SHORT COMMUNICATION

Multiple male morphs in the leaf-footed bug *Mictis longicornis* (Hemiptera: Coreidae)

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Abstract

Species within the coreid clade (Hemiptera: Coreidae) can often be observed competing in intrasexual competitions over access to mates and territories. Coreids that partake in these competitions typically possess sexually dimorphic hind legs that are used to strike and squeeze their rivals. In addition to their weaponized legs, some coreid species also possess sexually dimorphic abdominal tubercles, which are assumed to be sexually selected weapons. Still, much remains unknown about the morphology of these structures. Here, using the species *Mictis longicornis* Westwood, we investigate the frequency distribution and static allometry of abdominal thickness, a measure that includes tubercle length. Furthermore, we also investigate the morphological relationship between abdominal tubercles and weaponized hind legs. We find that male abdominal thickness is best explained by a bimodal distribution, thereby describing the first observed male polymorphism in the coreid clade; a phenomenon typically associated with alternative reproductive tactics. Additionally, we find that major males are characterized primarily by having large weaponized legs and abdominal tubercles, which further suggests that abdominal tubercles are used in male–male competition.

Key words: abdominal tubercle, dimorphism, Mictini, polymorphism, sexual selection.

Leaf-footed bugs (Hemiptera: Coreidae), as their common name implies, are well known for their morphologically elaborate legs (Baranowski & Slater 1986; Emlen 2008). While some leaf-footed bugs have legs that are foliaceous in form, many come in an array of other shapes and sizes. In fact, enlarged femurs and elongated spines characterize some of the most elaborated coreid legs. Often, legs that take this form are sexually dimorphic and are used by males in intraspecific competition over access to territories and females (Mitchell 1980; Fujisaki 1981; Miyatake 1993; Eberhard 1998; Procter *et al.* 2012). Therefore, these exaggerated legs are considered to be sexually selected weapons. Due to their elaboration, coreid hind legs have been the subject of multiple morphological studies (Miyatake 1997; Eberhard 1998; Miller & Emlen 2010; Miller *et al.* 2016; Sasson *et al.* 2016). However, the morphology of other exaggerated coreid traits has remained relatively unexplored.

In addition to their elaborate hind legs, many species in the tribe Mictini also exhibit exaggerated abdominal tubercles (O'Shea & Schaefer 1980). Like their enlarged legs, many of these abdominal tubercles appear to be sexually dimorphic (O'Shea & Schaefer 1980) and are thought to play a role in male–male competition (Tatarnic & Spence 2013). Thus, abdominal tubercles are assumed to be sexually selected weapons. In the context of weaponry, these tubercles are used when rival males adopt an escalated fighting position that places them ventrally abdomen-to-abdomen (Tatarnic & Spence 2013; Z.E. personal observation). When males assume this position their abdominal tubercles press against each other while their weaponized hind legs can wrap around and attack their rival's dorsal side (Tatarnic & Spence 2013; Z.E. personal observation). Besides the role of tubercles in

male–male combat, there is also some evidence to suggest that abdominal tubercles may be used to court females (Tatarnic & Spence 2013), potentially placing them centrally on the weapon-ornament spectrum (McCullough *et al.* 2016). Despite these behavioral observations, the morphology of abdominal tubercles has remained largely undescribed. Here, we investigate the frequency distribution and static allometry of median abdominal tubercle size in the coreid *Mictis longicornis* Westwood, 1842. Additionally, given that male abdominal tubercles are putative weapons or a trait that facilitates weapon use (i.e. weapon-support trait; Okada *et al.* 2012), we also investigate their morphological relationship with their weaponized hind legs.

To investigate the morphology of median abdominal tubercles in *M. longicornis* we used both wild-caught (*n* = 22; collected in 2016–2017) and Lee Kong Chian Natural History Museum specimens (*n* = 68). All specimens were collected in Singapore across many sites. Wild-caught specimens were measured to the nearest hundredth of a millimeter (maximum precision 0.01 mm) using Mitutoyo digital calipers. To prevent damage, museum specimens were measured with an ocular micrometer to the nearest tenth of a millimeter (maximum precision 0.1 mm). For each specimen, we recorded the sex and measured the maximum size of the hind right femur, pronotal width, and abdominal thickness. We used maximum abdominal thickness, which captures the projection length of the tubercle, because we wished to decrease subjective biases in identifying whether or not a tubercle was present (Fig. 1). We did not observe any major differences between the means of wild-caught and museum specimens for pronotal width (males, $F_{1, 49} = 1.003$, $P = 0.321$; females, $F_{1, 37} = 0.71$, $P = 0.405$), abdominal thickness (minor males, $F_{1, 10} = 1.075$, $P = 0.324$; major males, $F_{1, 37} = 0.759$, $P = 0.389$; females, $F_{1, 37} = 0.004$, $P = 0.95$), or hind right femur size (males, $F_{1, 49} = 0.071$, $P = 0.791$; females, $F_{1, 37} = 0.127$, $P = 0.724$). Thus, below, we present the results of the combined data (*n* = 90; 51 males and 39 females).

To investigate the distribution of abdominal thickness for males and females, we used the package *mixsmsn* (Prates & Cabral 2009) implemented in R v3.3.1 (R Core Team 2016) to fit finite mixture models that had 1, 2, or 3 skew-normal distributions to the data (Painting *et al.* 2015). We decided to include bi- and tri-modal distributions (i.e. 2 and 3 skew-normal distributions) because weapon size has previously been observed, in other taxa, to take bimodal and tri-modal forms (Rowland & Emlen 2009; Painting *et al.* 2015). All three models were then compared against one another using both Akaike and Bayesian information criterion (AIC and BIC, respectively) to determine the number of
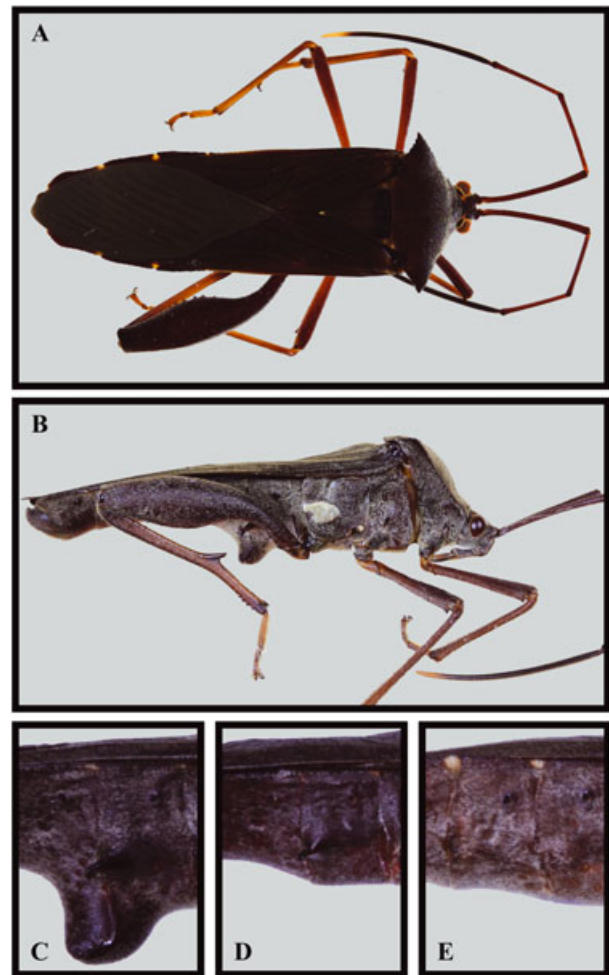

Figure 1 Habitus images of *Mictis longicornis*. Images A and B are, respectively, a dorsal and lateral habitus of a major male. Images C–E represent some of the variation in abdominal thickness. Specifically, image C is a representative of a major male morph, image D is a representative of a minor male morph, and image E is a representative of a female.

modalities that best explained the data. We considered the model with the lowest BIC and AIC values to be the best fitting model. However, models were only considered to have a significantly different fit from one another if their values differed by a score of 2 or more (Raftery 1995). If the data was explained by more than one skew-normal distribution (i.e. more than one modality) we then considered the trait to be polymorphic. We found that male abdominal thickness was best fit by two skew-normal distributions (Table 1; Fig. 2). Furthermore, both the AIC and BIC values of the bimodal model differed from the other models by a score of more than 2 (Table 1), allowing us to reject the unimodal and tri-modal models. Thus, we classify male abdominal tubercles as being dimorphic, having both major and minor males. Females, on the other hand, had an abdominal thickness

Table 1 Modality in the distributions of male and female abdominal thickness in *Mictis longicornis*

Number of Distributions	Male AIC	Male BIC	Female AIC	Female BIC
1	136.77	142.57	28.87[†]	33.86[†]
2	126.08[†]	139.6[†]	35.61	47.94
3	133.16	154.44	42.03	60.29

[†] Best fit models: male abdominal thickness was best explained by a bimodal distribution, while female abdominal thickness was best explained by a unimodal distribution.
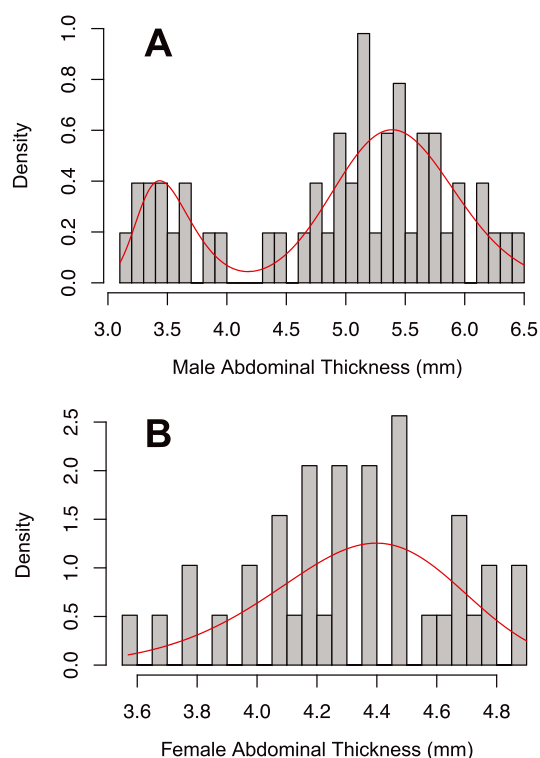

Figure 2 Distributions of abdominal thickness for (A) males and (B) females of *Mictis longicornis*. Male abdominal thickness was best explained by a bimodal distribution, while female abdominal thickness was best explained by a unimodal distribution (see also Table 1).

that was best explained by a unimodal distribution (Table 1; Fig. 2). As depicted in Figure 1, major males have an overt abdominal protrusion, while minor males and females appear to be completely missing this trait.

Since we found the males to be dimorphic, we assigned males into morphs based on a threshold abdominal size (Kotiaho & Tomkins 2001) before investigating the static allometry of abdominal thickness for males and females. Once morphs were assigned, we log-transformed our data (Huxley 1932) and calculated allometric slopes (i.e. the scaling relationship between a trait and body size) using ordinary least squares (Kilmer & Rodríguez 2017) in the R package *smatr* (Warton *et al.* 2012). For studies of

allometry, statistical theory recommends the use of ordinary least squares (OLS) regressions over the use of reduced major axis (RMA) regressions because a RMA regression does not describe (with some rare exceptions) the functional scaling relationship between a trait and body size (Kilmer & Rodriguez 2017). We then ran ANCOVAs to compare the OLS regressions (i.e. allometric slopes) of minor males, major males, and females. Major males possessed an allometric slope of 1.027 ± 0.407 for abdominal thickness, while minor males possessed an allometric slope of 0.406 ± 0.744. However, neither slope significantly deviated from isometry (major males, $r = 0.022$, df = 37, $P = 0.892$; minor males, $r = -0.490$, df = 10, $P = 0.106$). Conversely, with a slope of 0.488 ± 0.333, females possessed an allometry that deviated significantly from isometry ($r = -0.456$, df = 37, $P = 0.003$), making female abdominal thickness negatively allometric. Compared to one another (minor males *vs.* major males *vs.* females; Fig. 3), we only found that the allometry of abdominal thickness differed significantly between major males and females ($F_{1, 74} = 4.349$, $P = 0.040$). While the allometric slopes also appeared to differ between major males and minor males, both relationships are quite variable and hence this was not statistically supported ($F_{1, 47} = 2.375$, $P = 0.130$). We believe this result may stem, in part, from having small sample sizes, especially within the minor morph classification ($n = 12$).

Before investigating the relationship between weaponized hind legs and abdominal tubercles, we also investigated the frequency distribution and static allometry of *M. longicornis* hind leg size using the same methods described above. Hind leg width for both males and females was best explained by a unimodal distribution. Thus, we did not take male morphological classification (i.e. major *vs.* minor morphs) into consideration when we calculated hind leg allometry. Male hind legs had a steep positive allometry (estimated slope = 1.54 ± 0.411) that deviated from isometry ($r = 0.353$, df = 49, $P = 0.011$). Female hind legs, on the other hand, did not significantly deviate from isometry (estimated slope = 0.775 ± 0.395; $r = -0.186$, df = 37, $P = 0.258$), but the female slope did significantly differ from the male hind leg allometry ($F_{1, 86} = 16.681$, $P < 0.001$; Fig. 3).

Finally, using just males, we investigated the relationship between abdominal tubercles and weaponized hind legs. When we directly regressed one against the other we visually observed a sigmoidal scaling relationship (Fig. 4), which suggests that tubercle presence is linked to weaponized leg size. Therefore, we further investigated whether leg size or body size was better at classifying major and minor morphs. Specifically, since
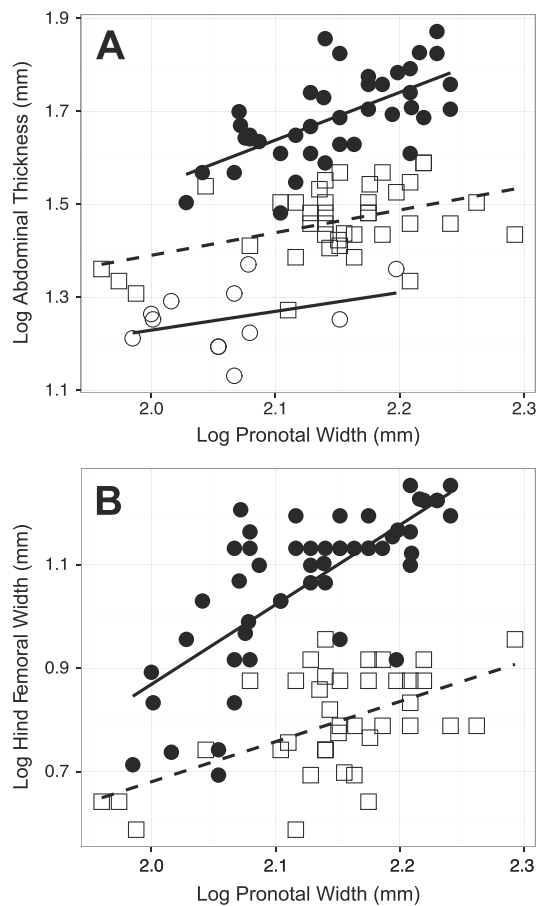
Figure 3 Abdominal thickness and hind femoral width allometry. Panel A depicts abdominal thickness allometry for major males (closed circles), females (squares), and minor males (open circles). While the allometric slopes (calculated using ordinary least squares regressions) of minor males and females were similar, only the allometry of female abdominal thickness statistically differed from the allometry of major male abdominal thickness. We believe that this result is a consequence of small sample sizes for our minor morphs classification ($n = 12$). Panel B depicts hind femoral width allometry for males (circles) and females (squares), which significantly differed from one another.
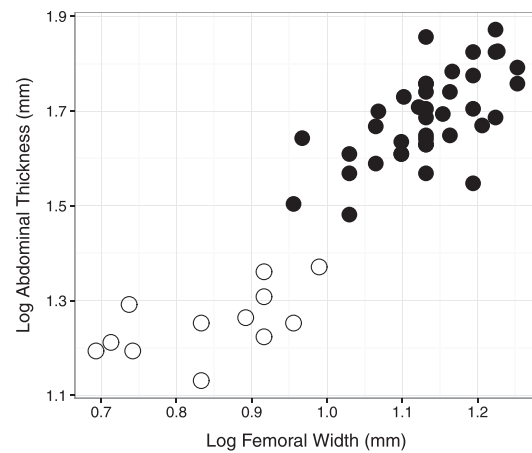

Figure 4 Relationship between weaponized hind femurs and abdominal thickness. Closed circles represent major males, while open circles represent minor males.

In summary, our morphological analyses of *M. longicornis* have shown that there are two classes of males, major and minor. Major males are primarily characterized by having a median abdominal tubercle and large weaponized legs. Minor males, on the other hand, lack a median abdominal tubercle and have small hind legs. Thus, here, we are the first to describe the occurrence of male polymorphism in the coreid clade; a phenomenon typically associated with alternative reproductive tactics.

To elaborate, we quantified dimorphism in *M. longicornis* median abdominal tubercles, a trait assumed to be under sexual selection. When it comes to direct competition, a small or absent sexually selected trait should provide little to no benefit (Buzatto *et al.* 2014). Therefore, for the presence of discrete morphs to be maintained, minor males are expected to find alternative ways to secure reproductive success (Goldsmith 1987; Gross 1996; Emlen 1997; Oliveira *et al.* 2008; Buzatto *et al.* 2014). Consequently, male dimorphism is frequently associated with alternative reproductive tactics. Horned dung beetles provide a clear example of this association (Rasmussen 1994; Emlen 1997; Moczek & Emlen 2000). In this clade, when male dimorphism is present, horned (major) males can be observed guarding tunnel entrances where they fight rivals over access to females (Rasmussen 1994; Emlen 1997). Alternatively, avoiding direct male–male combat, hornless (minor) males use a sneaking behavior to bypass the guarding male and obtain a mating opportunity (Rasmussen 1994; Emlen 1997). Like horned dung beetles, we hypothesize that *M. longicornis* minor males use a mating tactic that avoids direct competition. Additionally, building off previous abdominal tubercle research in Mictini (*M. profana*; Tatarnic & Spencer 2013), we hypothesize that

we *a priori* had decided which morph a male should be assigned, we used a linear discriminant analysis in the R package *MASS* (Venables & Ripley 2002) to investigate whether log-transformed body size or log-transformed weaponized leg size was a better predictor of whether or not a male would have an abdominal tubercle. Of these two variables, we found weaponized leg size to be better at classifying major and minor morphs (linear discriminant coefficient of 15.10, compared to −6.42). Additionally, we used a jackknife resampling technique to cross validate the model (i.e. leave-one-out cross validation) and found that hind femur size correctly classified male morphs 94.12% of the time, while body size correctly classified morphs only 74.51% of the time.

M. longicornis major males use a mating strategy that includes both hind legs and abdominal tubercles. We further hypothesize that these abdominal tubercles are important to male–male competition since we found a strong association between weaponized legs and abdominal tubercles.

In light of these findings, future research on abdominal tubercles could substantially contribute to our understanding of sexually selected weaponry and mating behavior evolution within coreids. Specifically, future work should investigate how abdominal protrusion morphology, and associated behaviors, have evolved throughout the tribe Mictini. Additionally, the association between weaponized legs and abdominal protrusions makes the Mictini an intriguing group for future weapon development studies.

ACKNOWLEDGMENTS

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