	Ramsay, 1886	Angat & Mt. Cagua, Philippines	Yes

Table 2-2. Spearman's correlation coefficients (r_s) and significance (P) of correlations between measurements of male adanal suckers and tritonymphal docking papillae in the genera *Neodectes* spp., *Proctophyllodes* spp., and *Proterothrix* spp. (Analgoidea: Proctophyllodidae).

Female Tritonymph Morphology	Male Morphology	Adanal Sucker Distal Width			Adanal Sucker Basal Width			Adanal Sucker Length		
		n	r_s	P	n	r_s	P	n	r_s	P
Docking papillae width		19	0.47	0.85	18	0.43	0.07	17	-0.43	0.06
Docking papillae medial length		19	0.36	0.13	18	0.2	0.42	17	0.38	0.13
Docking papillae lateral length		19	0.06	0.81	18	-0.21	0.41	17	0.24	0.35





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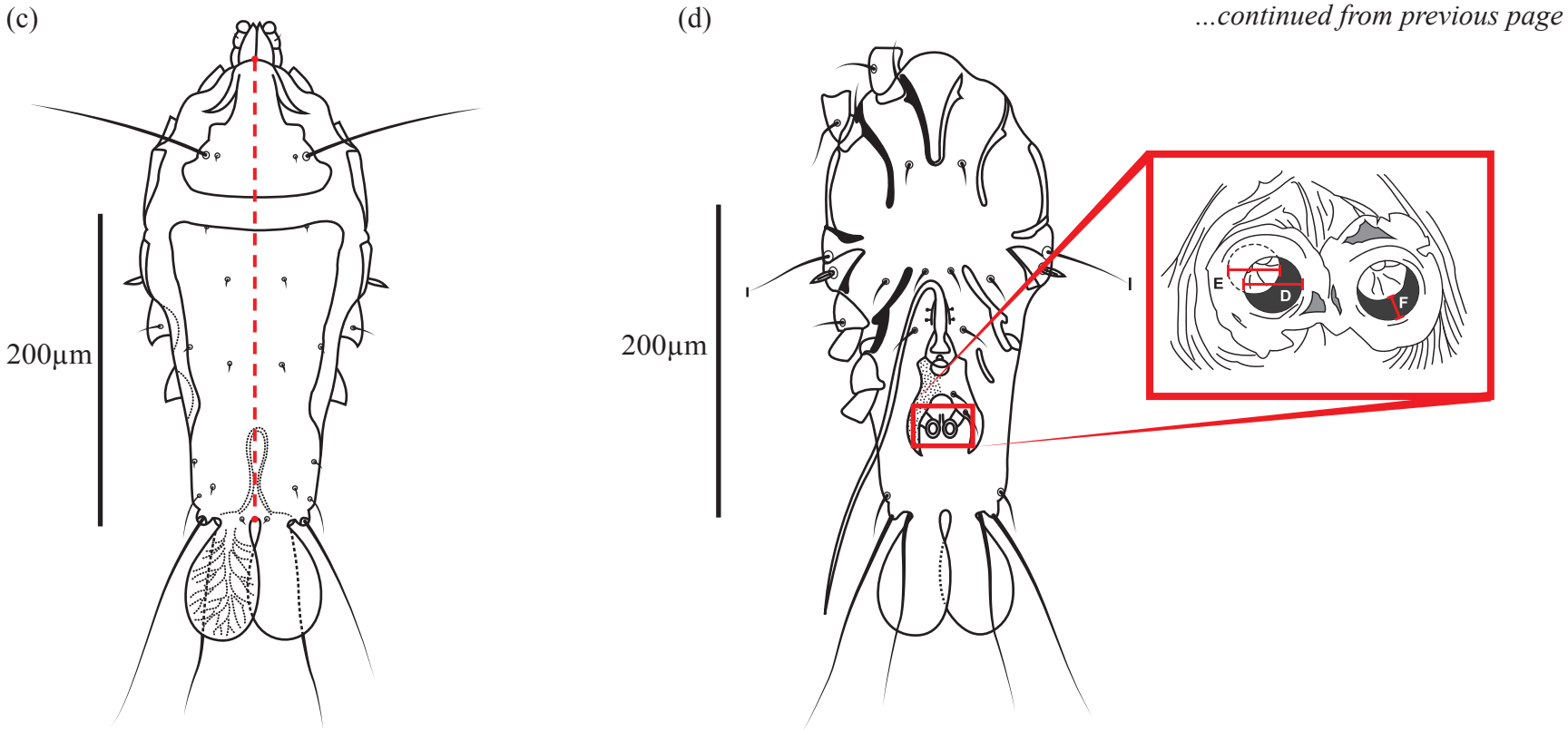

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Figure 2-1. Length of the idiosoma in *Proctophyllodes* spp. in (a) female tritonymphs, (b) adult females, (c) adult males dorsal, (d) adult males ventral. Measurements (dashed line) were taken from the margin of the prodorsum to the posterior margin of the body. In female tritonymphs we measured the lateral length (A), medial length (B) and width (C) of the docking papillae. In males (d; ventral), we measured the distal (D) and basal (E) widths of the adanal suckers as well as sucker depth (F). Line drawings are modeled after *Proctophyllodes glandarinus* (Koch) for adults and from *Proctophyllodes pari* Atyeo & Braasch for tritonymphs (Atyeo & Braasch, 1996). Diagrams of the docking papillae and adanal suckers are drawn after scanning electron images published in Witaliński *et al.* (1992).

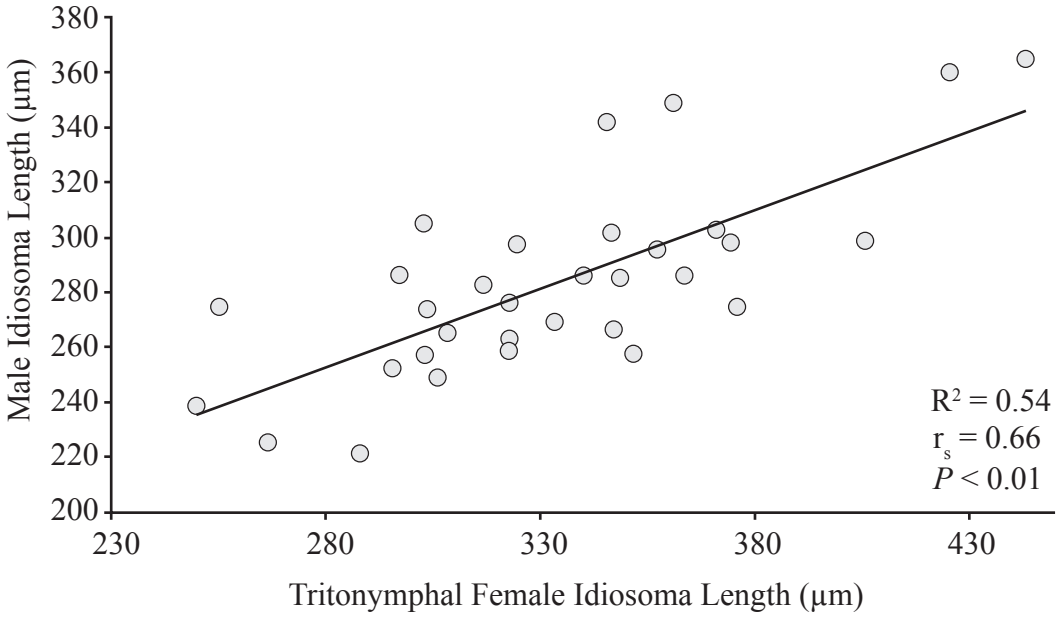
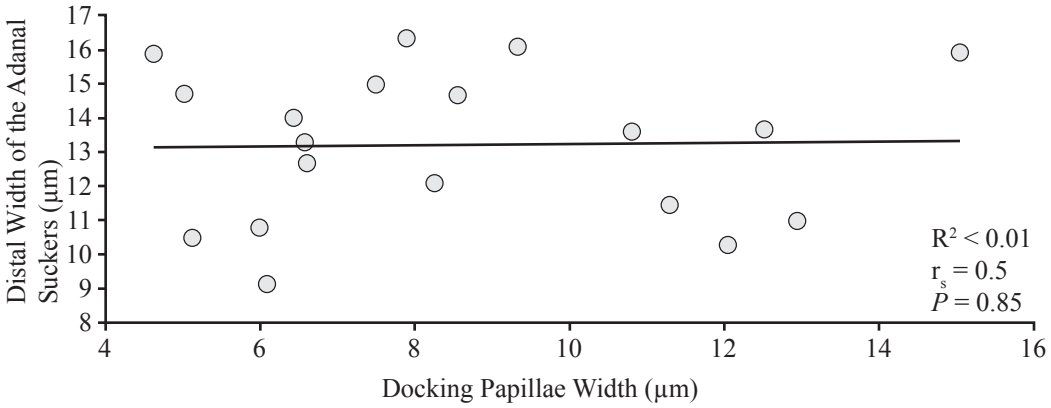
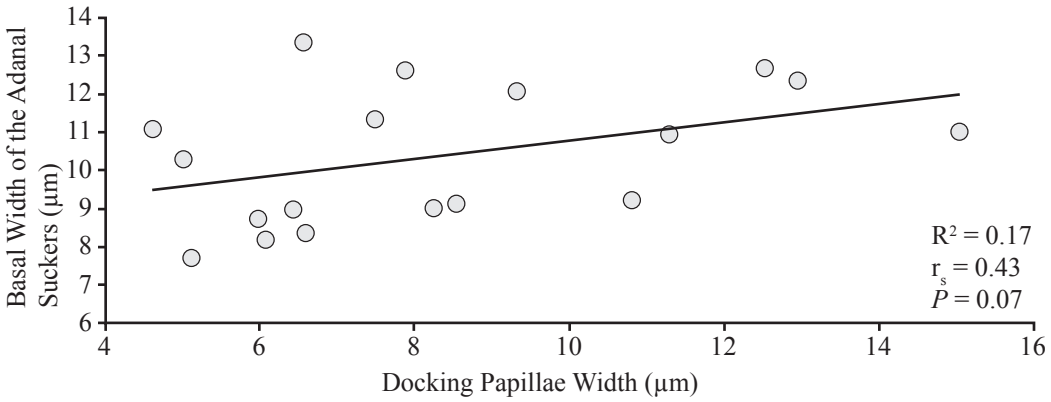


Figure 2-2. Correlations between adult male and tritonymphal female body length (μm) in *Neodectes* spp., *Proctophyllodes* spp., and *Proterothrix* spp. (Astigmata: Proctophyllodidae). Length of the idiosoma was measured from the anterior margin of the prodorsum to the posterior region of the body excluding the opisthosomal lamellae in males. Body size was positively correlated between the sexes (r_s = 0.66, n = 32, $P < 0.01$) whereby larger tritonymphal females were paired with larger conspecific males.

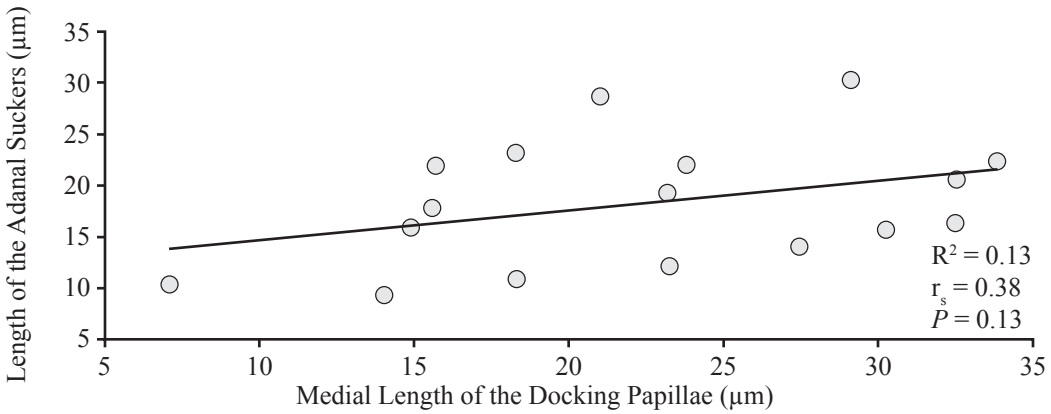
(a)



(b)



(c)



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(d)

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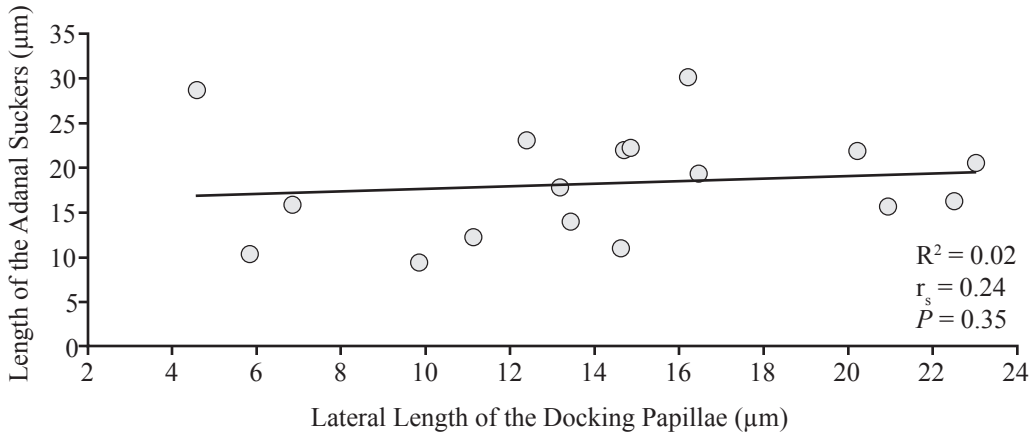

Figure 2-3. Correlations in morphologies between adult male and tritonymphal female *Neodectes* spp., *Proctophyllodes* spp. and *Proterothrix* spp. feather mites (Astigmata: Proctophyllodidae). Correlations are illustrated between widths (a-b) and lengths (c-d) of the male adanal suckers and the female docking papillae.

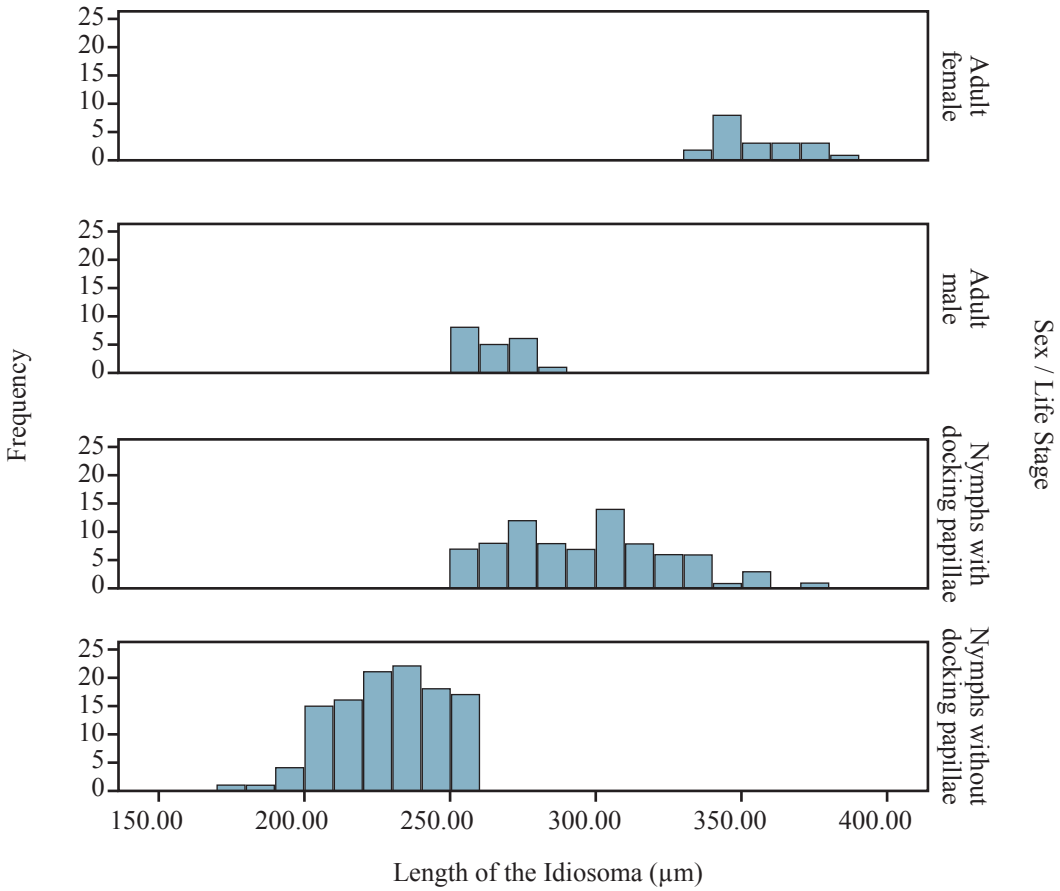

Figure 2-4. Size distributions of *Proctophyllodes troncatus* Robin adult females, adult males and nymphs with and without docking papillae. Body size was measured as the length of the idiosoma (µm) from the anterior margin of the prodorsum to the posterior region of the body excluding the terminal hyaline appendages in adult females and the opisthosomal lamellae in males. Nymphs were categorized as having docking papillae or lacking docking papillae. Adult females were significantly larger than adult males ($t_{38} = 22.6$, $P < 0.001$). Similarly, nymphs with docking papillae were larger on average than were those without docking papillae ($t_{194} = 20.6$, $P < 0.001$).

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Chapter 3. Correlated morphologies in genital and non-genital contact structures in *Trouessartia* spp. feather mites (Astigmata: Analgoidea)

3.1 Abstract

When males and females have opposing interests in reproduction, competition to gain control of fertilization can promote the evolution of sexually antagonistic morphologies and behaviours. Although male genitalia are often more variable in form than the genitalia of their conspecific females, in members of the feather mite genus *Trouessartia* (Astigmata: Analgoidea), females also display diversity in genitalic form. In these species the spermaduct of females extends externally past the supranal concavity to various lengths. Females of this genus also exhibit a greater degree of ornamentation on the dorsal hysteronotal shield than do males. As males attach to females via a pair of ventral adanal suckers in this region of ornamentation, we hypothesized that the female's ornamentation may serve to interfere with the male's attachment during copulation. Here we use the avian host phylogeny of *Trouessartia* spp. feather mites to determine whether variation in length of the female external spermaduct correlates with male genitalic morphology, as well as whether these structures are illustrative of patterns consistent with sexually antagonistic coevolution. We also determine whether patterns of female dorsal ornamentation suggest a coevolutionary arms race with male attachment structures. Our results indicate that females with longer external spermaducts are typically paired with males of comparatively larger genitalic organs. However, we did not detect correlations between male and female non-genitalic contact structures. Further analyses using mite rather than host phylogeny are required to determine whether directional selection is favouring these potentially resistant female traits.

3.2 Introduction

In sexual species, both males and females have a vested interest in the fitness gained from the successful completion of mating; however, reproductive investment is often disproportionate between the sexes. As a result, the sex with

higher gametic or parental investment will often be more selective of its mating partner (Parker *et al.,* 1979). Females commonly invest more in their offspring than do males (e.g., anisogamy, Bateman, 1948); given this, females are often the limiting sex (Trivers, 1972). This differential investment between the sexes can promote sexual conflict, whereby each sex acts to further its own interests. In some cases this struggle to gain control of fertilization can be at the cost of the opposite sex (Parker *et al*., 1979; Arnqvist & Rowe, 2005; Rönn *et al.,* 2007; Madjidian *et al*., 2012). Costs to females from undesired matings include reduction in their own reproductive success (Alexander *et al*., 1997), damage to the reproductive tract (Siva-Jothy, 2006) and increased predation (Rowe, 1994).

The genitalic traits of males are among the most rapidly evolving morphologies in internally fertilizing animals and are often more variable than female genitalia (Eberhard, 1985). This rapid diversification in male genitalia is hypothesized to arise via sexual selection (Eberhard, 2010a) acting through either cryptic female choice (Eberhard, 1985) or sexually antagonistic coevolution (SAC; Arnqvist & Rowe, 2005). However, these two hypotheses are not mutually exclusive (Hosken & Stockley, 2004; Eberhard, 2010b) and the generality of SAC across taxa has been questioned (Eberhard, 2004). In instances of sexual conflict, the development of sexually antagonistic morphologies and behaviours may arise as the sexes compete to gain control of fertilization (Arnqvist & Rowe, 2005). These adaptations can be quickly matched by counter-adaptations in the opposite sex, thus creating an arms race between the sexes (Parker *et al*., 1979; Arnqvist & Rowe, 2002a; Arnqvist & Rowe, 2005; Brennan *et al*., 2007; Rönn *et al.,* 2007; Perry & Rowe, 2011). When these adaptations are associated with genitalic structures, SAC can result in the diversification of reproductive morphologies, (Sota & Tanabe, 2010). Sexually antagonistic coevolution in reproductive structures has been documented in both vertebrates (Brennan *et al*., 2007) and invertebrates (Koene & Schulenburg, 2005), and has more recently been associated with traumatic insemination and harmful male genitalia in arthropods (Rönn *et al.,* 2007; Tatarnic & Cassis, 2010; Kamimura, 2012).

In addition to SAC in genitalic structures, sexual conflict may also influence non-genitalic contact structures involved in mate acquisition (Arnqvist & Rowe, 2002a). These structures can range from the sucker-like bursa of male nematodes (Ahmad & Jairajpuri, 1981) to the cerci of dragonflies (McPeek *et al*., 2009). Non-genital contact devices employed by males to grasp females often correspond to the dimensions of the females' receptive structures (Arnqvist & Rowe, 2002a; Huber, 2003; McPeek *et al*., 2009). An excellent example of antagonistic coevolution in grasping structures occurs in some diving beetles (Coleoptera: Dytiscidae), where males possess tarsal suction cups to grasp females and females have evolved modified dorsal macropunctures and setose furrows at these attachment sites to impair male attachment (Bergsten & Miller, 2007). In response, males have adapted elaborate suction-cup morphologies to counteract these female modifications. Similar patterns have been reported in the male grasping and female antigrasping structures of water striders (Arnqvist & Rowe, 2002b).

Studies evaluating correlated genitalic structures are often difficult to perform due to the internal nature of most female genitalia. However, some parts of the genitalia of female feather mites (Acari: Astigmata) are sclerotized (Proctor, 2003) and readily visible through the body wall in slide-mounted specimens, making them ideal for studying genitalic traits. Similar to other astigmatan mites, male feather mites possess a sclerotized aedeagus (copulatory organ), which females of most species receive in their copulatory pore; this pore opens posteriorly at the tip of the female's internal sclerotized spermaduct (Proctor, 2003). In some species of Astigmata, this spermaduct has elongated and extends externally from the female's body wall (see Klimov & Sidorchuk, 2011). Members of the vane-dwelling feather mite genus *Trouessartia* (Analgoidea: Trouessartiidae) are of particular interest as the spermaduct extends from the female's body terminus to various lengths (Santana, 1976). This external spermaduct is often supported by an interlobar membrane which extends between a pair of triangular lobes at the posterior of the hysterosoma (Santana, 1976) (Figure 3-1a). Such variation in the female genitalic form

contrasts with the observation that females typically have less variable genitalia than males (Eberhard, 1985; Huber, 2010; but see Polihronakis, 2006; Puniamoorthy *et al*., 2010).

Mating in feather mites occurs with the male and female oriented in opposite directions (Walter & Proctor, 1999) with the male's venter apposed to the female's dorsum (Proctor, 2003). In the majority of feather mites, insemination is achieved through insertion of the male's aedeagus into the female's copulatory pore and into the distal portion of her internal spermaduct (Proctor, 2003); however, in some species with external spermaducts (e.g. Pterolichoidea: Crypturoptidae) the male instead receives the tip of the spermaduct inside his aedeagus (Gaud & Atyeo, 1996). Whether *Trouessartia* spp. mate in a fashion similar to the Crypturoptidae is unknown, although Santana (1976) concluded that based on the lack of an intromittent structure associated with the genitalic sclerites, the male likely receives the female's external spermaduct inside his genitalic apparatus. This potential 'reversal' of copulatory roles may be a result of SAC (Klimov & Sidorchuk, 2011).

Female *Trouessartia* spp. not only possess an external spermaduct, but they also display a greater degree of dorsal ornamentation than do their conspecific males. This ornamentation is composed of lacunae (pits) on the dorsal side of the posterior hysterosoma (Figure 3-1a), which is in the approximate region of where the male's adanal suckers affix. It is also in this region that the female's *h1* setae are located. Similar to the external spermaduct, the length and area of the *h1* setae vary dramatically from hair-like microsetae to spearhead-like macrosetae (Santana, 1976). The larger setae may also play a role in thwarting unwanted mating attempts, similar to the upwards-pointing abdominal spines of some female water striders (Arnqvist & Rowe, 1995).

In this study we aim to answer three questions relating to correlated evolution of male and female structures. First, given the diversity of female genitalic form, we hypothesize that the elongation of the external spermaduct may have coevolved antagonistically, and we therefore expect to see a correlation between dimensions of male and female genitalia. Second, if female

ornamentation hinders male attachment, we hypothesize that the degree of dorsal ornamentation in females will correlate with male adanal sucker size. We also predict a similar relationship between the size of the adanal suckers and the size of the female's *h1* setae. Third, we incorporate phylogenetic analyses to elucidate whether there is evidence for directional selection acting on external spermaduct length, ornamentation and *h1* seta size.

3.3 Materials and methods

3.3.1 Host sampling

Avian hosts were collected worldwide from Canada, Europe, Australia, China and the Philippines (see Acknowledgements). Symbionts were removed from their host birds in various ways dependent on the region of capture. Birds collected from China and the Philippines were mist-netted and symbionts were removed by eye from dead birds. For European-caught birds, feathers were plucked from live hosts and stored in 70% ethanol for later inspection. In Australia, specimens were sampled from either the Western Australian Museum (WAM) or the Queensland Museum by one of the authors (HP). Birds from the WAM were sampled in two ways: for dry skins, bird bodies were ruffled over a sheet of white paper and the mites were removed with fine forceps and placed into 80% ethanol; if birds were preserved in ethanol, symbionts were removed from the bottom of the container using a pipette. Birds from the Queensland Museum in Australia were sampled in a similar manner to the dry study skins at the WAM. Mites from most Canadian birds were from Alberta. For these hosts (which were mainly window- and roadkills), bodies were stored at -20°C until processing by HP or KB. Frozen birds were thawed and symbionts were collected via washing. Each bird was placed into a container with ~15 mL of 95% ethanol, ~10 mL of Palmolive® dish detergent and an adequate volume of water to submerge the bird. The birds were massaged in the solution to ensure that symbionts were removed from the wing feathers, retrices and body. Each bird was rinsed over a Fisher-Scientific 53-µm mesh filter and the washing liquid was poured through the same filter. Symbionts were washed from the

mesh sieve with 80% ethanol and then stored in 75 mL screw-cap containers for a minimum of one week before sorting to allow the symbionts to rehydrate and sink. We examined washings for symbionts using a Leica MZ16 dissecting microscope at 20-25x magnification.

For all hosts, mites belonging to the family Trouessartiidae (including *Trouessartia* spp., *Allanalges* spp., *Arthrogynalges* spp., *Calcealges* spp., *Neocalcealges* spp.) and Thysanoscercidae (*Thysanocercus* spp.) (possible sister taxon to Trouessartiidae, pers. comm. B.M. OConnor, University of Michigan) were removed from ethanol, cleared from 1-48 h in 85% lactic acid and mounted in polyvinyl alcohol medium (6371A, BioQuip Products, Rancho Dominguez, California). Slides were placed on a 40ºC slide warmer for a minimum of 4 days. Once cured, each slide was examined using a Leica DMLB compound microscope with differential interference contrast at 200-400x magnification. Most *Trouessartia* species other than those from Europe are undescribed. A subset of specimens in good condition were prepared for scanning electron microscopy by dehydration followed by gold-coating with a Nanotek SEMprep 2 sputter coater, and imaged using a JEOL 6301F Field Emission Scanning Electron Microscope. Family, genus and species-level identifications were made using relevant literature (Santana, 1976; Gaud & Atyeo, 1996; OConnor *et al*., 2005). Most *Trouessartia* species outside of Europe and Africa have not yet been described, and so are listed as *Trouessartia* sp. Exemplars of all examined taxa are deposited in the E.H. Strickland Entomological Museum at the University of Alberta. A list of the sampled hosts with taxonomic authorities and mite associates is provided in Appendix 3-1.

3.3.2 Measurements

To assess correlation of morphological characteristics between the sexes, we took digital images of male and female mites using Image Capture software (Apple Computer, Cupertino, CA, USA) and a Canon PowerShot S40. Images were taken at 200 and 400x magnification and were uploaded to ImageJ (National Institutes of Health, Bethesda, Maryland). For both sexes we

measured the area of the hysterosonotal shield to give an estimate of body size, as well as the area of dorsal ornamentation and the proportion of the hysterosonotal shield covered with ornamentation (Figure 3-1a). We measured the area of five haphazardly selected lacunae oriented on the longitudinal axis and five on the lateral axis of the hysteronotal shield (total n = 10). In addition, we measured the length and area of the *h1* setae located dorso-posteriorly on the hysterosoma. For females, we measured the length of the total external spermaduct, the length of the posterior interlobar membrane and the length of the spermaduct extending externally past this membrane. For males, we measured the genitalic area (Figure 3-1c) as well as the proportion of the hysterosonotal shield occupied by the genitalia. Finally, we measured areas of both adanal suckers and took the average value of the two (see Appendices 3-2 and 3-3 for raw measures). For an estimate of measurement error, we measured one adult female and one adult male *Trouessartia* sp. from *Dicrurus balicassius* (Linnaeus) ten times to determine the degree of variation in measurements as indicated by the coefficient of variation (Zar, 2010).

3.3.3 Multivariate and correlation analyses

We performed a semi-strong hybrid multidimensional scaling (SSH-MDS) ordination analysis in PATN 3.11 (Belbin & Collins, 2006) using the Gower Metric (Gower, 1971) to explore associations among morphological characters. Characters used in the ordination are given in Table 3-1. For our ordination we performed a three-dimension MDS with 50 iterations and 1000 random starts. The correlation of variables with the ordination was determined by the Monte-Carlo Attributes in Ordination (MCAO). We plotted all intrinsic variables that were significant at $P < 0.05$, which resulted in excluding the female characters 3, 4, 5, 6, 9, 10 and the male characters 3, 4, 5, 6, 7, 9, 11 (see Table 3-1).

We tested for normality in our data using the Shapiro-Wilk test in SPSS version 20 (SPSS Inc., Chicago, IL, USA) which has been shown to be powerful with many types of distributions and sample sizes (Razali & Wah, 2011). As most of the morphologies we measured were non-normally distributed, we used

the Wilcoxon signed-rank test to determine whether the sexes differed in body size, the degree of dorsal ornamentation and *h1* setae size (Zar, 2010). For correlation analyses, we evaluated both untransformed and $\log_{10}$ transformed data to decipher whether differences were evident between the two data sets. We performed correlation analyses for male and female morphological characters in SPSS version 20 (SPSS Inc., Chicaco, IL, USA) using the non-parametric Spearman's rank correlation coefficient as several of our characters remained non-normally distributed after transformation (Bonett & Wright, 2000; Gel *et al*., 2007). For correlation analyses between female external spermaducts post interlobar membrane and male genitalic size, we removed species whose females lacked external spermaducts post membrane to remove the influence of zero counts driving the correlation. As Spearman correlation analyses do not take into account the influence of phylogenetic relatedness, we used these analyses as a preliminary indication of correlated structures and accounted for phylogenetic influence using host-based phylogenetically independent contrasts (Felsenstein, 1985) as outlined below.

3.3.4 Phylogenetic analyses

There are no published phylogenies for members of the Trouessartiidae, nor is there taxonomic substructure within *Trouessartia* (e.g., there are no named subgenera) that might have served as a proxy for phylogeny. Furthermore, we were unable to collect molecular information from our mites as the majority of specimens we acquired from Australia, China, and the Philippines were already mounted on slides, and those from Alberta were highly degraded due to being collected from hosts that had been found dead. However, several studies have indicated monophyly of feather mite families (Ehrnsberger *et al*., 2001; Dabert, 2003). As a proxy for the mite phylogeny, we used sequences obtained from their avian hosts acquired from GenBank (accession numbers are provided in Appendix 3-4). This assumes that host phylogeny provides a better estimate for mite relationships than does a haphazard arrangement of mite species (Dabert *et al*., 2001; Mironov & Waulthy, 2010). We included three mitochondrial

markers: cytochrome c oxidase subunit 1 (CO1), cytochrome b (Cytb), NADH dehydrogenase subunit 2 (ND2); and one nuclear marker: recombination activating-protein (RAG-1) from 97 avian hosts. Of the 97 host species, 16 had one marker in GenBank, 31 had two markers, 32 had three, and 16 had all four. Although sequences for all genes for all taxa could not be acquired, Fulton and Strobeck (2006) have shown that the amount of missing data does not strongly affect phylogenetic resolution and is more reliant on containing adequate informative characters. Similarly, Wiens (2006) suggests that including taxa with up to 75% missing data may not negatively influence the phylogeny's accuracy. Of the included taxa, three members of the Apodidae were designated as outgroups: *Chaetura spinicaudus* (Temminck)*, Collacalia esculenta* (Linnaeus), and *Streptoprocne rutila* (Vieillot). The Apodidae were chosen as the outgroups as they were host to the Thysanocercidae which are hypothesized to be closely related to the Trouessartiidae (Dabert & Mironov, 1999), and because they belong to an order of birds (Apodiformes) different from the order to which all but one of our trouessartiid hosts belong (Passeriformes, the exceptions being *Chrysococcyx lucidus* (Gmelin) from the Cuculiformes, *Veniliornis cassini* (Malherbe) and *Veniliornis nigriceps* (Orbigny) from the Piciformes). In instances where genetic information was unavailable for a particular host species, we used a closely related species as a proxy for host relationships. There were two such substitutions: *Climacteris rufus* Gould was used instead of *Climacteris melanurus* Gould and *Chaetura chapmani* Helimayr was used in place of *C. spinicaudus*. Two host birds were omitted from the phylogenetic analyses as genetic information was lacking for these species, and because our analyses included at least one other species within these genera: *Cyornis herioti* Ramsay and *Pitta brachyura* (Linnaeus).

All sequences were aligned independently using the ClustalW 2.0.12 algorithm (Larkin *et al*., 2007) via Mesquite v.2.71 (Maddison & Maddison, 2011) using default parameters. Data sets for the 97 avian taxa were subsequently corrected by eye based on sequence similarity among closely related species. To determine whether sequences could be concatenated, we

performed the incongruence length difference (ILD; Farris *et al*., 1994) test under equally weighted data sets. The ILD test was executed in PAUP v.4.0b10 (Swofford, 2002) on informative characters using simple taxon addition heuristic searches of 1,000 data repartitions with TBR branch-swapping. Pair-wise comparisons were made between all sequence combinations and the combined data set. We identified congruence between data sets when the test result was greater than 0.05 (i.e., accepting the null hypothesis of congruence).

As data sets proved to be congruent, we concatenated all four sequences into one data set. Maximum likelihood (ML) and Bayesian inference (BI) were used in tree construction to look for consistent patterns in phylogenetic relationships across different confidence measures (Alfaro *et al*., 2003). Aligned characters were treated as unordered with five possible states (four nucleotides and gap), with gaps identified as missing data (Simmons & Ochoterena, 2000). We tested for phylogenetic signal using the g1 test for skewness (Hillis & Huelsenbeck, 1992) in PAUP with random trees.

For ML and BI methods, we determined a model of DNA sequence evolution using the Akaike information criterion (AIC; Akaike, 1974) implemented in jModelTest 0.1.1 (Guindon & Gascuel, 2003; Posada, 2008). The most appropriate substitution model for the combined data set was GTR+I+Γ. Likelihood analyses were conducted in RAxML v. 7.2.7 (Stamatakis, 2006) using the CIPRES Science Gateway v. 3.1 (Miller *et al*., 2010). We ran the partitioned data sets under the GTRGAMMA model with parameters separately estimated for each partition. We estimated statistical support for branching patterns concurrently with the RAxML tree search using rapid bootstrapping of 1,000 replicates (Stamatakis *et al*., 2008). We performed partitioned BI analyses in MrBayes 3.2.1 (Ronquist *et al*., 2012) with default parameters to obtain posterior probabilities. To do this we ran two independent runs of four chains for 5,000,000 generations with sampling every 1,000 generations. Convergence was determined when the standard deviation of split frequencies reached a value less than 0.01, potential scale reduction factor values approximated 1 and effective sampling size greater than 200. We

removed the first 20% burn-in generations and obtained a 50% majority-rule consensus tree from the remaining topologies.

Species within the genus *Trouessartia* are related to each other by different degrees. Therefore, morphological measurements from these taxa may not be independent data points when analyzed by comparative tests (Harvey & Pagel, 1991; Garland *et al*., 1999). Using our fully resolved ML tree, we assessed the correlation of male and female continuous traits (Garland *et al*., 1992) using phylogenetically independent contrasts (Felsenstein, 1985) to create a set of comparisons that have been corrected for similarities from shared evolutionary history (Harvey & Pagel, 1991). Analyses of independent contrasts were performed in the PDAP plug-in (Midford *et al*., 2010) in Mesquite. We tested the assumptions of independent contrasts by examining the regression of absolute values for each character against the standard deviations for that character (Harvey & Pagel, 1991; Garland *et al*., 1992) to assure that the standardized contrasts were independent of their standard deviations (Garland *et al*., 1992). Independent contrasts were calculated for the morphological characters pertaining to male and female genitalia and those associated with our *a priori* hypotheses of sexually antagonistic attachment morphologies (characters 3, 7, 8, 9, 10, 11; Table 3-1). Finally, we mapped these morphological characters onto our Bayesian consensus tree using parsimony reconstruction with squared change assumption (Rogers, 1984). We used parsimony as our characters were continuous and we used the squared change assumption as our phylogeny included polytomies (Maddison & Maddison, 2011). For visualization, taxa in which only one sex was known were pruned from the trees.

3.4 Results

3.4.1 Measurements

Across taxa, female mites had significantly larger hysteronotal shields than males (Wilcoxon signed ranks: $Z = -5.55$, n = 53, $P < 0.001$). Females also had a greater proportion of their hysteronotal shields covered with ornamentation (Z

= - 6.27, n = 53, $P < 0.001$) and longer *h1* setae ($Z = - 4.9$, n = 53, $P < 0.001$) than males. The total length of the female external spermaduct (n = 68) ranged from absent (= 0 µm) to 99.71 µm while the length of the spermaduct extending caudad past the interlobar membrane was up to 54.25 µm (see Fig. 2 for exemplars). Females of 38 spp. of *Trouessartia* (out of 68) had external spermaducts past the interlobar membrane. Although there were far fewer non-*Trouessartia* spp. measured (females: n = 19), none possessed a spermaduct external to the body wall. In *Trouessartia* males (n = 66), the genitalic apparatus ranged from 251.48 to 2967.93 μm^2 and from 1% to 11% of the hysteronotal shield size. Adanal sucker area ranged from 50.82 to 338.63 μm^2.

Measurement error (mean ± standard deviation, coefficient of variation) as indicated by our repeated measurements revealed little variation. For adult females: hysteronotal shield area (31791μm^2 ± 767, CV = 0.02), ornamentation area (16079μm^2 ± 553, CV = 0.03) total external spermaduct length (33.06µm ± 2.27, CV = 0.07), and *h1* seta length (11.65µm ± 0.6, CV = 0.05). For adult males: hysteronotal shield area (32660μm^2 ± 617, CV = 0.02), aedeagus size (1134.72μm^2 ± 72.18, CV = 0.06) and adanal sucker size (201.16μm^2 ± 7.96, CV = 0.04).

3.4.2 Morphology of Trouessartia *spp. genitalia and observations*

Images produced by the light microscope revealed considerable variation in genitalic size among male mites (Figure 3-3a,b). The male genitalic organ in all taxa was located between the levels of trochanters III and IV. Curvature of the male genitalia was evident in most specimens, but the degree of this curvature depends upon the orientation of the individual on the slide. This lateral curvature was likely an artifact of the flattening of the specimen on the slide; in live mites the genitalic sclerites are likely symmetrically arranged within the male's genital compartment and curved upwards away from the midline of the body. The appearance and positions of the *c1* and *c2* setae in relation to the genitalia were taxon dependent. Observations of the male genitalia using SEM revealed several further aspects of this anatomy. The first is that the male genitalia appear to lie

behind an external hatch-like apparatus (Figure 3-3c,d). Furthermore, the genitalia seems to be eversible (Figure 3-3e,f) with the eversible component comprised of two halves; these genitalic sclerites are visible through the opening that lies in the center of this hatch-like apparatus.

External spermaducts in females were present in all 68 *Trouessartia* spp. representatives, while external spermaducts past the interlobar membrane were less common. These results are similar to Santana (1976) whose monograph of 71 species of *Trouessartia* documents external spermaducts extending to the margin of the interlobar membrane in approximately 90% of taxa. In the remaining taxa, Santana notes that the spermaduct ends within the interlobar membrane posterior to the supranal concavity.

3.4.3 Multivariate and correlation analyses

Figure 3-4 displays the results of the SSH-MDS ordination (stress = 0.114) including vectors for those characters that contributed significantly to the topology of the ordination. Members of the *Trouessartia* grouped apart from the outgroup genera (both *Thysanocercus* and those within the Trouessartiidae). The total number of birds from which both sexes of *Trouessartia* spp. were retrieved was 53, while there were six birds from which both sexes of outgroup taxa were collected. Based on arrangement of the significant vectors in three-dimensional space (Figure 3-4a), male and female hysteronotal shield size were positively correlated, as were length of the female external spermaduct past the interlobar membrane and male genitalic size. In contrast, the degree of dorsal ornamentation in males was not strongly associated with any of the other vectors, which supported our observations that females were commonly more ornamented than males.

To examine the directions and strength of these morphological relationships independently, we used correlation analyses. The majority of our measured morphologies for both males and females were non-normal. Log_{10} transformation of our *Trouessartia* spp. morphological data failed to achieve normality for female ornamentation area (Shapiro-Wilk: $P = 0.03$), female *h1*

area ($P = 0.08$) and female *h1* length ($P = 0.037$). We analyzed both transformed and untransformed data to determine whether differences existed between the two sets of analyses. As the majority of correlations were performed with at least one data set which was non-normally distributed, we used the Spearman's correlation coefficient for both the untransformed and $\log_{10}$ transformed data. Hysteronotal shield size was strongly correlated for males and females both in the untransformed ($r_s = 0.65$, n = 53, $P < 0.001$) and $\log_{10}$ transformed analyses ($r_s = 0.66$, n = 53, $P < 0.001$) indicating that larger males tended to be associated with larger females (Figure 3-5a). For both untransformed and $\log_{10}$ transformed data, male genitalia size was highly correlated with the total length of the female external spermaduct ($r_s = 0.38$, n = 53, $P = 0.005$) (Figure 3-5b) as well as the length of the spermaduct extending past the interlobar membrane ($r_s > 0.59$, n = 29, $P < 0.003$) (Figure 3-5c). While the size of the male's genitalia expressed as a proportion of the male's hysteronotal shield did not correlate with the total length of the female external spermaduct ($r_s = 0.24$, n = 53, $P = 0.14$) and the spermaduct post membrane ($r_s = 0.55$, n = 29, $P = 0.28$) in the untransformed data, it did correlate with these characters in the $\log_{10}$ transformed data (total external spermaduct: $r_s = 0.35$, n = 29, $P = 0.01$; spermaduct post membrane: $r_s = 0.57$, n = 29, $P = 0.001$). In contrast, male sucker area was not correlated with female ornamentation area in either data set ($r_s > 0.19$, n = 53, $P > 0.12$); nor did male sucker area correlate with measures of the *h1* setae in either data set for both *h1* area ($r_s = -0.51$, n = 53; $P = 0.72$), and length ($r_s = -0.82$, n = 53, $P = 0.56$).

3.4.4 Phylogenetic analyses of host birds

We found CO1 sequences for 50 avian hosts (1551 bp aligned, including gaps), Cytb sequences for 86 taxa (1143 bp aligned, including gaps), ND2 sequences for 70 taxa (1041 bp aligned, including gaps), and RAG-1 sequences for 41 taxa (2872 bp aligned, including gaps). As results of the ILD test implied congruence between all pair-wise comparisons ($P > 0.17$) and congruence in the concatenated data ($P = 0.9$), we concatenated our alignments. Moreover,

independent likelihood topologies had similar structure with less resolution. The concatenated data set was 6607 bp with 2683 parsimony informative, 3657 constant and 267 autapomorphic sites and contained significant phylogenetic signal (g1 = - 0.45, $P < 0.01$).

Both ML and BI yielded similar phylogenies with the Bayesian phylogeny generally indicating higher branch support values (see Appendix 3-5 for the best ML tree with branch lengths). The Bayesian analysis resulted in an effective sampling size of 1340. After collapsing branches with less than 70% ML bootstrap support and < 0.9 BI posterior probabilities, there were no instances in which the best resolved tree through ML methods contradicted the Bayesian consensus tree. In most cases, differences were evident where Bayesian posterior probabilities indicated higher branch support (see Alfero *et al*., 2003), or either Bayesian or ML bootstrapping provided support where the other did not. As trees were in strong agreement between methods, we used our 50% majority rule Bayesian tree with ML bootstrap support mapped onto the top of each relevant branch and with posterior probabilities indicated below (Figure 3-6).

3.4.5 Independent Contrasts and Correlated Evolution

As neither male nor female morphologies correlated with their standard deviations, we determined that our untransformed data met the assumptions of phylogenetically independent contrasts (Garland *et al*., 1992). Independent contrast analyses returned significant correlations between male genitalia size and total length of the female external spermaduct ($t_{55} = 4.43$, $P < 0.001$), and male genitalia size and length of the female external spermaduct extending past the interlobar membrane ($t_{55} = 2.38$, $P = 0.02$) (Figure 3-7). Male adanal sucker area and the degree of female ornamentation were not significantly correlated ($t_{55} = -1.7$, $P = 0.09$), nor were sucker size and female *h1* area and length ($t_{55} =$ 0.53 – 1.0, $P > 0.32$).

Figure 3-8a shows relationships between male aedeagus size and female total external spermaduct length mapped onto the host phylogeny. Spermaducts

extending past the supranal concavity were present in all *Trouessartia* spp., with spermaducts past the interlobar membrane occurring several times independently. Similar results from the Spearman's analyses and independent contrasts show that spermaduct size was positively correlated with the size of the male's aedeagus. In contrast, male sucker size was not correlated with the degree of dorsal ornamentation (Fig. 8b) or *h1* dimensions in females.

3.5 Discussion

3.5.1 Correlations between male and female genitalia

Genitalic morphologies, particularly those of males, have long been used in species identification due to their tendency to diverge rapidly (Eberhard, 1985) while their role as indicators of antagonistic correlated evolution has been recently discussed in the literature (Eberhard, 2004; Rönn *et al*., 2007; Eberhard *et al*., 2010 a,b). Male genitalia are often highly variable while females illustrate relatively little variation in genitalic form across closely related taxa (Eberhard, 1985). However, in *Trouessartia* spp. feather mites, females demonstrate considerable variation across taxa in the elongation of the external spermaduct. If the extension of the female's spermaduct is involved in a correlated evolution of genitalic traits, then the elongation of the spermaduct should correlate with male genitalic measures. In accordance with this hypothesis, we found that male genitalic size was positively correlated with the length of the female external spermaduct. Moreover independent contrasts indicated that these structures have coevolved (assuming that mite phylogeny parallels that of the host birds). However, in both correlation and independent contrast analyses, the R^2 values were low, indicating that much variation in genitalic size is unexplained. Size measurements are not the only way to detect antagonistic coevolution. Many studies evaluating genetalic correlations have used indices of complexity (Tatarnic & Cassis, 2010) or specific morphologies associated with genitalic harm and traumatic insemination (e.g., male genitalic spines: Rönn *et al.,* 2007; Kamimura, 2012). On a finer scale, multiple modifications in structural components of the genitalia may be coevolving in feather mites. We did not

measure these components of the male genitalia independently. Additional measurements of the male genital organ (e.g., genital discs, pregenital apodeme) might lend further explanation to these patterns.

External copulatory tubes have been reported in numerous astigmatan mite taxa including in some non-feather mite taxa Chaetodactylidae (e.g., *Chaetodactylus osmiae* (Dufour); Klimov & OConnor, 2008) and Rosensteiniidae (OConnor & Reisen, 1978); and in the feather mite families Caudiferidae (Gaud & Atyeo, 1996), Crypturoptidae (Gaud *et al*., 1973), Eustathiidae (Peterson *et al*., 1980), as well as several genera within the Pterolichidae (Atyeo, 1992) and Thoracosathesidae (OConnor, 2009). Klimov and Sidorchuk (2011) suggest that these structures have evolved through antagonistic interactions between the sexes and may act as barriers to unfavourable males. If this were a directional process still visible in the phylogeny of the Trouessartiidae (see below for caveat), female spermaducts would be shortest in the basal regions of the tree and would be longer in more recently derived taxa. In line with our hypothesis, many of the *Trouessartia* spp. in the basal region of the tree had relatively short external spermaducts, while *Trouessartia* spp. in more derived regions had moderate to long external spermaducts, with the longest external spermaducts from hosts in the Emberizidae, Fringillidae and Turdidae. Contrary to our predictions, the *Trouessartia* spp. female with the longest external spermaduct was sampled from *Climacteris melanurus* Gould near to the outgroup taxa. Although a clear progression in our phylogeny from short to long external spermaducts was not observed, this may have been affected by the lack of resolution throughout most of the tree. Although Fulton and Strobeck (2006) found that missing data did not greatly influence the topology of supertree and supermatrix analyses, this relies on having sufficient informative characters (Wiens, 2003, 2006). Adding genetic sequences for those host taxa with only one or two of four sequences might result in higher support values and lend greater support to the phylogeny. However, the resolved relationships among host birds are in line with several studies of avian phylogenetics (see Sheldon *et al*., 2005; Pasquet *et al*., 2007;

Johannson *et al*., 2008; Lovette *et al*., 2010). Moreover, though *Trouessartia* spp. are believed to primarily be monoxenous (Santana, 1976; Lombert, 1988), on the host families Corvidae and Sturnidae, mite species are known to occur on up to three different related host species (Santana, 1976); thus a phylogeny based on mite DNA would be preferred. Our hypothesis also relies on the idea that the direction of selection is 'frozen' phylogenetically such that earlier derivative taxa will show a state of genitalic evolution that occurs early in the coevolutionary process, and that more recently derived taxa show later stages. If the process is rapid, however, then it could easily take place within the lifespan of a species irrespective of its location on a tree, and there would be no reason to expect to see the pattern mirrored phylogenetically in the states of male and female genitalia across many species. Nevertheless, some studies (e.g., Tatarnic & Cassis, 2010) have found evidence of an evolutionary progression.

Although our phylogenetic studies do not support antagonistic coevolution as a driving force in the evolution of male and female genitalia in *Trouessartia* spp., the female's external spermaduct may still actively contribute to mate choice and sexual selection. Klimov and Sidorchuck (2011) suggest that the extended copulatory tubes in some feather mites may demonstrate precopulatory female choice. In feather mites, males often engage in precopulatory guarding of female nymphs (Witalińsky *et al*., 1992) and presumably mate with the newly moulted adult female upon eclosion, which would appear to minimize the female's ability to select among potential mates. Precopulatory guarding of the female tritonymph by adult males seems to be absent in the *Trouessartia* (B. OConnor pers. comm., and HP pers. obvs.), which may indicate that females utilize the external spermaduct to discourage precopulatory guarding (Klimov & Sidorchuk, 2011). Furthermore, if the external spermaduct allows for females to take more control over fertilization through insertion into the male genitalia, the external spermaduct may be used as a means to select among males (Klimov & Sidorchuk, 2011).

3.5.2 Non-genitalic contact structures

The lack of correlation between the male adanal suckers and the degree of female ornamentation and *h1* seta dimensions opposed what we expected for morphologies evolving under antagonistic coevolution. Although numerous studies report a lack of correlation in contact devices (e.g. symphypleone collembolans in Eberhard, 2004; Eberhard, 1985) variations in size and density of the dorsal lacunae of female *Trouessartia* spp. are reminiscent of the dorsal macropunctures present on females of some dytiscid water beetles (Bergsten & Miller, 2007). Contrary to the correlated relationships between sucker adaptations and female dorsal morphology in dytiscid beetles, in *Trouessartia* spp., the variations in male sucker size did not match the extent to which females varied in dorsal ornamentation. These dorsal lacunae may be "cooperative" instead of "antagonistic" (Eberhard, 2004), however, we believe these structures to be cooperative. First, the grooves and pits listed as cooperative in Eberhard (2004) allow for insertion or attachment into the groove by the male grasping structure. In *Trouessartia* spp., the adanal suckers have an area significantly greater than the area of the largest dorsal lacunae of the female. Second, as the adanal suckers attach through negative pressure (Witalińsky *et al*., 1992), it seems logical that these dorsal lacunae would serve not to encourage but rather to disrupt attachment as is seen in diving beetles (Bergsten & Miller, 2007). Moreover, if dorsal lacunae are cooperative, we would still expect to see a correlation between these grasping structures (McPeek *et al*., 2009).

A lack of correlation between adanal sucker area and the dimensions of the female's *h1* setae is further indication that these contact structures may not affect successful coupling; though they may influence copulation in a way that was not measureable in our study. Eberhard (2004) suggests that resistance structures which can be employed facultatively (e.g., mobile structures such as erectable spines) work best in antagonistic interactions. The anatomy of the *h1* setae in *Trouessartia* spp. is currently unclear; however, it is possible that the female's musculature allow for these setae to become erect thus impeding

coupling. Further studies evaluating the musculature of these mites are required before the role of these setae in antagonistic interactions can be asserted. Though clearing in lactic acid removes the visibility of soft tissues, examining uncleared specimens would provide additional information as to the mechanisms of these structures. Additionally, glands may play a role in copulation which we did not analyze. Phoretic deutonymphs in many uropodid mites employ glandular secretions to attach to their host (Bajerlein & Witalínski, 2012), and Fain *et al*. (1984) suggest that glandular secretions may aid in male-female coupling in fur mites (Astigmata: Chirodiscidae). If glandular secretions are employed by adult male *Trouessartia* to assist in affixing to females, then the elaborations in dorsal ornamentation in females may evolve in response to these glandular secretions rather than to surface area of suckers.

3.5.3 Future considerations

Although there have been suggestions as to the ways in which male and female genitalia in *Trouessartia* interact (Santana, 1976; OConnor, 2009), it is still unclear as to whether the male receives the female's spermaduct in his genital opening. In the Crypturoptidae, the male's aedeagus has moved between the first set of coxae, and is believed to receive the female's external spermaduct (Gaud & Atyeo, 1996). Although this movement of the male's genitalic opening is not evident in *Trouessartia* spp., the male's genitalia may still receive the female's spermaduct. In our study we found one pair of adults in copula. Through SEM examination of this couple it appears that the male's genital organ clasps the female's external spermaduct between its two halves. This bipartition of the male genitalia was also evident in several other males we examined by SEM, though whether or not the external spermaduct fits within a groove in the male's aedeagus (OConnor, 2009) requires further examination. To fully understand the relationship between the female spermaduct and male aedeagus in *Trouessartia* spp., it would be ideal to observe live mites and their mating. This, however, is difficult as feather mites require their hosts to complete their life cycle (Clayton & Walther, 1997). Increased sampling may result in the collection of additional

mating pairs. Similarly, studies evaluating male genitalic structure in depth would further elucidate how the male aedeagus interacts with the female spermaduct.

Several components of our study will require additional research before they can be incorporated. First, we used host phylogeny as a proxy for mite phylogeny as there currently is not a published phylogeny for *Trouessartia* spp. By using host phylogeny, we assume that these two groups share similar phylogenetic patterns and that cospeciation of mites and their host birds has driven their evolution (Gaud & Atyeo, 1979). Furthermore, many of the taxa in our study are new species; as such, we were unable to identify them using the available keys. Although feather mites are known to be fairly species specific (Dabert & Mironov, 1999), Santana (1976) has documented more than one species of *Trouessartia* from a single bird species. In this regard, it is possible that we treated male and female mites from a single host as conspecifics when they were not. To improve the certainty of our results, these species must first be described.

As a final consideration, there were several instances where contamination may have occurred due to these collection methods. For birds mist-netted in the field, cross-contamination of symbionts between hosts may have occurred if the same tools were used for measuring bird morphometrics. For birds stored in drawers at the Australian Museums, there may have been cross-contamination if birds were moved or reorganized, or if the container had been reused from a previous preservation. These potential contaminations emphasize the necessity for symbiont identification and published descriptions.

3.5.4 Conclusion

In *Trouessartia* spp., the length of the female external spermaduct correlates with the overall size of the male's aedeagus. Although females with longer external spermaducts tend to be paired with males of comparatively large genitalia, we did not discover directional selection on genitalic size as represented in our phylogenetic analyses. As mapped on host phylogeny, it

appears that throughout the genus *Trouessartia*, females have evolved longer external spermaducts independently several times. Whether this elongation of the external spermaduct plays a role in sexually antagonistic coevolution is unclear. However, our analyses of female ornamentation and *h1* seta size did not reveal any evidence for correlated evolution of these traits with the surface area of male adanal suckers. To better determine the role of these morphologies in sexual interactions, there must be further investigations into the mating behaviours of these mites, as well as detailed evaluations of these morphologies. We suggest that *Trouessartia* is an ideal group to study coevolution due to the diversity of female structures and their potentially antagonistic associations with male morphologies.

3.6 Acknowledgments

Thanks to Sarah Bush (University of Utah) for sending the mites from China and the Philippines, Terry Galloway (University of Manitoba) for those from Manitoba, and both Sofia Fernandéz (University Complutense of Madrid) and Ismael Galván (Centre National de la Recherche Scientifique, Paris) for mites from Spain. We also extend our thanks to J. Newton and B. Doty for their assistance with this manuscript. Bodies of birds from Alberta were held under Alberta Sustainable Resource Development and Canadian Wildlife Service permits to HP. This research was supported by a Natural Sciences and Engineering Research Council of Canada Discovery Grant to HP.

Table 3-1. Male and female morphological characters measured and analyzed using multivariate and correlation analysis

Number	Female Character	Male Character
1	Hysteronotal area (μm^2)	Hysteronotal area (μm^2)
2	Ornamentation area (μm^2)	Ornamentation area (μm^2)
3	Proportion of hysteronotal shield with ornamentation	Proportion of hysteronotal shield with ornamentation
4	Average size of lacunae on longitudinal axis of the hysteronotal shield (μm^2)	Average size of lacunae on longitudinal axis of the hysteronotal shield (μm^2)
5	Average size of lacunae on lateral axis of the hysteronotal shield (μm^2)	Average size of lacunae on lateral axis of the hysteronotal shield (μm^2)
6	Average size of lacunae (μm^2)	Average size of lacunae (μm^2)
7	Size of *h1* setae (μm^2)	Size of *h1* setae (μm^2)
8	Length of *h1* setae (μm)	Length of *h1* setae (μm)
9	Length of total female external spermaduct (μm)	Size of male adanal sucker (μm^2)
10	Length of the total female external spermaduct (μm)	Size of male genitalia (μm^2)
11	Length of the posterior interlobar membrane (μm)	Proportion of the male's body devoted to the genitalia

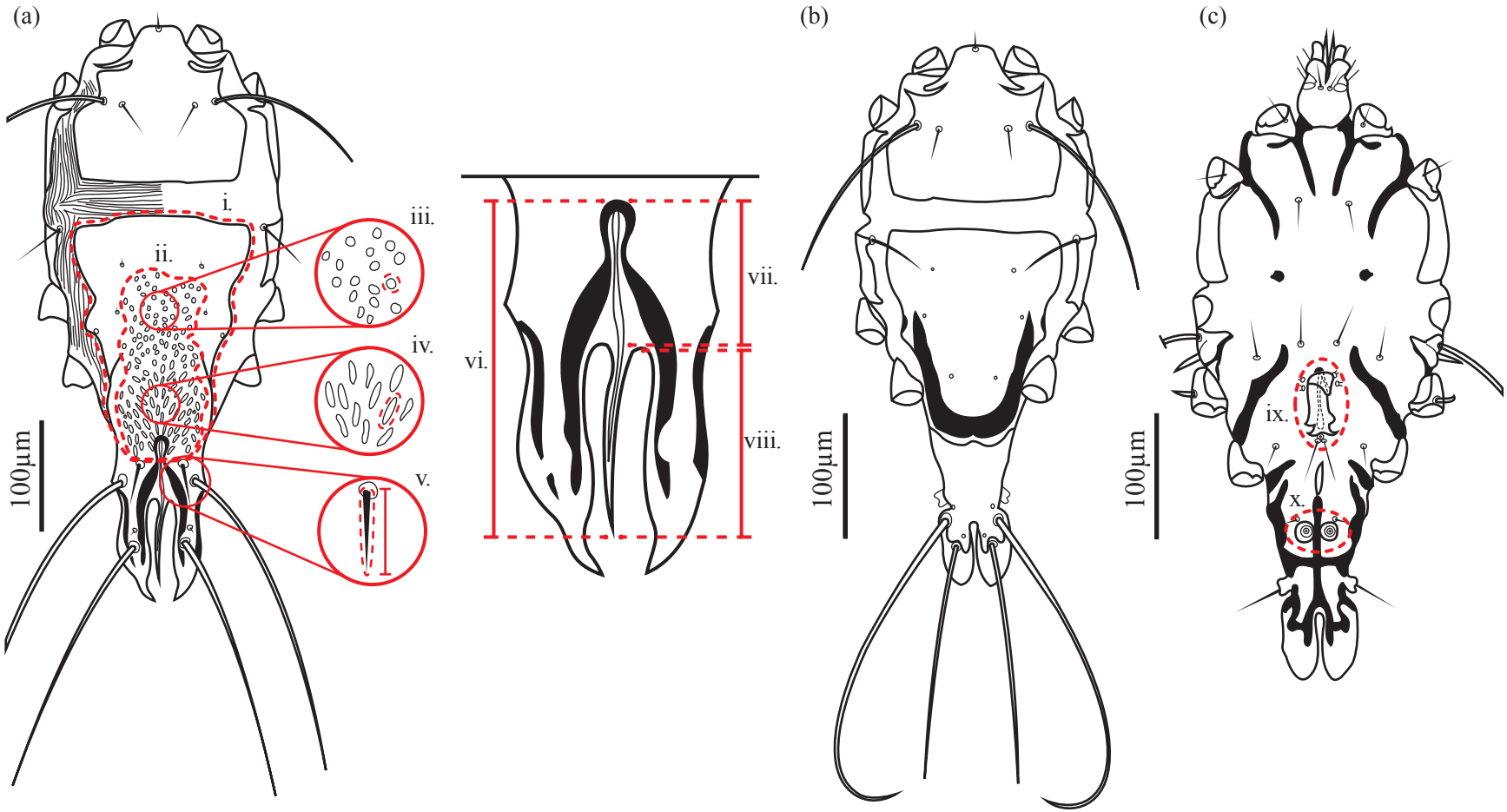

(a)
i.
ii.
iii.
iv.
v.
100µm
vi.
vii.
viii.
(b)
100µm
(c)
ix.
x.
100µm

Figure 3-1. (previous page) Feather mite body measurements (μm) for adult female (a) and adult male dorsal (b) and ventral (c) *Trouessartia* spp. Both males and females were measured for the area of the hysteronotal shield (i) and area of the hysteronotal shield containing ornamentation (ii). The average size of lacunae along the longitudinal axis (iii) and lateral axis (iv) was measured, as well as the average measurement for these two sets of lacunae combined. Males and females were measured for the length and area of the dorsal *h1* setae (v). For females, we measured the total length of the female external spermaduct (vi), the length of the interlobar membrane (vii) and the length of the spermaduct extending past the posterior interlobar membrane (viii). For males, we measured the area of the aedeagus (ix) and average area of the ventral adanal suckers (x). Illustration drawn after *Trouessartia geospiza* OConnor, Foufopoulos & Lipton (OConnor *et al*., 2005).

(a)
10 µm
(b)
10 µm
(c)
10 µm

Figure 3-2. (previous page) Scanning electron micrographs taken of the dorsal sides of female *Trouessartia* spp. obtained from these avian hosts (a) *Progne subis* (Linnaeus) (b) *Pyrrhula leucogenis* Ogilvie-Grant and (c) *Hirundo rustica* Linnaeus. External spermaducts are indicated with an arrow.

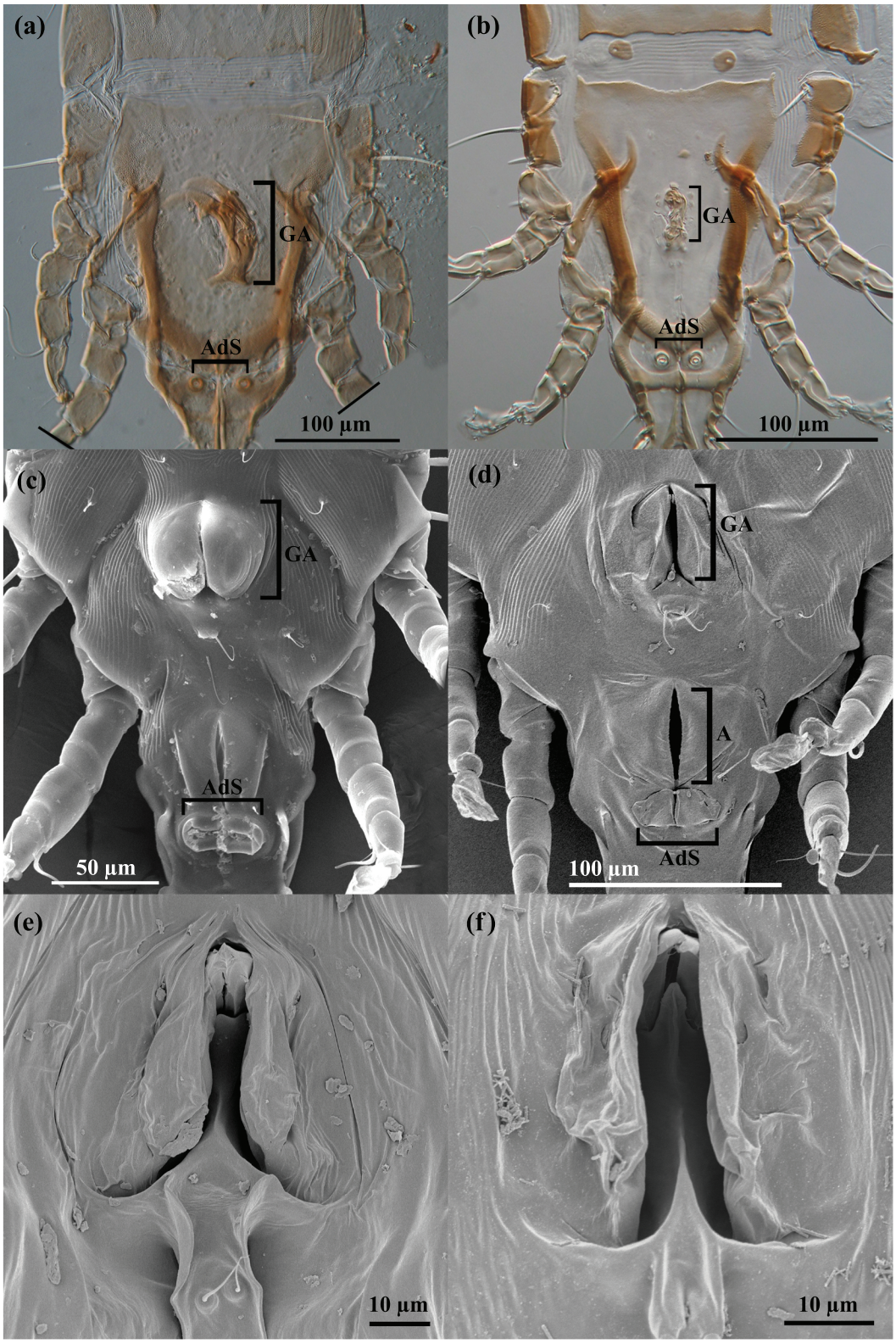
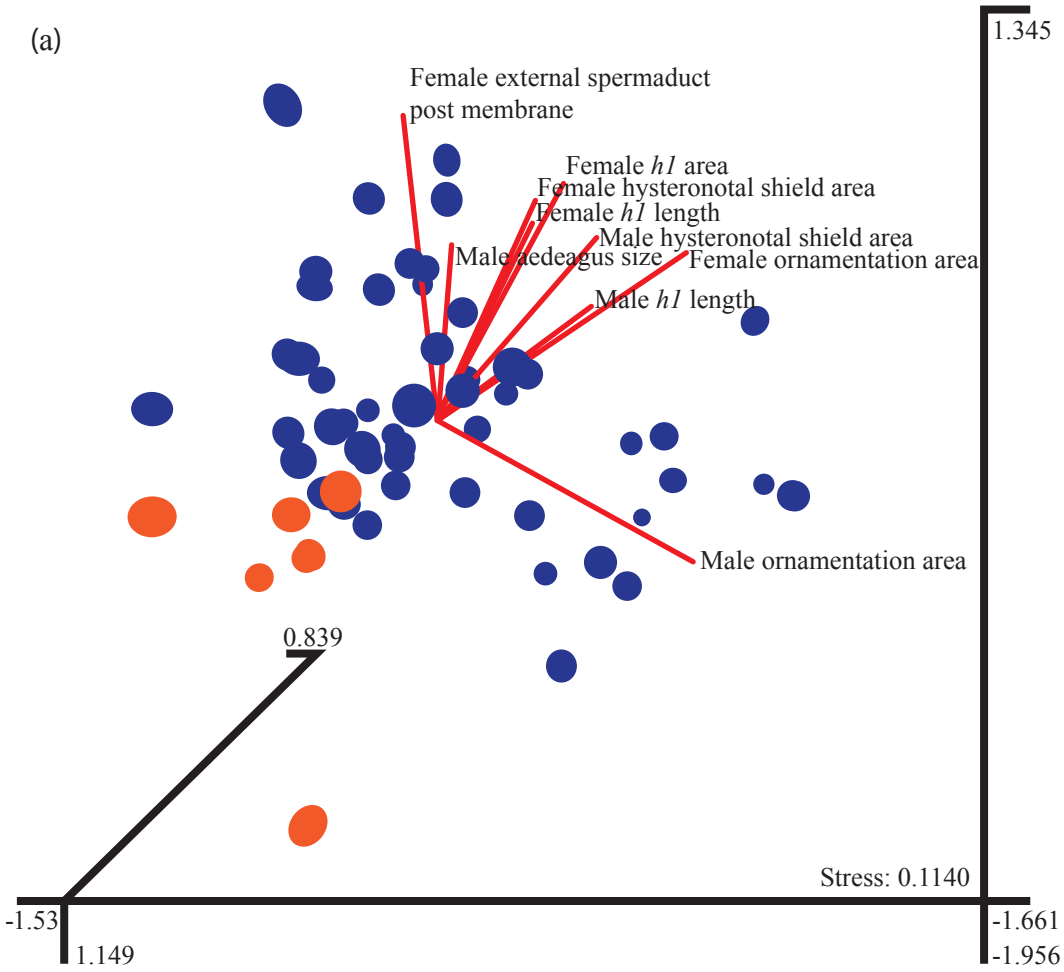

(a)
GA
AdS
100 µm
(b)
GA
AdS
100 µm
(c)
GA
AdS
50 µm
(d)
GA
A
AdS
100 µm
(e)
10 µm
(f)
10 µm

Figure 3-3. (previous page) Light microscopy images (a,b) and scanning electron mircrographs (SEM) taken of the male genital apparatus (GA), anus (A), and adanal suckers (AdS). Light microscope images are taken of males from the hosts (a) *Sialia sialis* (Linnaeus) and (b) *Stachyridopsis ruficeps* (Blyth) while SEM images are taken of mites removed from (c) *Cyornis herioti* Ramsay (d) *Pyrrhula leucogenis* Ogilvie-Grant (e) *Dicrurus balicassius* (Linnaeus) and (f) *Turdus merula* (Linnaeus). In figures (c) and (d) the male genital apparatus appears to be enclosed behind a hatch-like cover, while in figures (e) and (f) the genital sclerites are visible through the genital opening.



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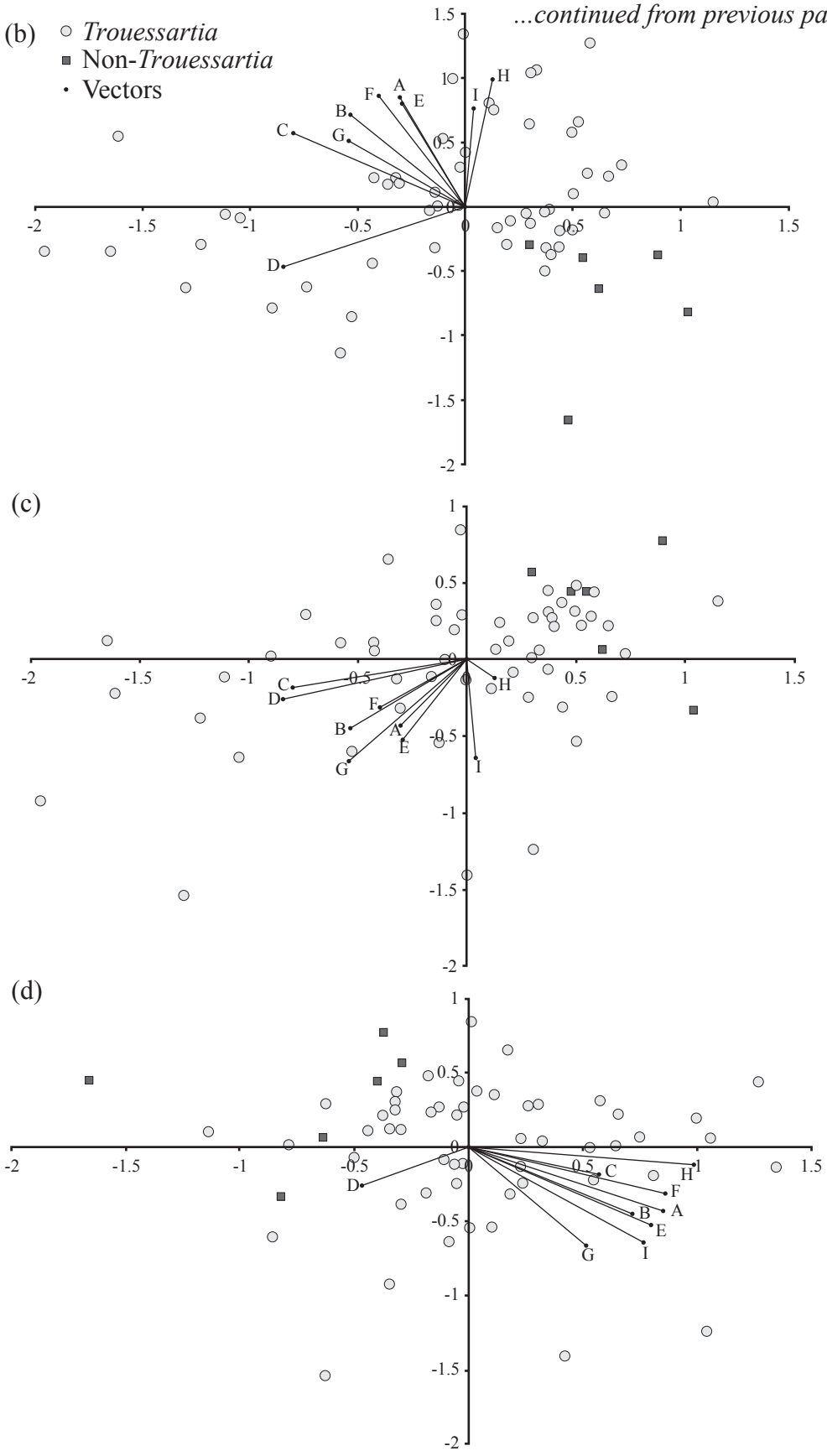
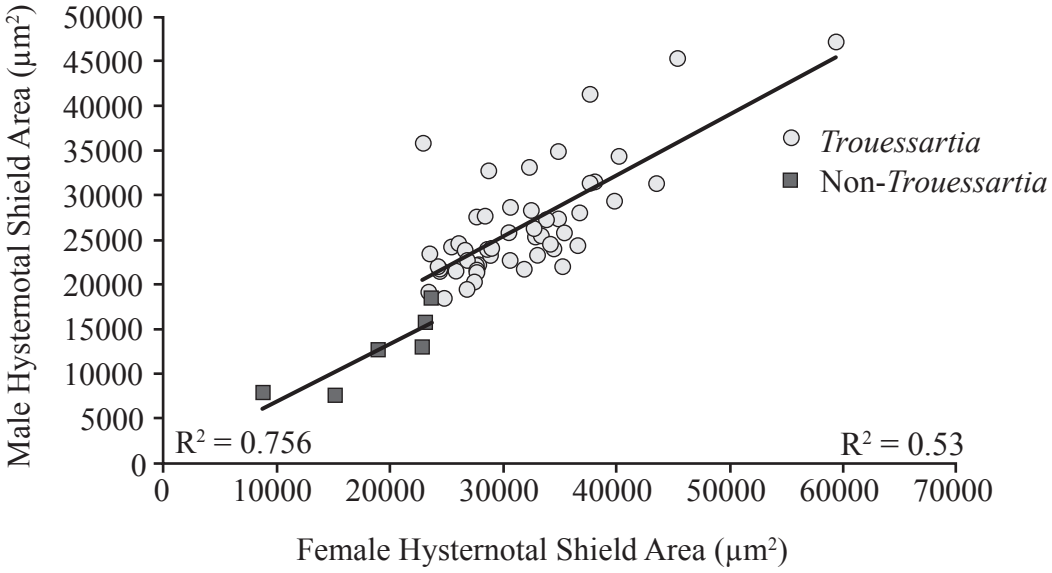
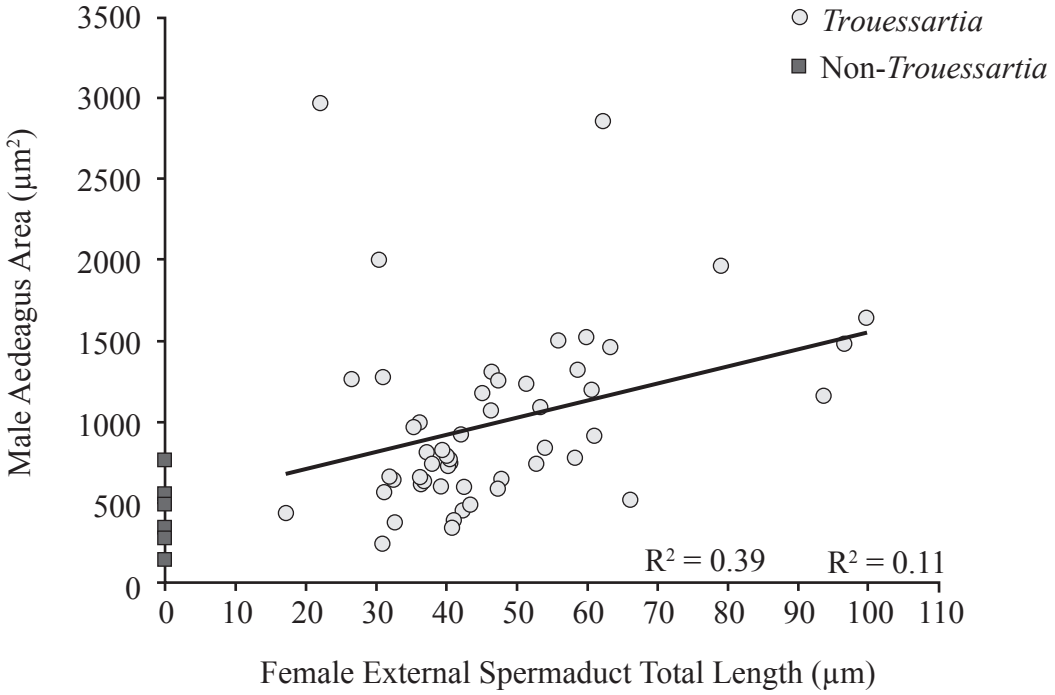

(b)
Trouessartia
Non-Trouessartia
Vectors
...continued from previous page
A
B
C
D
E
F
G
H
I
(c)
(d)

Figure 3-4. (previous page) SSH-MDS ordination of male and female morphological characters. A three-dimensional ordination is displayed in (a). Species within the genus *Trouessartia* are indicated in blue, while outgroup taxa (*Thysanocercus* spp. and non-*Trouessartia* spp. within the Trouessartiidae) are depicted in orange. Two- dimensional representations of these ordinations are shown as (b) axes 1 and 2, (c) axes 1 and 3, (d) axes 2 and 3. Plotted vectors were those that significantly contributed to the topology of the ordination at $P < 0.05$. Lettering for each vector is as follows: A - female hysteronotal area; B - male hysteronotal area; C - female ornamentation area; D - male ornamentation area; E - female *h1* length; F - female *h1* area; G - male *h1* length; H - female external spermaduct length posterior to the interlobar membrane; I - male aedeagus size.

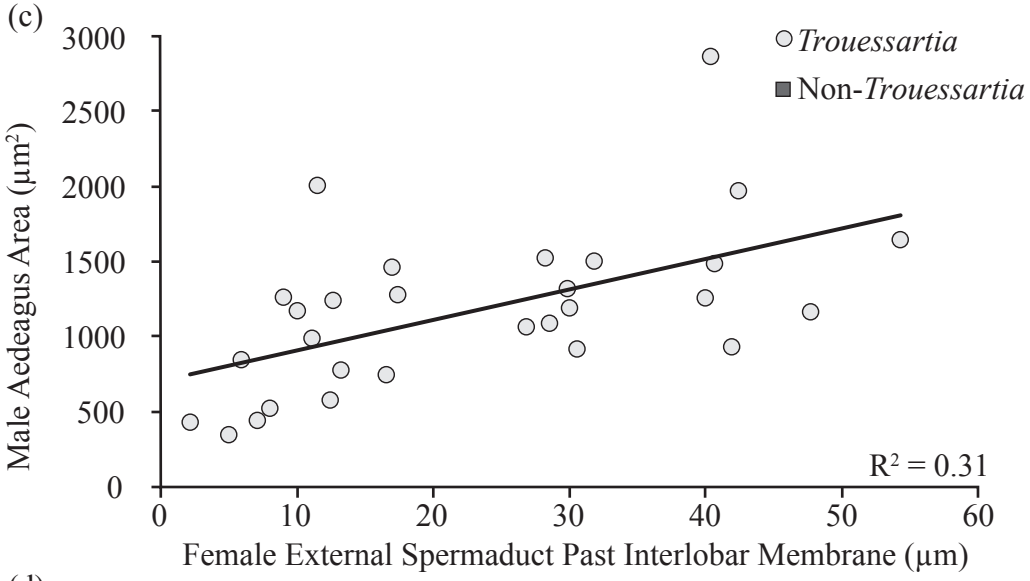
(a)


Male Hysternotal Shield Area (µm²)
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
0
10000
20000
30000
40000
50000
60000
70000
Female Hysternotal Shield Area (µm²)
Trouessartia
Non-Trouessartia
R² = 0.756
R² = 0.53

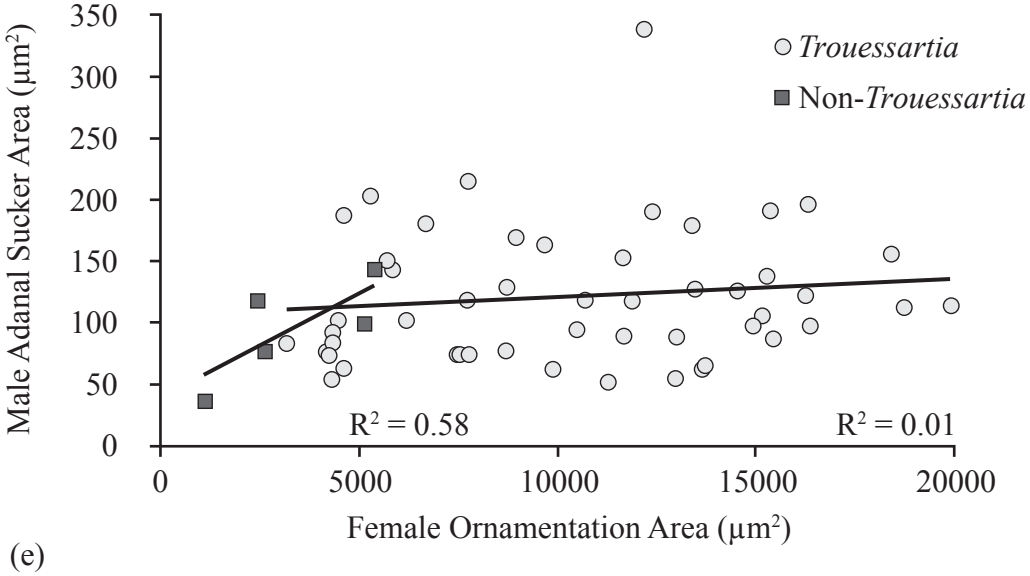
(b)


Male Aedeagus Area (µm²)
3500
3000
2500
2000
1500
1000
500
0
0
10
20
30
40
50
60
70
80
90
100
110
Female External Spermaduct Total Length (µm)
Trouessartia
Non-Trouessartia
R² = 0.39
R² = 0.11

(c)


Trouessartia
Non-Trouessartia
Male Aedeagus Area (µm²)
3000
2500
2000
1500
1000
500
0
0
10
20
30
40
50
60
R² = 0.31
Female External Spermaduct Past Interlobar Membrane (µm)

(d)


Trouessartia
Non-Trouessartia
Male Adanal Sucker Area (µm²)
350
300
250
200
150
100
50
0
0
5000
10000
15000
20000
R² = 0.58
R² = 0.01
Female Ornamentation Area (µm²)

(e)

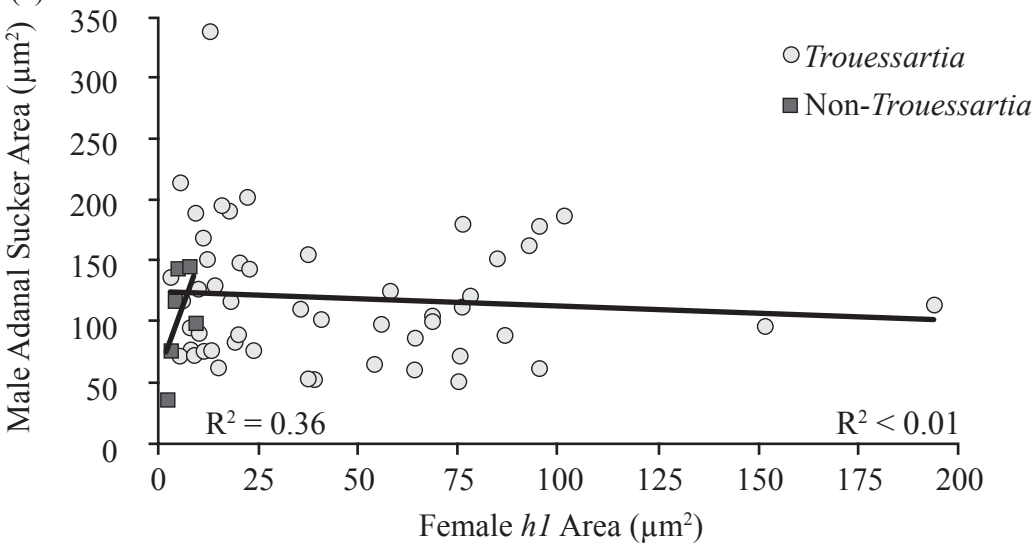

Trouessartia
Non-Trouessartia
Male Adanal Sucker Area (µm²)
350
300
250
200
150
100
50
0
0
25
50
75
100
125
150
175
200
R² = 0.36
R² < 0.01
Female h1 Area (µm²)

Figure 3-5. (previous page) Correlations between untransformed morphological characters in *Trouessartia* spp. (indicated as circles) and non-*Trouessartia* spp. outgroups (indicated as squares). Significance of correlations for *Trouessartia* spp.: (a) $r_s = 0.65$, n = 53, $P < 0.001$*; (b) $r_s = 0.38$, n = 53, $P = 0.005$*; (c) $r_s > 0.59$, n = 29, $P < 0.003$*); (d) $r_s = 0.22$, n = 53, $P = 0.12$; (e) $r_s = -0.51$, n = 53; $P = 0.72$. See results for further description of correlation analyses.

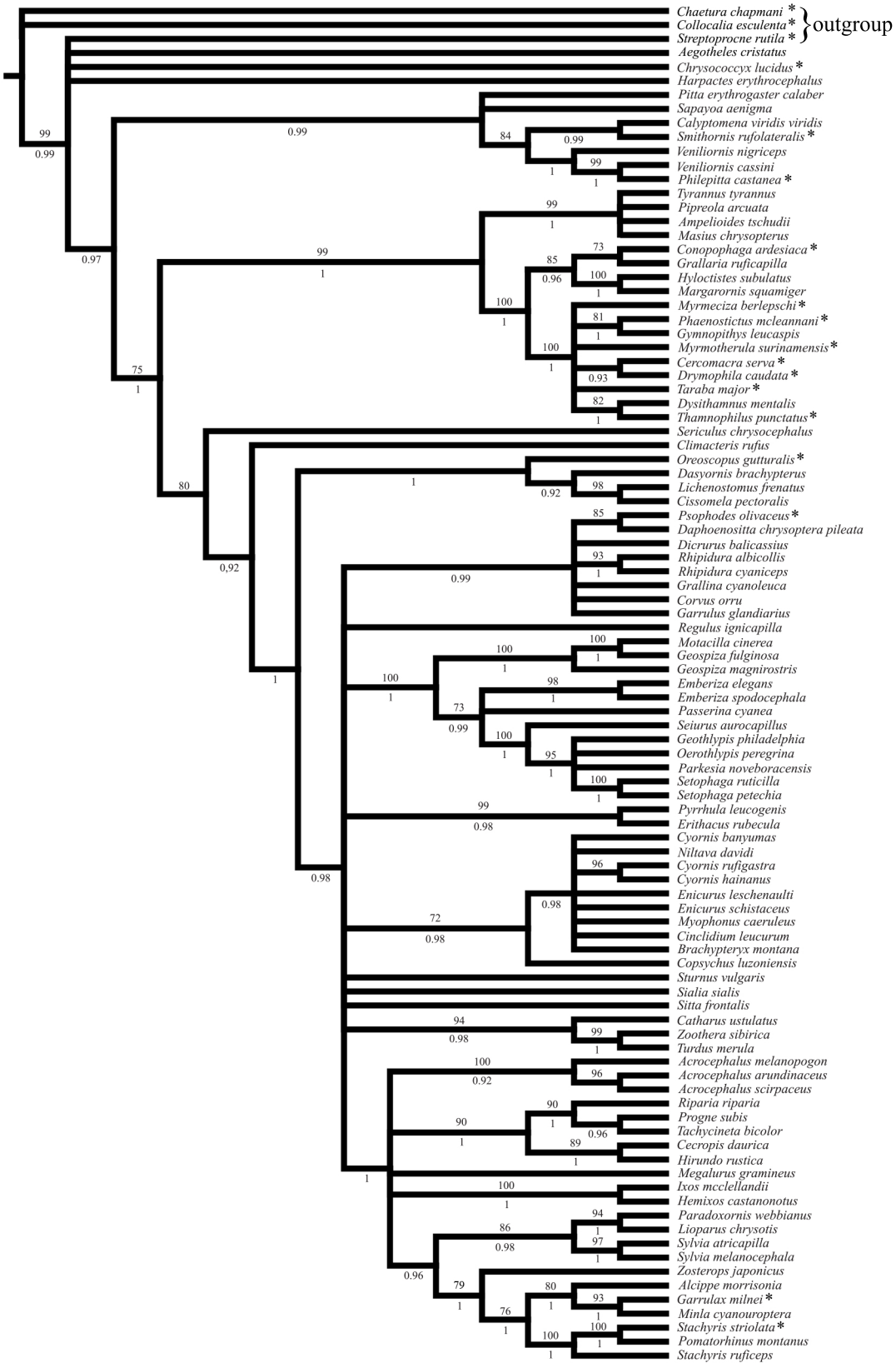
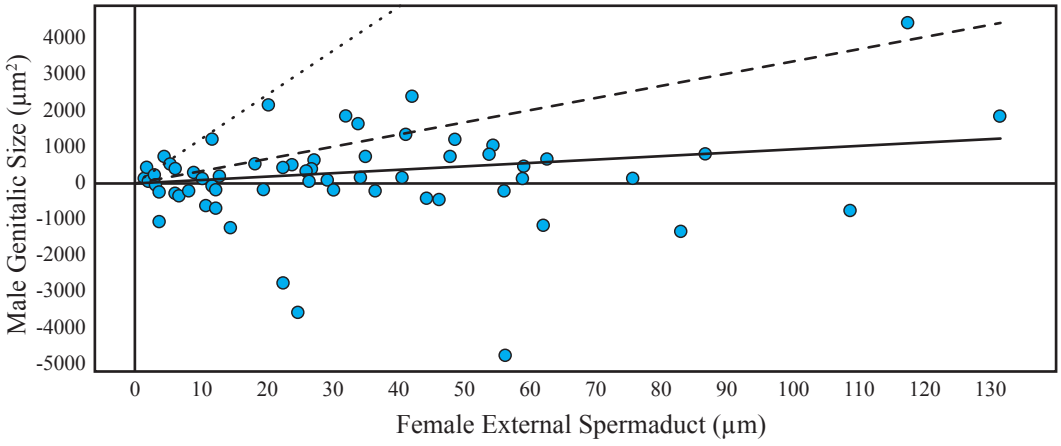
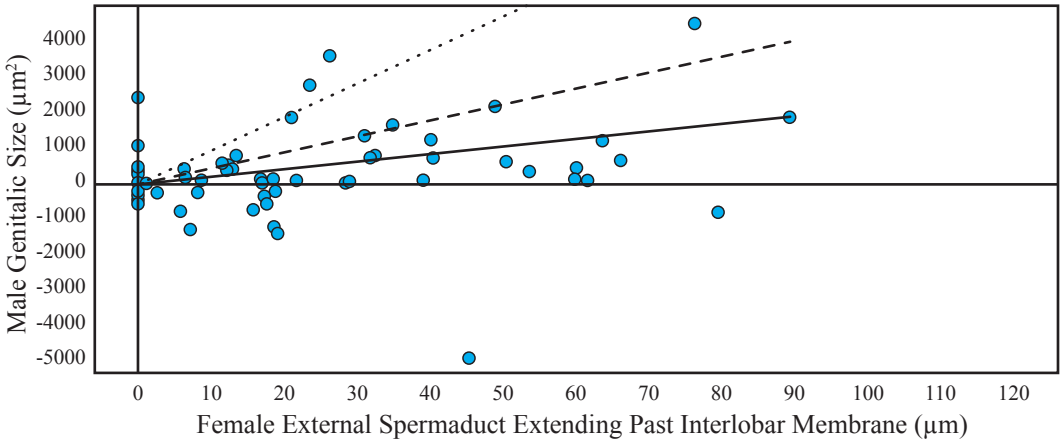

Chaetura chapmani *
Collocalia esculenta *
Streptoprocne rutila *
outgroup
Aegotheles cristatus
Chrysococcyx lucidus *
Harpactes erythrocephalus
Pitta erythrogaster calaber
Sapayoa aenigma
Calyptomena viridis viridis
Smithornis rufolateralis *
Veniliornis nigriceps
Veniliornis cassini
Philepitta castanea *
Tyrannus tyrannus
Pipreola arcuata
Ampelioides tschudii
Masius chrysopterus
Conopophaga ardesiaca *
Grallaria ruficapilla
Hyloctistes subulatus
Margarornis squamiger
Myrmeciza berlepschi *
Phaenostictus mcleannani *
Gymnopithys leucaspis
Myrmotherula surinamensis *
Cercomacra serva *
Drymophila caudata *
Taraba major *
Dysithamnus mentalis
Thamnophilus punctatus *
Sericulus chrysocephalus
Climacteris rufus
Oreoscopus gutturalis *
Dasyornis brachypterus
Lichenostomus frenatus
Cissomela pectoralis
Psophodes olivaceus *
Daphoenositta chrysoptera pileata
Dicrurus balicassius
Rhipidura albicollis
Rhipidura cyaniceps
Grallina cyanoleuca
Corvus orru
Garrulus glandiarius
Regulus ignicapilla
Motacilla cinerea
Geospiza fulginosa
Geospiza magnirostris
Emberiza elegans
Emberiza spodocephala
Passerina cyanea
Seiurus aurocapillus
Geothlypis philadelphia
Oerothlypis peregrina
Parkesia noveboracensis
Setophaga ruticilla
Setophaga petechia
Pyrrhula leucogenis
Erithacus rubecula
Cyornis banyumas
Niltava davidi
Cyornis rufigastra
Cyornis hainanus
Enicurus leschenaulti
Enicurus schistaceus
Myophonus caeruleus
Cinclidium leucurum
Brachypteryx montana
Copsychus luzoniensis
Sturnus vulgaris
Sialia sialis
Sitta frontalis
Catharus ustulatus
Zoothera sibirica
Turdus merula
Acrocephalus melanopogon
Acrocephalus arundinaceus
Acrocephalus scirpaceus
Riparia riparia
Progne subis
Tachycineta bicolor
Cecropis daurica
Hirundo rustica
Megalurus gramineus
Ixos mcclellandii
Hemixos castanonotus
Paradoxornis webbianus
Lioparus chrysotis
Sylvia atricapilla
Sylvia melanocephala
Zosterops japonicus
Alcippe morrisonia
Garrulax milnei *
Minla cyanouroptera
Stachyris striolata *
Pomatorhinus montanus
Stachyris ruficeps

Figure 3-6. (previous page) Bayesian 50% majority rule consensus tree of 97 avian hosts. The phylogeny is resolved using three mitochondrial (CO1, Cytb, ND2) and one nuclear (RAG-1) gene sequences. Maximum likelihood (ML) produced a nearly identical tree (see results). Values are ML bootstrap support (1000 replicates) above 70% (above branches) and Bayesian posterior probabilities > 0.9 (below branches). Hosts with non-*Trouessartia* spp. feather mites are indicated with an asterisk.

(a)


Male Genitalic Size (µm²)
4000
3000
2000
1000
0
-1000
-2000
-3000
-4000
-5000
0
10
20
30
40
50
60
70
80
90
100
110
120
130
Female External Spermaduct (µm)

(b)


Male Genitalic Size (µm²)
4000
3000
2000
1000
0
-1000
-2000
-3000
-4000
-5000
0
10
20
30
40
50
60
70
80
90
100
110
120
Female External Spermaduct Extending Past Interlobar Membrane (µm)

(c)

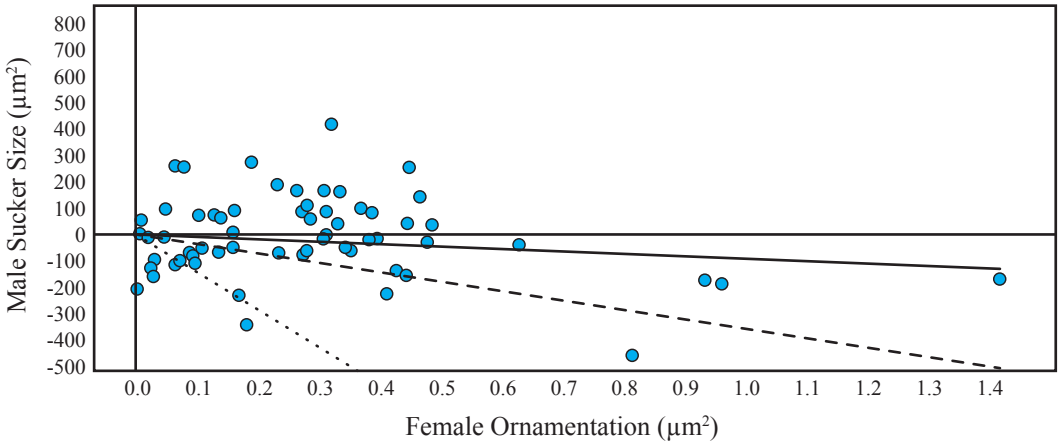
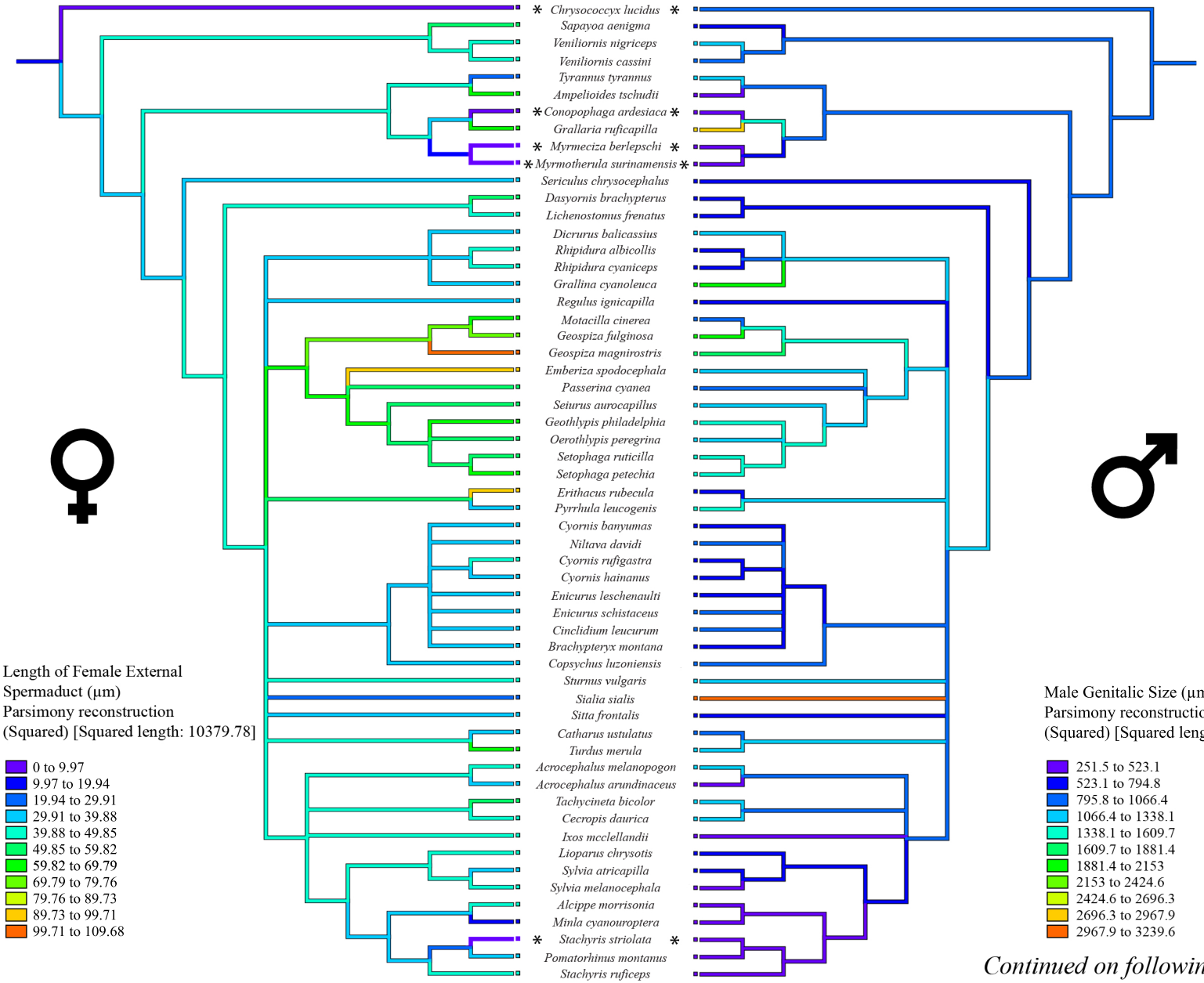

Male Sucker Size (µm²)
800
700
600
500
400
300
200
100
0
-100
-200
-300
-400
-500
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
1.2
1.3
1.4
Female Ornamentation (µm²)

Figure 3-7. (previous page) Positivized independent contrasts of (a) male aedeagus size (μm^2) vs. female external spermaduct length (μm) (number of contrasts = 56, degrees of freedom = 55, $R^2 = 0.078$, $t = 2.15$, $P = 0.036$; (b) male aedeagus size (μm^2) vs. female external spermaduct length extending past the posterior interlobar membrane (μm) (number of contrasts = 56, degrees of freedom = 55, $R^2 = 0.23$, $t = 4.05$, $P < 0.001$; (c) male adanal sucker size (μm^2) vs. the proportion of the female's dorsal hysteronotal shield covered in ornamentation (number of contrasts = 56, degrees of freedom = 55, $R^2 = 0.06$, $t = -1.95$, $P = 0.056$ (2-tailed), 0.028 (1-tailed)); dashed line = reduced major axis; dotted line = major axis; solid line = ordinary least squares.

(a)



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(b)

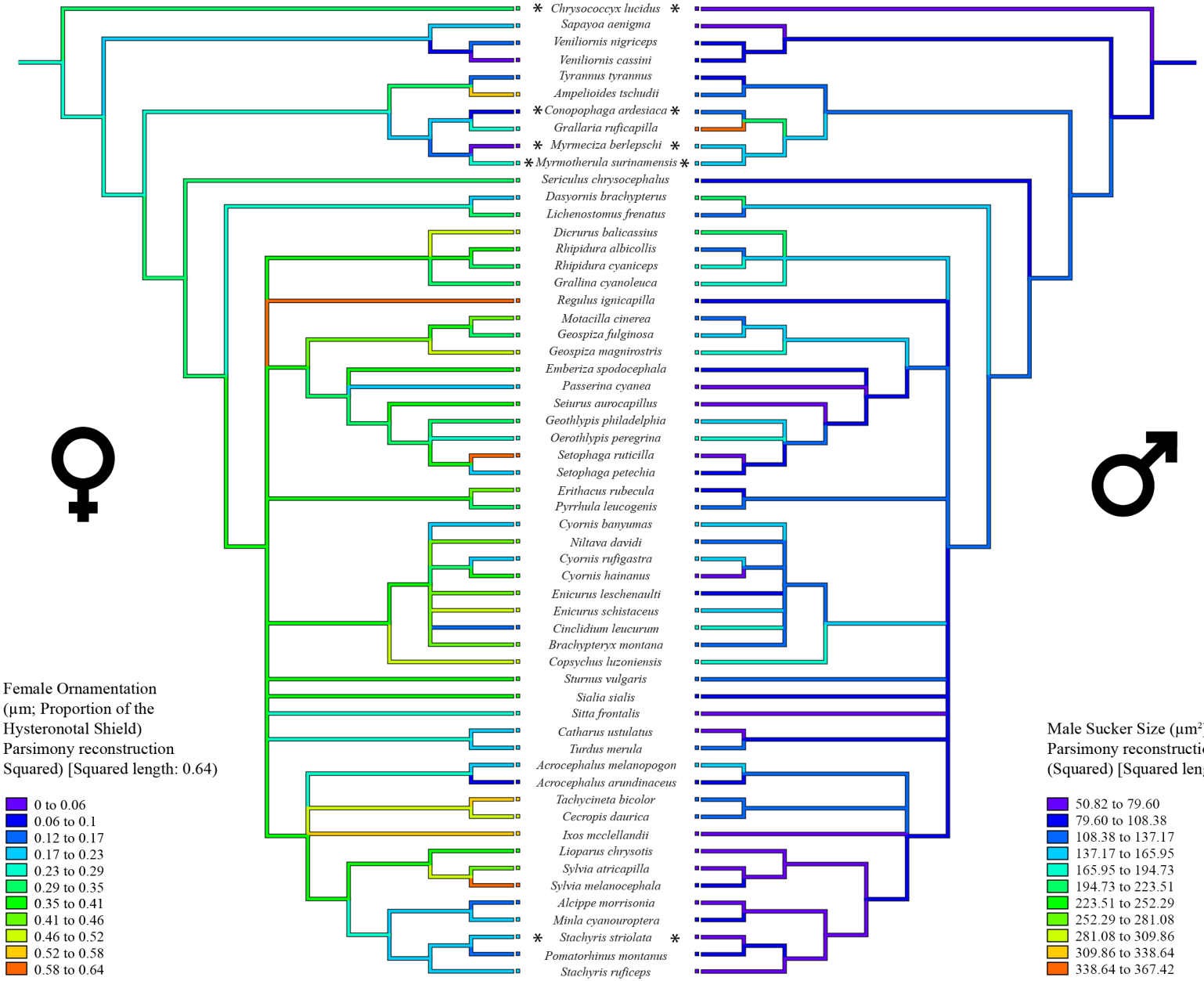
Chrysococcyx lucidus
Sapayoa aenigma
Veniliornis nigriceps
Veniliornis cassini
Tyrannus tyrannus
Ampelioides tschudii
Conopophaga ardesiaca
Grallaria ruficapilla
Myrmeciza berlepschi
Myrmotherula surinamensis
Sericulus chrysocephalus
Dasyornis brachypterus
Lichenostomus frenatus
Dicrurus balicassius
Rhipidura albicollis
Rhipidura cyaniceps
Grallina cyanoleuca
Regulus ignicapilla
Motacilla cinerea
Geospiza fulginosa
Geospiza magnirostris
Emberiza spodocephala
Passerina cyanea
Seiurus aurocapillus
Geothlypis philadelphia
Oerothlypis peregrina
Setophaga ruticilla
Setophaga petechia
Erithacus rubecula
Pyrrhula leucogenis
Cyornis banyumas
Niltava davidi
Cyornis rufigastra
Cyornis hainanus
Enicurus leschenaulti
Enicurus schistaceus
Cinclidium leucurum
Brachypteryx montana
Copsychus luzoniensis
Sturnus vulgaris
Sialia sialis
Sitta frontalis
Catharus ustulatus
Turdus merula
Acrocephalus melanopogon
Acrocephalus arundinaceus
Tachycineta bicolor
Cecropis daurica
Ixos mcclellandii
Lioparus chrysotis
Sylvia atricapilla
Sylvia melanocephala
Alcippe morrisonia
Minla cyanouroptera
Stachyris striolata
Pomatorhinus montanus
Stachyris ruficeps
♀
♂
Female Ornamentation
(µm; Proportion of the
Hysteronotal Shield)
Parsimony reconstruction
Squared) [Squared length: 0.64]
0 to 0.06
0.06 to 0.1
0.12 to 0.17
0.17 to 0.23
0.23 to 0.29
0.29 to 0.35
0.35 to 0.41
0.41 to 0.46
0.46 to 0.52
0.52 to 0.58
0.58 to 0.64
Male Sucker Size (µm²)
Parsimony reconstruction
(Squared) [Squared length: 75922.7]
50.82 to 79.60
79.60 to 108.38
108.38 to 137.17
137.17 to 165.95
165.95 to 194.73
194.73 to 223.51
223.51 to 252.29
252.29 to 281.08
281.08 to 309.86
309.86 to 338.64
338.64 to 367.42

Figure 3-8. (previous page) Bayesian 50% majority rule consensus tree of host birds with morphological characters of female and male feather mites mapped onto the topology. (a) The total female external spermaduct length (μm) is mapped on the left and male genitalic size (μm^2) on the right. (b) The proportion of the female hysteronotal shield covered in ornamentation (μm^2) is mapped on the left vs. male adanal sucker size (μm^2) on the right. Mite taxa for which only one sex was available were removed from the tree. Hosts with non-*Trouessartia* spp. feather mites are indicated with an asterisk.

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Appendix 3-1. List of the 99 captured hosts and their location of capture. Feather mites associated with these hosts are identified in most cases to genus.

Host Taxonomy			Capture Location	Mite Taxonomy
Family	**Species**	**Taxonomic Authority**		**Species**
Outgroup				
Apodidae	*Chaetura spinicaudus*	(Temminck, 1839)	Cali, Colombia	*Thysanocercus* sp.
	Collocalia esculenta	(Linnaeus, 1758)	Mt. Cagua, Philippines	*Thysanocercus* sp.
	Streptoprocne rutile	(Vieillot, 1817)	Peru	*Thysanocercus* sp.
Ingroup				
Acanthizidae	*Oreoscopus gutturalis*	(De Vis, 1889)	Mt. Spec, Australia [1]	*Allanalges* sp.
Acrocephalidae	*Acrocephalus arundinaceus*	(Linnaeus, 1758)	Spain	*Trouessartia trouessarti* Oudemans
	Acrocephalus melanopogon	(Temminck, 1823)	Spain	*T. trouessarti* Oudemans
	Acrocephalus scirpaceus	(Hermann, 1804)	Spain	*T. trouessarti* Oudemans
Aegothelidae	*Aegotheles cristatus*	(Shaw, 1790)	Manypeaks, Australia [2]	*Trouessartia* sp.
Calyptomenidae	*Smithornis rufolateralis rufolateralis*	Gray, 1864	Congo River, Lukolela, Democratic Republic of the Congo [3]	*Calcealges* sp.
	Calyptomena viridis viridis	Raffles, 1822	Malacca, Malaysia [3]	*Trouessartia* sp.
Cardinalidae	*Passerina cyanea*	(Linnaeus, 1766)	Mount Berry, Georgia	*Trouessartia* sp.
Climacteridae	*Climacteris melanurus*	Gould, 1841	Wyndham-East Kimberley, Australia [1]	*Trouessartia* sp.
Conophagidae	*Conopophaga ardesiaca*	D'Orbigny & Lafresnaye, 1837	Peru	*Calcealges* sp.
Corvidae	*Corvus orru*	Bonaparte, 1850	Queensland, Australia	*Trouessartia* sp.
	Garrulus glandarius	(Linnaeus, 1758)	Kuan Kuoshui Nature Reserve, China	*Trouessartia* sp.
Cotingidae	*Ampelioides tschudii*	(Gray, 1846)	Cali, Colombia	*Trouessartia* sp.
	Pipreola arcuata	(Lafresnaye, 1843)	Cali, Colombia	*Trouessartia* sp.
Cuculidae	*Chrysococcyx lucidus*	(Gmelin, 1788)	Brisbon, Australia	*Allanalges* sp.
Dasyornithidae	*Dasyornis brachypterus*	(Latham, 1802)	Australia [2]	*Trouessartia* sp.
Dicruridae	*Dicrurus balicassius*	(Linnaeus, 1766)	Aurora National Park, Philippines	*Trouessartia* sp.
Emberizidae	*Emberiza elegans*	Temminck, 1836	Kuan Kuoshui Nature Reserve, China	*Trouessartia* sp.

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Host Taxonomy			Capture Location	Mite Taxonomy
Family	**Species**	**Taxonomic Authority**		**Species**
	Emberiza spodocephala	Pallas, 1776	Shuipu village and Kuan Kuoshui Nature Reserve, China	*Trouessartia* sp.
	Geospiza fuliginosa	Gould, 1837	Galapagos	*Trouessartia geospiza* OConnor, Foufopoulos & Lipton
	Geospiza magnirostris	Gould, 1837	Galapagos	*Trouessartia geospiza*
Fringillidae	*Pyrrhula leucogenis*	Ogilvie-Grant, 1895	Aurora National Park, Philippines	*Trouessartia* sp.
Furnariidae	*Hyloctistes subulatus*	(Spix, 1824)	Cali, Colombia	*Trouessartia* sp.
	Margarornis squamiger	(D'Orbigny & Lafresnaye, 1838)	Cali, Colombia	*Trouessartia* sp.
Grallariidae	*Grallaria ruficapilla*	Lafresnaye, 1842	Cali, Colombia	*Trouessartia* sp.
Hirundinidae	*Cecropis daurica*	(Linnaeus, 1771)	Mt. Cagua, Philippines	*Trouessartia* nr. *appendiculata (*Berlese) *Trouessartia* sp. keys to *corolligera* Gaud
	Hirundo rustica	Linnaeus, 1758	Spain	*Trouessartia crucifera* Gaud
	Progne subis	(Linnaeus, 1758)	Alberta and Manitoba, Canada	*Trouessartia* sp.
	Riparia riparia	(Linnaeus, 1758)	Alberta, Canada	*Trouessartia* sp.
	Tachycineta bicolor	(Vieillot, 1808)	Alberta, Canada	*Trouessartia* sp.
Leiothrichidae	*Garrulax milnei*	(David, 1874)	Kuan Kuoshui Nature Reserve, China	*Neocalcealges* sp.
	Minla cyanouroptera	(Hodgson, 1838)	Kuan Kuoshui Nature Reserve, China	*Trouessartia* sp.
Locustellidae	*Megalurus gramineus*	(Gould, 1845)	Manjimup, Australia [1]	*Trouessartia* sp.
Meliphagidae	*Cissomela pectoralis*	(Gould, 1841)	Derby-West Kimberley, Australia [1]	*Trouessartia* sp.
	Lichenostomus frenatus	(Ramsay, 1874)	Lake Eacham, Australia	*Trouessartia* sp.
Monarchidae	*Grallina cyanoleuca*	(Latham, 1802)	Derby-West Kimberley and Victoria, Australia [1]	*Trouessartia* sp.
Motacillidae	*Motacilla cinerea*	Tunstall, 1771	Kuan Kuoshui Nature Reserve, China	*Trouessartia* sp.

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Host Taxonomy			Capture Location	Mite Taxonomy
Family	**Species**	**Taxonomic Authority**		**Species**
Muscicapidae	*Brachypteryx montana*	Horsfield, 1821	Aurora Memorial National Park, Philippines	*Trouessartia* sp.
	Cinclidium leucurum	(Hodgson, 1845)	Shiwandashan Nature Reserve, China	*Trouessartia* sp.
	Copsychus luzoniensis	(Kittlitz, 1832)	Aurora Luzon Island, Philippines	*Trouessartia* sp.
	Cyornis banyumas	(Horsfield, 1821)	Jing Xin County Nature Reserve and Kuan Kuoshui Nature Reserve, China	*Trouessartia* sp.
	Cyornis hainanus	(Ogilvie-Grant, 1900)	Shiwandashan Nature Reserve, China	*Trouessartia* sp.
	Cyornis herioti	Ramsay 1886	Angat, Philippines	*Trouessartia* sp.
	Cyornis rufigaster	(Raffles, 1822)	Burdeos, Philippines	*Trouessartia* sp.
	Enicurus leschenaulti	(Vieillot, 1818)	Kuan Kuoshui Nature Reserve, Canada	*Trouessartia* sp.
	Enicurus schistaceus	(Hodgson, 1836)	Shiwandashan Nature Reserve and Shuipu village, China	*Trouessartia* sp.
	Erithacus rubecula	(Linnaeus, 1758)	Cádiz, Spain	*Trouessartia* sp.
	Myophonus caeruleus	Scopoli, 1786	Kuan Kuoshui Nature Reserve, China	*Trouessartia* sp.
	Niltava davidi	La Touche, 1907	Kuan Kuoshui Nature Reserve, China	*Trouessartia* sp.
Neosittidae	*Daphoenositta chrysoptera pileata*	Gould, 1838	Dumbleyung, Australia [1]	*Trouessartia* sp.
Parulidae	*Geothlypis philadelphia*	(Wilson, 1810)	Alberta, Canada	*Trouessartia* sp.
	Oreothlypis peregrina	(Wilson, 1811)	Edmonton, Alberta, Canada	*Trouessartia* sp.
	Parkesia noveboracensis	(Gmelin, 1789)	Edmonton, Alberta, Canada	*Trouessartia* sp.
	Seiurus aurocapillus	(Linnaeus, 1766)	Alberta, Canada	*Trouessartia* sp.
	Setophaga petechia	(Linnaeus, 1766)	Barrhead, Edmonton, Hinton and Millet, Alberta, Canada	*Trouessartia* sp.
	Setophaga ruticilla	(Linnaeus, 1758)	Alberta, Canada	*Trouessartia* sp.
Pellorneidae	*Alcippe morrisonia*	Swinhoe, 1863	Kuan Kuoshui Nature Reserve, China	*Trouessartia* sp.
Philepettidae	*Philepitta castanea*	(Statius Muller, 1776)	Australia [2]	*Arthrogynalges biovoidatus* Orwig 1968
Picidae	*Veniliornis cassini*	(Malherbe, 1862)	Cali, Colombia	*Trouessartia* sp.
	Veniliornis nigriceps	(Orbigny, 1840)	Cali, Colombia	*Trouessartia* sp.

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Host Taxonomy			Capture Location	Mite Taxonomy
Family	**Species**	**Taxonomic Authority**		**Species**
Pipridae	*Masius chrysopterus*	(Lafresnaye, 1843)	Cali, Colombia	*Trouessartia* sp.
Pittidae	*Pitta brachyura*	Linnaeus 1766	Mysore state, Bangalore, India	*Trouessartia* sp.
	Pitta erythrogaster	Temminck, 1823	Celebes, Indonesia [3]	*Trouessartia* sp.
Psophodidae	*Psophodes olivaceus*	(Latham, 1802)	Australia [2]	*Calcealges* sp.
Ptilonorhynchidae	*Sericulus chrysocephalus*	(Lewin, 1808)	Australia [2]	*Trouessartia* sp.
Pycnonotidae	*Hemixos castanonotus*	Swinhoe, 1870	Shiwandashan Nature Reserve, China	*Trouessartia* sp.
	Ixos mcclellandii	(Horsfield, 1840)	Jing Xin County Nature Reserve, China	*Trouessartia* sp.
Regulidae	*Regulus ignicapillus*	(Temminck, 1820)	Cádiz, Spain	*Trouessartia* sp.
Rhipiduridae	*Rhipidura albicollis*	(Vieillot, 1818)	Jing Xin County Nature Reserve, China	*Trouessartia* sp.
	Rhipidura cyaniceps	(Cassin, 1855)	Zabali Camp, Philippines	*Trouessartia* sp.
Sapayoidae	*Sapayoa aenigma*	Hartert, 1903	Gamboa, Panama[1]; Cali and Rio Uva [3] Colombia	*Trouessartia* sp.
Sittidae	*Sitta frontalis*	Swainson, 1820	Aurora Memorial National Park, Philippines	*Trouessartia* sp.
Sturnidae	*Sturnus vulgaris*	Linnaeus, 1758	Alberta, Canada	*Trouessartia rosterii* (Berlese)
Sylviidae	*Lioparus chrysotis*	(Blyth, 1845)	Kuan Kuoshui Nature Reserve, China	*Trouessartia* sp.
	Paradoxornis webbianus	(Gould, 1852)	Shuipu Village, China	*Trouessartia* sp.
	Sylvia atricapilla	(Linnaeus, 1758)	Cádiz, Spain	*Trouessartia* sp.
	Sylvia melanocephala	(Gmelin, 1789)	Cádiz, Spain	*Trouessartia* sp.
Thamnophilidae	*Cercomacra tyrannina*	(Sclater, 1855)	Cali, Colombia	*Calcealges* sp.
	Drymophila caudata	(Sclater, 1855)	Cali, Colombia	*Calcealges* sp.
	Dysithamnus mentalis	(Temminck, 1823)	Cali, Colombia	*Trouessartia* sp.
	Gymnopithys leucaspis	(Sclater, 1855)	Cali, Colombia	*Trouessartia* sp.
	Myrmeciza berlepschi	(Hartert, 1898)	Cali, Colombia	*Calcealges* sp.
	Myrmotherula surinamensis	(Gmelin, 1788)	Cali, Colombia	*Calcealges* sp.
	Phaenostictus mcleannani	(Lawrence, 1860)	Cali, Colombia	*Calcealges* sp.

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Host Taxonomy			Capture Location	Mite Taxonomy
Family	**Species**	**Taxonomic Authority**		**Species**
	Taraba major	(Vieillot, 1816)	Cali, Colombia	*Calcealges* sp.
	Thamnophilus punctatus	(Shaw, 1809)	Cali, Colombia	*Calcealges* sp.
Timaliidae	*Pomatorhinus montanus*	Horsfield, 1821	Bali, Indonesia [1]	*Trouessartia* sp.
	Stachyridopsis ruficeps	(Blyth, 1847)	Kuan Kuoshui Nature Reserve and Shuipu Village, China	*Trouessartia* sp.
	Stachyris striolata	(Muller, 1835)	Jing Xin County Nature Reserve, Canada	*Bicentralges* sp.
Trogonidae	*Harpactes erythrocephalus*	(Gould, 1834)	Jing Xin County Nature Reserve, Canada	*Trouessartia* sp.
Turdidae	*Catharus ustulatus*	(Nuttall, 1840)	Edmonton and Ministik Hills, Alberta, Canada	*Trouessartia* sp.
	Sialia sialis	(Linnaeus, 1758)	Georgia, USA	*Trouessartia* sp.
	Turdus merula	Linnaeus 1758	Cádiz, Spain	*Trouessartia* sp.
	Zoothera sibirica	(Pallas, 1776)	Jing Xin County Nature Reserve, Canada	*Trouessartia* sp.
Tyrannidae	*Tyrannus tyrannus*	(Linnaeus, 1758)	Alberta and Manitoba, Canada	*Trouessartia* sp.
Zosteropidae	*Zosterops japonicus*	Temminck & Schiegel, 1847	Shuipu Village, China	*Trouessartia* sp.

[1] Indicates specimens collected from the Western Australian Museum, Australia
[2] Indicates specimens collected from the Queensland Museum, Australia
[3] Indicates specimens collected from the American Museum of Natural History, New York

Appendix 3-2. Measurements of female feather mites (n = 68) from the families Trouessartiidae and Thysanocercidae (Acari: Astigmata) listed by their host bird.

Host Taxonomy		Female Measurements					
Family	**Species**	**Hysteronotal Shield (μm^2)**	**Ornamentation (μm^2)**	**Average *h1* Seta Length (μm)**	**Average *h1* Seta Area (μm^2)**	**External Spermaduct (μm)**	**Spermaduct Post Membrane (μm)**
Outgroup							
Apodidae	*Chaetura chapmani* *	13758.99	2889.61	5.00	5.00	n/a	n/a
	Collocalia esculenta *	11570.28	6232.48	3.00	3.00	n/a	n/a
	Streptoprocne rutila *	17775.59	7056.72	5.00	5.00	n/a	n/a
Ingroup							
Acanthizidae	*Oreoscopus gutturalis* *	7549.55	n/a	5.00	5.00	n/a	n/a
Acrocephalidae	*Acrocephalus arundinaceus*	33019.58	3189.13	9.98	9.89	32.69	n/a
	Acrocephalus melanopogon	26124.93	5676.61	14.34	20.31	46.45	n/a
	Acrocephalus scirpaceus	25702.45	3496.00	7.07	8.86	31.15	n/a
Aegothelidae	*Aegotheles cristatus*	32797.28	11880.13	1.00	1.00	35.15	9.78
Calyptomenidae	*Smithornis rufolateralis* *	10378.68	576.88	5.00	5.00	n/a	n/a
Cardinalidae	*Passerina cyanea*	23384.79	4244.81	25.77	75.25	53.85	5.92
Climacteridae	*Climacteris rufus*	36273.04	9403.04	26.38	68.73	103.00	67.00
Conophagidae	*Conopophaga ardesiaca* *	23157.60	2431.07	4.00	4.00	n/a	n/a
Corvidae	*Garrulus glandarius*	44944.86	8450.85	4.78	5.81	20.73	n/a
Cotingidae	*Ampelioides tschudii*	28410.63	15269.71	3.00	3.00	66.00	8.00
	Pipreola arcuata	45096.70	10918.22	19.00	30.00	79.70	44.55
Cuculidae	*Chrysococcyx lucidus* *	8756.63	2643.04	3.00	3.00	n/a	n/a
Dasyornithidae	*Dasyornis brachypterus*	43554.48	7754.40	3.70	5.39	58.22	13.24
Dicruridae	*Dicrurus balicassius*	32289.43	16299.01	12.57	15.68	30.90	17.39
Emberizidae	*Emberiza spodocephala*	31861.61	11657.83	29.32	86.44	93.64	47.64
	Geospiza fuliginosa	34101.49	11660.90	21.74	84.58	78.94	42.40
	Geospiza magnirostris	27673.02	13395.45	29.73	95.17	99.71	54.25

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Family	Species	Hysteronotal Shield (µm²)	Ornamentation (µm²)	Average *h1* Seta Length (µm)	Average *h1* Seta Area (µm²)	External Spermaduct (µm)	Spermaduct Post Membrane (µm)
Fringillidae	*Pyrrhula leucogenis*	59371.00	18736.29	29.92	75.83	96.57	40.63
Furnariidae	*Margarornis squamiger*	33952.65	n/a	10.00	10.00	40.00	n/a
Grallariidae	*Grallaria ruficapilla*	45385.62	12204.21	13.00	13.00	62.07	40.36
Hirundinidae	*Cecropis daurica*	22935.05	11911.36	5.00	5.00	46.20	26.85
	Hirundo rustica	30911.18	7581.92	31.26	101.74	53.77	28.88
	Riparia riparia	32715.03	9033.95	30.71	64.03	54.08	26.26
	Tachycineta bicolor	36739.25	19892.22	46.30	193.85	51.25	12.70
Leiothrichidae	*Garrulax milnei* *	18516.17	n/a	6.00	6.00	n/a	n/a
	Minla cyanouroptera	24437.09	4482.65	12.56	40.43	17.18	2.19
Locustellidae	*Megalurus gramineus*	38841.37	9917.30	7.00	7.00	32.31	n/a
Meliphagidae	*Lichenostomus frenatus*	28662.57	8717.58	14.00	14.00	47.66	n/a
Monarchidae	*Grallina cyanoleuca*	37640.46	12354.15	6.75	9.09	30.23	11.53
Motacillidae	*Motacilla cinerea*	33758.66	14548.81	22.00	58.08	60.91	30.57
Muscicapidae	*Brachypteryx montana*	35375.58	16262.60	22.30	77.95	36.24	n/a
	Cinclidium leucurum	34420.63	4631.67	40.00	101.29	35.00	n/a
	Copsychus luzoniensis	32867.57	15368.36	12.73	17.66	37.10	n/a
	Cyornis banyumas	29063.35	5751.68	10.00	12.14	37.79	n/a
	Cyornis hainanus	24535.55	8682.39	9.86	13.56	31.92	n/a
	Cyornis herioti	27789.75	5286.57	15.00	22.00	40.48	n/a
	Cyornis rufigaster	25830.71	5830.45	12.70	22.32	39.91	n/a
	Enicurus leschenaulti	39734.04	16372.61	22.59	55.87	32.54	n/a
	Enicurus schistaceus	38064.09	18404.62	22.00	37.61	39.66	n/a
	Erithacus rubecula	32935.05	15208.20	22.27	68.46	36.86	n/a
	Niltava davidi	36612.98	15220.80	18.00	35.58	39.21	n/a
Parulidae	*Geothlypis philadelphia*	30648.81	9657.28	28.34	92.53	59.86	28.21
	Oreothlypis peregrina	27445.12	6667.11	26.36	75.92	58.61	29.85
	Parkesia noveboracensis	26371.43	5996.90	29.35	82.07	50.37	23.95

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Family	Species	Hysteronotal Shield (µm^2)	Ornamentation (µm^2)	Average *h1* Seta Length (µm)	Average *h1* Seta Area (µm^2)	External Spermaduct (µm)	Spermaduct Post Membrane (µm)
	Seiurus aurocapillus	27430.29	9906.93	33.00	95.03	53.27	28.50
	Setophaga petechia	27420.84	6214.95	26.48	68.46	63.32	16.99
	Setophaga ruticilla	23542.59	13641.57	25.14	63.92	55.91	31.79
Pellorneidae	*Alcippe morrisonia*	26764.88	4338.80	14.29	38.83	40.86	4.98
Philepettidae	*Philepitta castanea* *	27722.57	10295.83	12.00	12.00	30.00	n/a
Picidae	*Veniliornis cassini*	28576.78	n/a	9.00	9.00	41.89	41.89
	Veniliornis nigriceps	37717.11	4380.36	15.00	19.05	47.30	40.00
Pittidae	*Pitta brachyura*	45391.87	18784.431	30.00	110.55	69.19	44.91
Psophodidae	*Psophodes olivaceus* *	8297.99	2450.05	4.00	4.00	n/a	n/a
Ptilonorhynchidae	*Sericulus chrysocephalus*	32427.27	10474.05	8.00	8.00	36.13	n/a
Pycnonotidae	*Hemixos castanonotus*	23903.11	5306.76	12.00	20.00	40.01	n/a
	Ixos mcclellandii	25466.59	13793.89	27.00	54.00	42.32	7.07
Regulidae	*Regulus ignicapillus*	25839.42	14909.43	40.00	151.55	32.14	n/a
Rhipiduridae	*Rhipidura albicollis*	30648.28	10721.94	11.84	18.18	42.52	n/a
	Rhipidura cyaniceps	26530.72	8967.45	11.00	20.55	39.94	n/a
Sapayoidae	*Sapayoa aenigma*	34830.57	7783.58	8.00	8.00	52.77	16.60
Sittidae	*Sitta frontalis*	27695.14	7502.26	9.00	9.00	31.10	n/a
Sturnidae	*Sturnus vulgaris*	33322.50	13439.18	10.00	10.00	45.00	10.00
Sylviidae	*Lioparus chrysotis*	28836.25	11303.75	22.34	75.06	47.17	12.41
	Paradoxornis webbianus	31742.24	5352.58	24.53	78.00	22.14	n/a
	Sylvia atricapilla	30437.96	12972.85	18.87	37.63	39.12	n/a
	Sylvia melanocephala	26825.08	15428.50	19.58	63.94	43.40	n/a
Thamnophilidae	*Cercomacra tyrannina* *	22829.36	n/a	7.00	7.00	n/a	n/a
	Drymophila caudata *	20166.08	310.08	5.00	5.00	n/a	n/a
	Gymnopithys leucaspis	28269.22	11513.67	35.00	80.00	55.00	30.00
	Myrmeciza berlepschi *	22809.26	n/a	8.00	8.00	n/a	n/a
	Myrmotherula surinamensis *	18987.64	5401.79	5.00	5.00	n/a	n/a

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Family	Species	Hysteronotal Shield (µm^2)	Ornamentation (µm^2)	Average *h1* Seta Length (µm)	Average *h1* Seta Area (µm^2)	External Spermaduct (µm)	Spermaduct Post Membrane (µm)
	Taraba major *	18085.95	n/a	7.00	7.00	n/a	n/a
Timaliidae	*Pomatorhinus montanus*	24363.58	4202.41	16.00	24.00	30.82	n/a
	Stachyridopsis ruficeps	24759.98	4624.51	15.00	15.00	41.02	n/a
	Stachyris striolata *	23594.18	5124.50	9.00	9.00	n/a	n/a
Turdidae	*Catharus ustulatus*	35264.13	7560.74	5.00	5.00	36.02	11.10
	Sialia sialis	34884.12	13003.36	20.00	30.00	22.00	0.00
	Turdus merula	40197.13	7719.51	6.00	6.00	60.67	30.00
	Zoothera sibirica	39166.98	10019.05	6.00	6.00	58.47	38.69
Tyrannidae	*Tyrannus tyrannus*	32689.13	4359.22	10.00	10.00	26.52	9.01

* Indicates outgroup taxa (non-*Trouessartia* spp.) See Appendix 4-1 for a list of mites and their hosts.

Appendix 3-3. Measurements of male feather mites (n = 66) from the families Trouessartiidae and Thysanocercidae (Acari: Astigmata) listed by their host bird.

Host Taxonomy		Mite Measurements					
Family	**Species**	**Hysteronotal Shield (µm²)**	**Ornamentation (µm²)**	**Average *h1* Seta Length (µm)**	**Average *h1* Seta Area (µm²)**	**Average Adanal Sucker Area (µm)**	**Aedeagus Size (µm)**
Ingroup							
Acrocephalidae	*Acrocephalus arundinaceus*	23224.96	n/a	7.09	7.09	82.65	376.27
	Acrocephalus melanopogon	24609.88	n/a	12.31	12.31	147.97	1312.87
Calyptomenidae	*Calyptomena viridis viridis*	21134.32	n/a	15.00	15.00	123.08	552.91
Cardinalidae	*Passerina cyanea*	19220.30	n/a	11.00	11.00	73.12	844.30
Conophagidae	*Conopophaga ardesiaca* *	15643.22	n/a	9.00	9.00	117.48	516.81
Corvidae	*Corvus orru*	30853.54	n/a	16.00	16.00	202.86	1233.28
Cotingidae	*Ampelioides tschudii*	27648.38	n/a	15.00	15.00	136.99	522.51
Cuculidae	*Chrysococcyx lucidus* *	7758.02	n/a	3.00	3.00	76.17	874.31
Dasyornithidae	*Dasyornis brachypterus*	31303.81	n/a	4.00	4.00	213.91	780.10
Dicruridae	*Dicrurus balicassius*	33167.29	7126.27	18.00	18.00	196.12	1274.37
Emberizidae	*Emberiza elegans*	28833.91	n/a	18.00	18.00	207.78	699.92
	Emberiza spodocephala	21728.02	n/a	7.00	7.00	89.80	1163.69
	Geospiza fuliginosa	24642.52	n/a	11.00	11.00	152.52	1964.97
	Geospiza magnirostris	27540.22	n/a	19.00	19.00	180.01	1645.98
Fringillidae	*Pyrrhula leucogenis*	47150.95	n/a	5.00	5.00	111.75	1484.12
Furnariidae	*Hyloctistes subulatus*	35071.62	n/a	11.00	11.00	105.87	1713.27
Grallariidae	*Grallaria ruficapilla*	45273.59	n/a	12.00	12.00	338.64	2860.00
Hirundinidae	*Cecropis daurica*	35877.57	6836.54	4.00	4.00	117.69	1067.24
	Progne subis	25676.10	8971.89	13.00	13.00	427.31	1492.68
	Tachycineta bicolor	27962.24	5962.17	10.00	10.00	114.21	1239.17
Leiothrichidae	*Minla cyanouroptera*	21317.61	n/a	5.00	5.00	102.39	435.31
Meliphagidae	*Cissomela pectoralis*	18851.87	n/a	5.00	5.00	66.49	589.56

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Family	Species	Hysteronotal Shield (µm^2)	Ornamentation (µm^2)	Average *h1* Seta Length (µm)	Average *h1* Seta Area (µm^2)	Average Adanal Sucker Area (µm)	Aedeagus Size (µm)
	Lichenostomus frenatus	32784.34	5954.11	7.00	7.00	129.24	650.07
Monarchidae	*Grallina cyanoleuca*	41297.00	13438.75	21.00	21.00	189.10	2003.91
Motacillidae	*Motacilla cinerea*	27134.40	n/a	7.00	7.00	126.42	916.17
Muscicapidae	*Brachypteryx montana*	25765.33	n/a	12.00	12.00	122.48	598.09
	Cinclidium leucurum	23927.09	n/a	10.00	10.00	186.67	963.03
	Copsychus luzoniensis	26381.41	n/a	9.00	9.00	191.53	815.09
	Cyornis banyumas	24134.50	n/a	9.00	9.00	151.63	735.93
	Cyornis hainanus	21733.35	n/a	10.00	10.00	76.07	609.12
	Cyornis herioti	22169.712	n/a	6.00	6.00	202.43	742.23
	Cyornis rufigaster	21403.99	n/a	12.59	12.59	143.16	764.55
	Enicurus leschenaulti	29260.40	n/a	15.00	15.00	98.00	639.05
	Enicurus schistaceus	31559.28	6124.11	15.00	15.00	155.10	795.26
	Erithacus rubecula	25275.15	3317.58	12.39	12.39	105.95	635.50
	Myophonus caeruleus	35738.55	13941.53	10.00	10.00	70.89	598.75
	Niltava davidi	24286.92	n/a	5.00	5.00	111.12	830.58
Neosittidae	*Daphoenositta chrysoptera pileata*	30670.29	n/a	10.00	10.00	102.33	1052.96
Parulidae	*Geothlypis philadelphia*	28521.10	n/a	10.00	10.00	163.36	1525.65
	Oreothlypis peregrina	22050.46	n/a	10.00	10.00	180.35	1318.80
	Seiurus aurocapillus	21210.22	n/a	8.00	8.00	61.70	1089.32
	Setophaga petechial	20204.66	n/a	12.00	12.00	102.66	1458.47
	Setophaga ruticilla	23416.04	n/a	10.00	10.00	61.31	1502.44
Pellorneidae	*Alcippe morrisonia*	19357.58	n/a	4.90	4.90	53.15	345.12
Picidae	*Veniliornis cassini*	23997.95	n/a	9.00	9.00	85.05	930.26
	Veniliornis nigriceps	31403.91	n/a	12.00	12.00	83.78	1257.13
Pipridae	*Masius chrysopterus*	27958.47	6045.34	6.00	6.00	67.00	513.88
Pittidae	*Pitta erythrogaster*	24634.77	n/a	12.00	12.00	73.79	1014.80

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Family	Species	Hysteronotal Shield (µm^2)	Ornamentation (µm^2)	Average *h1* Seta Length (µm)	Average *h1* Seta Area (µm^2)	Average Adanal Sucker Area (µm)	Aedeagus Size (µm)
Ptilonorhynchidae	*Sericulus chrysocephalus*	28212.21	n/a	2.00	2.00	94.51	657.07
Pycnonotidae	*Ixos mcclellandii*	24158.19	6259.66	15.00	15.00	65.53	446.35
Regulidae	*Regulus ignicapillus*	21406.31	6763.28	19.00	19.00	97.43	653.21
Rhipiduridae	*Rhipidura albicollis*	22672.89	2468.03	12.00	12.00	117.17	598.46
	Rhipidura cyaniceps	23891.12	4911.42	8.00	8.00	169.03	718.87
Sapayoidae	*Sapayoa aenigma*	27238.93	n/a	8.00	8.00	74.33	739.78
Sittidae	*Sitta frontalis*	21194.39	n/a	7.00	7.00	74.06	562.50
Sturnidae	*Sturnus vulgaris*	25444.62	9828.44	10.00	10.00	127.87	1173.61
Sylviidae	*Lioparus chrysotis*	23244.58	n/a	6.00	6.00	50.82	582.21
	Sylvia atricapilla	25763.09	n/a	7.00	7.00	54.61	600.33
	Sylvia melanocephala	22577.39	n/a	15.00	15.00	87.62	488.97
Thamnophilidae	*Dysithamnus mentalis*	29159.79	n/a	6.00	6.00	84.32	523.07
	Myrmeciza berlepschi *	12998.08	n/a	9.00	9.00	145.98	326.74
	Myrmotherula surinamensis *	12732.43	n/a	6.00	6.00	143.47	478.94
	Phaenostictus mcleannani *	13785.50	n/a	15.00	15.00	225.04	300.50
	Thamnophilus punctatus *	14805.66	148.65	15.00	15.00	202.99	494.17
Timaliidae	*Pomatorhinus montanus*	21948.56	n/a	7.00	7.00	76.72	251.48
	Stachyridopsis ruficeps	18315.71	n/a	6.00	6.00	62.69	395.47
	Stachyris striolata *	18517.42	n/a	4.00	4.00	99.57	399.21
Trogonidae	*Harpactes erythrocephalus*	24471.07	3689.46	8.00	8.00	97.68	1053.33
Turdidae	*Catharus ustulatus*	21949.49	n/a	5.00	5.00	74.06	989.66
	Sialia sialis	34893.84	n/a	20.00	20.00	88.98	2967.93
	Turdus merula	34347.99	n/a	11.23	11.23	117.61	1195.06
Tyrannidae	*Tyrannus tyrannus*	26178.74	n/a	15.00	15.00	91.77	1261.85
Zosteropidae	*Zosterops japonicas*	17709.65	n/a	7.00	7.00	53.00	589.40

* Indicates outgroup taxa (non-*Trouessartia* spp.) See Appendix 4-1 for a list of mites and their hosts.

Appendix 3-4. Genbank accession numbers for avian hosts including the mitochondrial genes cytochrome b, cytochrome c oxidase subunit 1, NADH dehydrogenase subunit 2, and the nuclear recombination activating gene 1

Host Taxonomy		**Genbank Accession Number**			
Family	**Species**	**Cytochrome b**	**Cytochrome c oxidase subunit 1**	**NADH dehydrogenase subunit 2**	**Recombination activating gene 1**
Outgroup					
Apodidae	*Chaetura chapmani*	FJ588454.1		AY294539.1	
	Collocalia esculenta	AY135613.1		EF600707.1	
	Streptoprocne rutila		AY275859.1		
Ingroup					
Acanthizidae	*Oreoscopus gutturalis*	FJ821131.1	GU825762.1	GU825880.1	GU825814.1
Acrocephalidae	*Acrocephalus arundinaceus*	AJ004784.1	FR847226.1	GQ242092.1	
	Acrocephalus melanopogon	AJ004767.1	GQ481267.1		
	Acrocephalus scirpaceus	AM889139.1	AM889139.1	AM889139.1	
Aegothelidae	*Aegotheles cristatus*	EU344979.1	EU344979.1	EU344979.1	
Calyptomenidae	*Smithornis rufolateralis rufolateralis*	AY065727.1			AY057031.1
	Calyptomena viridis viridis				DQ320606.1
Cardinalidae	*Passerina cyanea*	AF301447.1	DQ434710.1	AF447296.1	
Climacteridae	*Climacteris rufus*	U58501.1		AY064746.1	AY037846.1
Conophagidae	*Conopophaga ardesiaca*				AY443271.1
Corvidae	*Corvus orru*	FJ499000.1			AY443277.1
	Garrulus glandarius	JN018413.1	JN018413.1	JN018413.1	
Cotingidae	*Ampelioides tschudii*	DQ470491.1			FJ501597.1
	Pipreola arcuata	DQ470508.1			
Cuculidae	*Chrysococcyx lucidus*	AF168109.1	AF168062.1	HM159188.1	
Dasyornithidae	*Dasyornis brachypterus*		GU825759.1		
Dicruridae	*Dicrurus balicassius*	EF449768.1		EF449674.1	
Emberizidae	*Emberiza elegans*	AY495391.1	EU847675.1		
	Emberiza spodocephala	DQ792768.1	GQ481838.1		

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Family	Species	Cytochrome b	Cytochrome c oxidase subunit 1	NADH dehydrogenase subunit 2	Recombination activating gene 1
	Geospiza fuliginosa	AF108786.1			
	Geospiza magnirostris	AF108778.1			
Fringillidae	*Pyrrhula leucogenis*	HQ284678.1			
Furnariidae	*Hyloctistes subulatus*	GQ140085.1			FJ461145.1
	Margarornis squamiger	HM125626.1		HM125597.1	AY065732.1
Grallariidae	*Grallaria ruficapilla*	AY370544.1		AY370581.1	FJ461215.1
Hirundinidae	*Cecropis daurica*	AY825977.1	GQ481533.1	AY826036.1	
	Hirundo rustica	GU460258.1	GU571202.1	GU460318.1	AY443290.1
	Progne subis	EU427742.1	FJ582624.1	AY825996.1	
	Riparia riparia	AF074578.1	FJ582635.1	AY826015.1	
	Tachycineta bicolor	GU460236.1	GU460339.1	AY136590.1	
Leiothrichidae	*Garrulax milnei*	EU447076.1	EU447031.1		EU447122.1
	Minla cyanouroptera	GU139515.1	GU139501.1	GU139529.1	FJ358114.1
Locustellidae	*Megalurus gramineus*	HQ333042.1		AY382397.1	
Meliphagidae	*Cissomela pectoralis*	AY488339.1		AY488268.1	
	Lichenostomus frenatus			HQ267669.1	
Monarchidae	*Grallina cyanoleuca*	AY443249.1		DQ084074.1	AY443288.1
Motacillidae	*Motacilla cinerea*	AF447370.1	GU571490.1	AY259443.1	AY057007.1
Muscicapidae	*Brachypteryx montana*	HM633264.1		GU358777.1	
	Cinclidium leucurum	HM633275.1		GU358786.1	
	Copsychus luzoniensis	HM633399.1	EU541451.1	HM120193.1	
	Cyornis banyumas	HM633287.1			
	Cyornis hainanus		EU541453.1		
	Cyornis rufigaster		EU541452.1		
	Enicurus leschenaulti	HM633292.1		GU358794.1	
	Enicurus schistaceus			GU358795.1	
	Erithacus rubecula	AY491533.1	GU571382.1	DQ466861.1	AY307191.1

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Family	Species	Cytochrome b	Cytochrome c oxidase subunit 1	NADH dehydrogenase subunit 2	Recombination activating gene 1
	Myophonus caeruleus	HM633345.1			
	Niltava davidi	EF081353.1	EF422245.1		
Neosittidae	*Daphoenositta chrysoptera pileata*	FJ821116.1			AY443281.1
Parulidae	*Geothlypis philadelphia*	FJ653079.1		FJ605351.1	
	Oreothlypis peregrina	GU932420.1	GU932133.1	GU932133.1	
	Parkesia noveboracensis	GU932367.1	AY650209.1	AF383117.1	
	Seiurus aurocapillus	GU932365.1	GU932043.1	GU932043.1	
	Setophaga petechia	EU815687.1	AY650222.1	AF383112.1	
	Setophaga ruticilla	EU815694.1	AY650182.1	AY650182.1	
Pellorneidae	*Alcippe morrisonia*	JF756765.1	EU447062.1	EF154826.1	JF756875.1
Philepettidae	*Philepitta castanea*	AY065726.1			AY057018.1
Picidae	*Veniliornis cassini*	AY927210.1	AY927190.1		
	Veniliornis nigriceps	AF389337.1	AF272598.1	DQ361287.1	
Pipridae	*Masius chrysopterus*		EF111035.1	GU985505.1	FJ501666.1
Pittidae	*Pitta erythrogaster*		EU541462.1		DQ320616.1
Psophodidae	*Psophodes olivaceus*	FJ821139.1		EF592322.1	FJ821069.1
Ptilonorhynchidae	*Sericulus chrysocephalus*	U10365.1		EU341425.1	EU341458.1
Pycnonotidae	*Hemixos castanonotus*			GU112647.1	
	Ixos mcclellandii	DQ008506.1		GQ242079.1	
Regulidae	*Regulus ignicapillus*	AY894888.1	GU572075.1		
Rhipiduridae	*Rhipidura albicollis*	AF096462.1		GQ145387.1	
	Rhipidura cyaniceps	AF096461.1		JN545983.1	
Sapayoidae	*Sapayoa aenigma*				DQ320609.1
Sittidae	*Sitta frontalis*	U63400.1			
Sturnidae	*Sturnus vulgaris*	AF285790.1	EF484212.1	HM159191.1	AY057032.1
Sylviidae	*Lioparus chrysotis*	JF756763.1	HM140300.1	JF756837.1	FJ358109.1
	Paradoxornis webbianus	JF756697.1	EF515796.1	JF756771.1	JF756879.1

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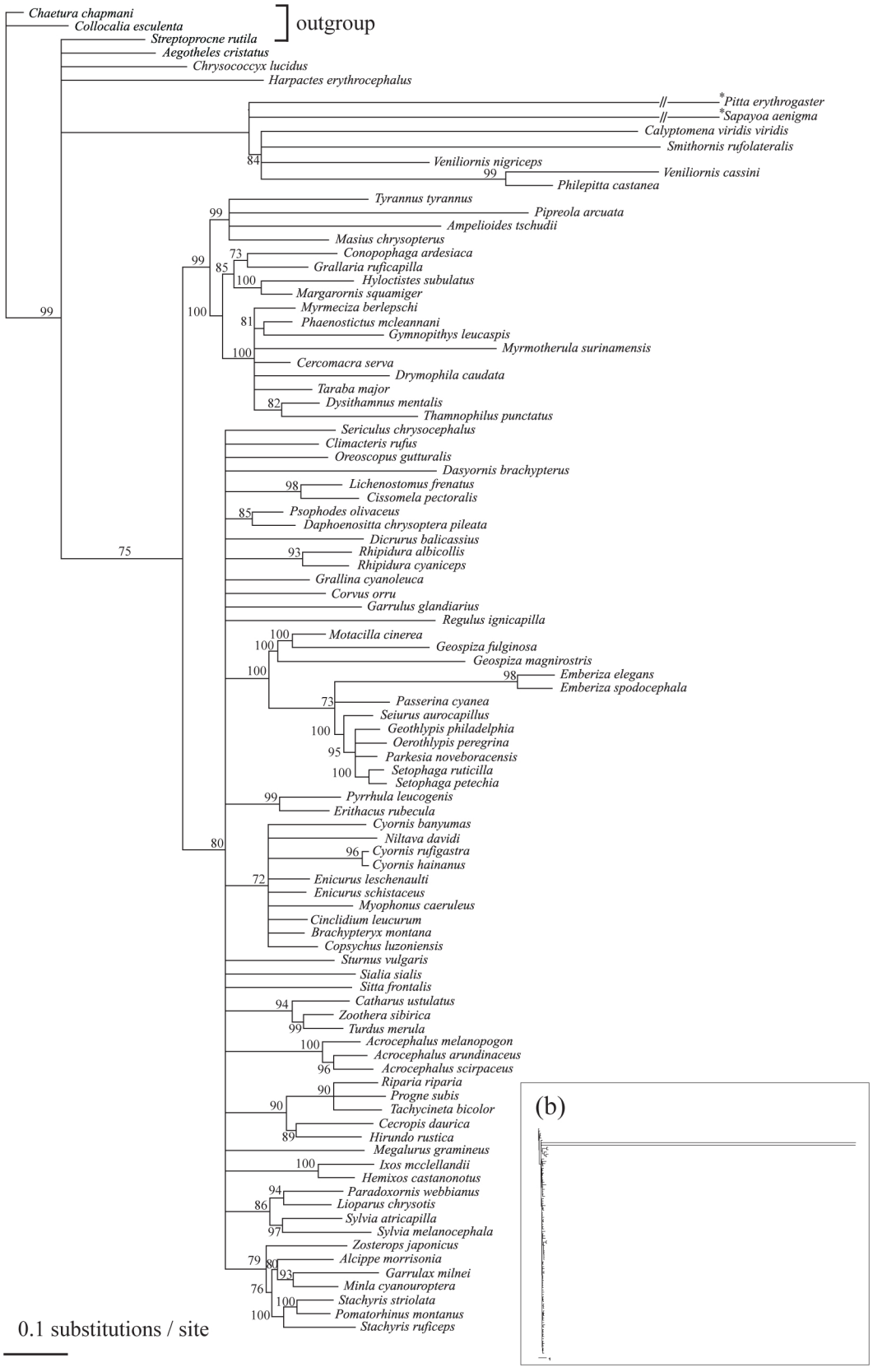
Family	Species	Cytochrome b	Cytochrome c oxidase subunit 1	NADH dehydrogenase subunit 2	Recombination activating gene 1
	Sylvia atricapilla	AM889140.1	AM889140.1	AM889140.1	EF568261.1
	Sylvia melanocephala	AJ534544.1	FJ465369.1	JF502339.1	
Thamnophilidae	*Cercomacra tyrannina*	EF639941.1		FJ175888.1	FJ461191.1
	Drymophila caudata	AF118173.1		AF118207.1	
	Dysithamnus mentalis	EF639945.1	FJ027520.1	EF640012.1	FJ461181.1
	Gymnopithys leucaspis	EF639995.1		EF640062.1	
	Myrmeciza berlepschi	EF639962.1		EF640029.1	FJ461203.1
	Myrmotherula surinamensis	GU215271.1			
	Phaenostictus mcleannani	EF639978.1		EF640045.1	FJ461210.1
	Taraba major	EF639986.1	FJ028394.1	EF640053.1	FJ461174.1
	Thamnophilus punctatus	EF030334.1	EU119787.1	EF030303.1	
Timaliidae	*Pomatorhinus montanus*	GU724383.1		GU724461.1	
	Stachyridopsis ruficeps	GU724400.1	EU447061.1	GU724478.1	EU447152.1
	Stachyris striolata	GU724401.1		GU724479.1	FJ358136.1
Trogonidae	*Harpactes erythrocephalus*			HQ380007.1	AY625242.1
Turdidae	*Catharus ustulatus*	EU619756.1	DQ434532.1	GU237101.1	AY443265.1
	Sialia sialis	HM633380.1	EU525498.1	GU358825.1	AY320001.1
	Turdus merula	AY286396.1	GU571670.1	AY752348.1	
	Zoothera sibirica	EU154690.1		AY752333.1	
Tyrannidae	*Tyrannus tyrannus*		JN801392.1		AF143739.1
Zosteropidae	*Zosterops japonicus*	HQ608850.1	HQ608875.1	GU724482.1	FJ358145.1


(a)
*40 substitutions / site
outgroup
Chaetura chapmani
Collocalia esculenta
Streptoprocne rutila
Aegotheles cristatus
Chrysococcyx lucidus
Harpactes erythrocephalus
*Pitta erythrogaster
*Sapayoa aenigma
Calyptomena viridis viridis
Smithornis rufolateralis
Veniliornis nigriceps
Veniliornis cassini
Philepitta castanea
Tyrannus tyrannus
Pipreola arcuata
Ampelioides tschudii
Masius chrysopterus
Conopophaga ardesiaca
Grallaria ruficapilla
Hylocti stes subulatus
Margarornis squamiger
Myrmeciza berlepschi
Phaenostictus mcleannani
Gymnopithys leucaspis
Myrmotherula surinamensis
Cercomacra serva
Drymophila caudata
Taraba major
Dysithamnus mentalis
Thamnophilus punctatus
Sericulus chrysocephalus
Climacteris rufus
Oreoscopus gutturalis
Dasyornis brachypterus
Lichenostomus frenatus
Cissomela pectoralis
Psophodes olivaceus
Daphoenositta chrysoptera pileata
Dicrurus balicassius
Rhipidura albicollis
Rhipidura cyaniceps
Grallina cyanoleuca
Corvus orru
Garrulus glandiarius
Regulus ignicapilla
Motacilla cinerea
Geospiza fulginosa
Geospiza magnirostris
Emberiza elegans
Emberiza spodocephala
Passerina cyanea
Seiurus aurocapillus
Geothlypis philadelphia
Oerothlypis peregrina
Parkesia noveboracensis
Setophaga ruticilla
Setophaga petechia
Pyrrhula leucogenis
Erithacus rubecula
Cyornis banyumas
Niltava davidi
Cyornis rufigastra
Cyornis hainanus
Enicurus leschenaulti
Enicurus schistaceus
Myophonus caeruleus
Cinclidium leucurum
Brachypteryx montana
Copsychus luzoniensis
Sturnus vulgaris
Sialia sialis
Sitta frontalis
Catharus ustulatus
Zoothera sibirica
Turdus merula
Acrocephalus melanopogon
Acrocephalus arundinaceus
Acrocephalus scirpaceus
Riparia riparia
Progne subis
Tachycineta bicolor
Cecropis daurica
Hirundo rustica
Megalurus gramineus
Ixos mcclellandii
Hemixos castanonotus
Paradoxornis webbianus
Lioparus chrysotis
Sylvia atricapilla
Sylvia melanocephala
Zosterops japonicus
Alcippe morrisonia
Garrulax milnei
Minla cyanouroptera
Stachyris striolata
Pomatorhinus montanus
Stachyris ruficeps
0.1 substitutions / site
(b)

Appendix 3-5. (previous page) Maximum likelihood (ML) tree showing relationships among 97 avian hosts of mites from the families Thysanocercidae and Trouessartiidae. Bootstrap support (1000 replicates) values >70% are shown above branches (a). Insert (b) represents the ML phylogeny with full branch lengths indicated for *Pitta erythrogaster* Temminck and *Sapayoa aenigma* Hartert in relation to the remainder of the avian taxa.

Chapter 4. General discussion and future directions

4.1 The path (un)travelled

Sexual dimorphism has long been recognized throughout animals in regard to both primary and secondary sexual characters (Andersson, 1994; Berglund, 1996; Emlen *et al*., 2005). Although the correlated evolution of male and female traits has been researched previously in several arthropod taxa (Insecta: *Callosobruchus*: Rönn *et al.,* 2007; *Coridromius*: Tatarnic & Cassis, 2010; *Drosophila*: Kamimura, 2012), prior to my thesis, very few studies had evaluated the degree of correlation between the sexes in genitalia (Atyeo & Braasch, 1966) and non-genitalic contact structures (Witaliński *et al*., 1992). My results illustrate the diversity in morphology that exists across feather mites and demonstrates that exceptions exist to many published trends.

4.2 Correlations in genitalia and non-genitalic contact structures

The original focus of my thesis, to analyze the potential correlations in genitalic structures between the sexes in *Trouessartia* spp., grew to include evaluations of non-genitalic contact structures in both the genus *Trouessartia* and family Proctophyllodidae. Although both projects involved correlative analyses, the theoretical bases for these studies were strongly opposing. In Chapter 2, I analyzed whether correlations between the male adanal suckers and the dorsal female docking papillae in the Proctophyllodidae were suggestive of "cooperative" structures used to promote coupling during precopulatory guarding (Witaliński *et al*., 1992). In contrast, I looked for correlated morphologies as an indication of sexually antagonistic coevolution (SAC) in *Trouessartia* spp. (Chapter 3). As correlated morphologies may indicate either cooperative or antagonistic coevolution, distinguishing between these two processes can be difficult. Eberhard (2004) suggests that antagonistic traits may be best recognized as those morphologies which can be employed facultatively to hinder unwanted males from mating. These female morphologies should not appear cooperative (e.g., grooves or pits which facilitate grasping by the male).

In *Trouessartia* spp., it is unknown whether the external spermaduct can be used facultatively; however, the absence of precopulatory guarding in this taxon (HP pers. obs.) and the potential for females to gain control over fertilization through insertion of the spermaduct into the male genital opening (Klimov & Sidorchuck, 2011) suggests that external spermaducts may be antagonistic. I hypothesized that the elongation of the female external spermaduct was used to hinder unwanted males from mating, and would result in counter-adaptations in the males' genitalia. I also predicted that dorsal ornamentation and the *h1* setae were used to disrupt the male's attachment via his adanal suckers - an interaction similar to that illustrated by dytiscid beetles (Bergsten & Miller, 2007). I used host phylogeny as proxy for mite phylogeny to look for directional trends in the evolution of these traits.

In the Proctophyllodidae, I did not find a correlation between the dimensions of the female docking papillae and the male adanal suckers. This opposes Witaliński *et al*. (1992) who reported strong similarities in size between these structures for other taxa of astigmatan mites. My results were unexpected, but not inexplicable. Firstly, Witaliński *et al*. (1992) suggest that during coupling the docking papillae swell within the adanal suckers. As the mites in my study were not in precopula when they were measured, measurements may not be representative of these structures when in precopula. Although, most of these mites were in precopula immediately prior to mounting, and thus this is likely not the only explanation. The findings of this study are largely preliminary, but do emphasize the need to observe live specimens to fully incorporate aspects of their reproductive biology. However, as feather mites require their host to complete their life cycle, direct observations of mites on their hosts are difficult (Haribal *et al*., 2011).

Similarly, the surface area of male adanal suckers and the degree of female dorsal ornamentation in *Trouessartia* spp. did not correlate in magnitude. I predicted that variations in ornamentation would be matched by adaptations in the male suckers to overcome their potential disruptive function. However, *Trouessartia* spp. fall amongst numerous other organisms that lack correlations

between the sexes in contact structures (Eberhard, 1985, 2001). The additional absence of correlation between the female *h1* setae and male adanal suckers was also unexpected. Whether the *h1* setae can be raised or moved facultatively to deter unwanted males (e.g. similar to erectable spines; Eberhard, 2004) in *Trouessartia* spp. is unknown. A further analysis of these structures must first be performed to determine their mechanics.

In contrast, female external spermaduct length was positively correlated with male genitalic size in *Trouessartia* spp. Thus, my first prediction for illustrating SAC was confirmed. My second prediction - that external spermaduct length would show directional trends - was not strongly supported. Though external spermaducts were recorded in all *Trouessartia* spp. in this study, there was not a clear progression from "short" to "long" spermaducts throughout the host phylogeny. Interestingly, external spermaducts extending past the interlobar membrane also appear to have evolved several times independently. The lack of strong evolutionary trends in genitalic morphology may be due to using the host phylogeny, which may not accurately reflect mite relationships. Though I cannot make assertions about directional selection acting on spermaduct length, I will venture that support for SAC in *Trouessartia* spp. is minimal. In concert with my study of dorsal ornamentation and *h1* setae, I see little evidence for an intersexual arms race in this genus. Additional information as to the reproductive biology and phylogenetic relationships of these mites is essential in further studies of SAC in this group.

4.3 Exceptions to the rule

I have considered sexual dimorphism and morphological differences between feather mites in both genitalic and non-genitalic contact structures, as well as the correlated evolution of these traits. What I have discerned from my results is that mites offer interesting exceptions to intersexual relationships that have been largely supported in other taxa. I believe there are two ways to regard these results. The first is that "exceptions" to the rule may be more common than reported; this may be due to there being relatively few studies evaluating these

concepts, or it may be due to potential publication bias. To my knowledge, only Eberhard (2004) has greatly contested SAC. Secondly, feather mites themselves might be the exception. Feather mites are highly specialized and very diverse, occupying numerous host habitats. Moreover, feather mites are only one small fraction of the diversity within the Acari (Krantz & Walter, 2009). This degree of diversity is uncommon in other taxa and makes mites excellent model organisms for the study of sexual selection and the correlated evolution of reproductive traits between the sexes.

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