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Behavior

Coreidae (Insecta: Hemiptera) Limb Loss and Autotomy

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Abstract

Autotomy, induced limb loss, is a trait that is effectively used throughout the animal kingdom to avoid predation and entrapment, and has independently evolved multiple times. Within the insect clade, species have been observed autotomizing legs, antennae, cerci, and caudal filaments. However, our knowledge of which species autotomize and the frequency of limb loss in natural populations is quite limited. Understanding autotomy's diversity can provide key insights into how this extreme trait has evolved and is a first step in understanding the costs and benefits of this behavior. Here, we quantify the frequency of leg loss and investigate the ability to autotomize in nine coreid (Insecta: Hemiptera: Coreidae) species (*Euthochtha galeator* F., *Anasa andresii* Guérin-Méneville, *Anasa tristis* DeGeer, *Narnia femorata* Stål, *Chelinidea vittiger* McAtee, *Leptoglossus phyllopus* L., *Acanthocephala declivis* Say, *Acanthocephala terminalis* Dallas, and *Acanthocephala femorata* F.), across five tribes. In wild populations of these species, limb absence ranged from 7.9 to 21.5%. In the lab, all nine species investigated were able to autotomize. These discoveries are particularly interesting because some coreids use their hind legs in intrasexual competitions for access to females; therefore, autotomy in coreids can mean permanently losing a sexually selected weapon. These observations provide a more complete understanding of autotomy's diversity while also developing new hypotheses regarding the interaction between autotomy, sexual selection, and natural selection.

Key words: Hemiptera, Coreidae, autotomy, elaborated hind limb, sexually selected weapon

Throughout the animal kingdom organisms can be observed releasing limbs (i.e., autotomizing) to avoid predation and entrapment, increasing their immediate survival (Maginnis 2006, Fleming et al. 2007). However, autotomizing can also have long-term consequences related to locomotion (Bateman and Fleming 2005), reproduction (Uetz et al. 1996), and longevity (Figiel et al. 1995). Still, the ability to escape by releasing a limb appears to be strongly selected for, as autotomy has independently evolved multiple times (Wasson et al. 2002, Fleming et al. 2007). Understanding autotomy's taxonomic diversity can provide key insights into how this extreme trait has evolved and can help us to understand the consequences of autotomy on life history, morphology, and behavior. While recent reviews have highlighted some of the taxa in which autotomy occurs (Fleming et al. 2007), gaps in our knowledge limit our understanding of autotomy's diversity. In an attempt to fill one of these gaps, we investigate whether insects within the Coreidae clade possess the ability to autotomize their legs.

Coreids (Hemiptera: Coreidae), commonly referred to as leaf-footed bugs, are known for their diverse hind leg morphology (Baranowski and Slater 1986, Brailovsky 2006, Emlen 2008), which can include enlarged femurs, tibial flags, and spines (Fig. 1). In some cases, elaborate legs are weapons (Mitchell 1980, Fujisaki 1981,

Miyatake 1993, Eberhard 1998, Procter et al. 2012), but in others, their only known function is for locomotion. The elaboration and unique function of hind legs in some species, but not in others, make this group of particular interest in the evolution of autotomy. For instance, losing a leg in some species may mean the loss of a sexually selected weapon (Mitchell 1980, Fujisaki 1981, Miyatake 1993, Eberhard 1998, Procter et al. 2012), and may thus have drastic effects on mating success (Smith 1992). Therefore, identifying patterns in the ability to autotomize can provide important insights into the evolution of coreid hind leg function.

Previously, missing limbs have been noted in wild coreid populations (Eberhard 1998). However, the prevalence of limb loss has yet to be quantified. Thus, our first aim was to identify the frequency of limb loss across multiple species of wild coreid populations. Furthermore, since other Hemipterans have been observed to autotomize limbs (Bulliére and Bulliére 1985, Fleming et al. 2007), our second aim was to identify whether coreids possessed this ability as well. Personal observations had revealed that males and females, for all of the investigated species, had limbs missing at the trochanter-femur joint (Z.E., personal observation). Therefore, we hypothesized that all nine species possessed the ability to autotomize and would do so at this joint.
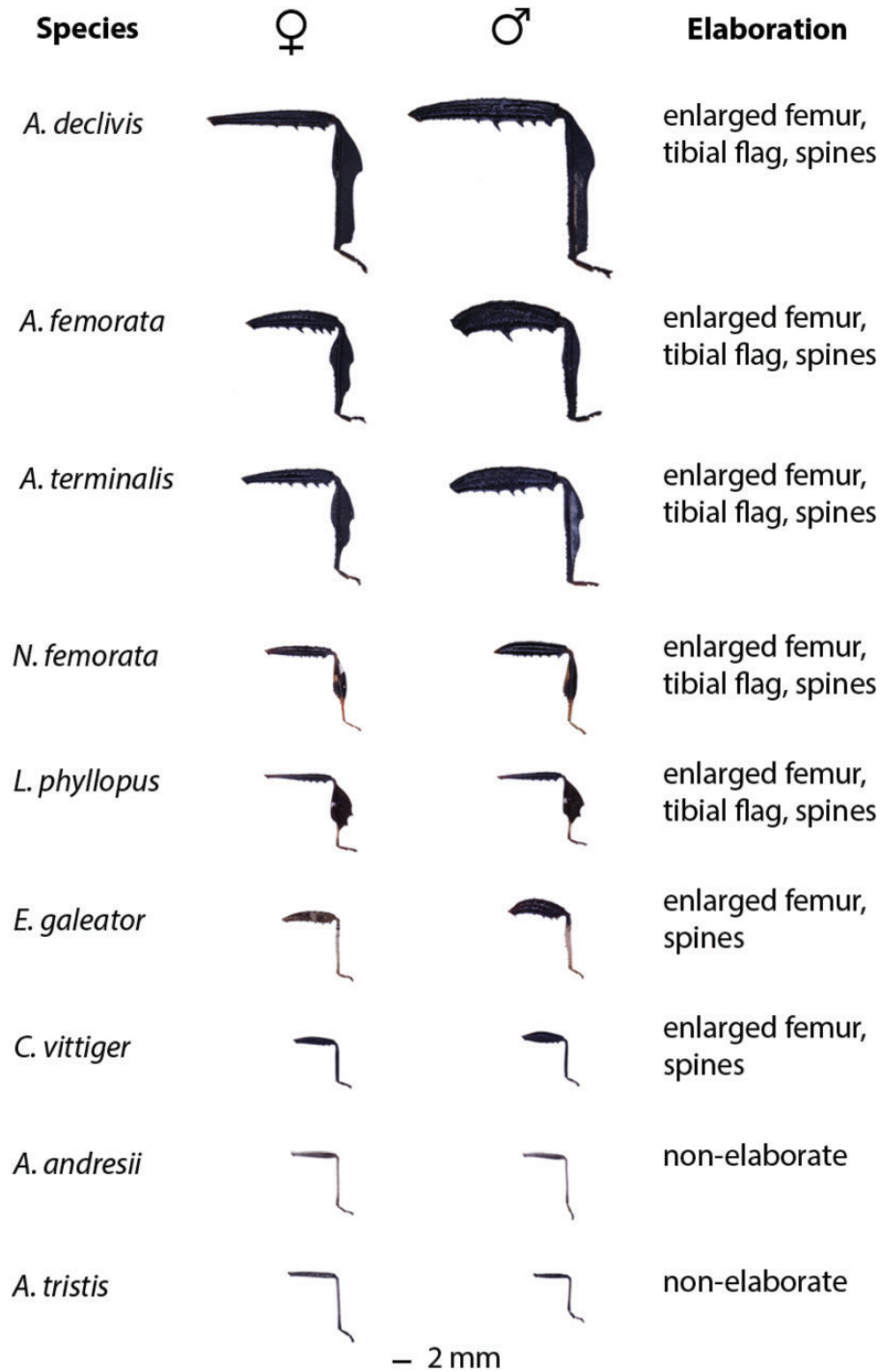
Fig. 1. Representative hind legs of each species in this study, to scale. Morphologically, across the coreid clade, hind legs vary in size, shape, and color. Hind legs can also vary between males (right) and females (left). To create the leg images above, legs were removed at the joint between the trochanter and femur.

Materials and Methods

In an attempt to capture some of the morphological, and potentially behavioral, diversity found within the coreid clade, we investigated the ability to autotomize across multiple genera (*Euthochtha galeator* F., *Anasa andresii* Guérin-Méneville, *Anasa tristis* DeGeer, *Narnia femorata* Stål, *Chelinidea vittiger* McAtee, *Leptoglossus phyllopus* L., *Acanthocephala declivis* Say, *Acanthocephala terminalis* Dallas, and *Acanthocephala femorata* F.). Coreid adults were collected opportunistically throughout north central Florida in 2015, from June to September. Upon collection, limb presence and limb location (i.e., front, middle, and hind) was recorded for each individual. A sample of intact individuals, $n = 20$ per species (10 males and 10 females), were then used to experimentally quantify autotomy, in each case within 48 h of capture. Autotomy induction tests were performed by gripping the insect's hind right femur with forceps, for up to an hour, while the insect was in contact with a

piece of wood (38 by 44 by 305 mm). By holding their leg, individuals could only escape by releasing their trapped limb (Supp. Video 1 [online only]). If individuals autotomized, the location at which autotomy occurred was recorded.

Historically, the term autotomy has been used in a variety of contexts that reflect the manner in which the appendage is lost (Wood and Wood 1932, Maginnis 2006, Fleming et al. 2007). Here, we define autotomy as limb loss that 1) morphologically occurs at a specific breakage plane, and is 2) behaviorally used to escape. Therefore, to identify the presence of autotomy in a species, at least two individuals would have to drop their entrapped hind leg. While one individual would sufficiently demonstrate the ability to induce limb loss, a second individual allows us to confirm whether limb loss is occurrent at a specific breakage plane.

In addition to autotomizing limbs to escape from predators, within arthropods, autotomy can also occur to escape from a fouled molt (Wood and Wood 1932, Smith 1992, Foelix 1996, Maginnis 2008). In the lab, limb loss is still observed in coreids, although it is not as common as limb loss in wild populations (Z. E., unpublished data). Furthermore, when lab-reared individuals are missing a limb, the missing limb is often found stuck within their previous molt (Fig. 2), suggesting that coreids, like other arthropods, also use autotomy to escape a fouled molt. However, it remains unclear how quickly an individual will autotomize a limb in this scenario. Nonetheless, since limb loss in coreids is also thought to be due to predation, we also noted whether or not each individual autotomized within the first 60 s.

To analyze our data, we used Fisher's exact tests or, if appropriate (i.e., if the expected values for each category were 5 or more), a chi-square test. All of our analyses were conducted using R statistical software, version 3.1.2 (R Core Team 2014).

Results

Missing Legs in Wild Populations

In each species of coreid, we observed individuals (at least one male and one female) with missing legs in wild populations (Fig. 3). However, the frequency of missing leg varied across species (Fisher's exact test, $P = 0.002$), as did the position (i.e., front, middle, and hind) of the missing legs. In general, the missing legs were disproportionately those in the hind leg position, which is also the location of the greatest limb elaborations in the species sampled (Table 1). *L. phyllopus* had the highest rate of limb absence at 21.5%, while *E. galeator* had the lowest observed rate at 7.9%. All of the species investigated, except *A. terminalis*, *E. galeator*, and *A. tristis*, had at least one individual missing two limbs, while *N. femorata*, *C. vittiger*, and *L. phyllopus* all had one individual missing three limbs (Fig. 3). We did not observe any individual to be missing four or more limbs. Furthermore, we observed a significant sex difference in the number of individuals missing limbs for *A. declivis* (Fisher's exact test, $P = 0.015$).

Experimental Induction of Autotomy

We found that all nine species had the ability to autotomize and did so at the trochanter–femur joint (the proximal break for all legs featured in Fig. 1). Behaviorally, autotomy also appeared to be stereotypic; individuals would raise their abdomen and then drop it, shearing their limb from their body (Supp. Video 1 [online only]). Although autotomy was stereotypic and widespread, species varied in their expression. Remarkably, in our "one-hour escape from entrapment" scenario, autotomy was observed for all 20 individuals we tested in *A. andresii*, *A. tristis*, *N. femorata*, *C. vittiger*, and *L. phyllopus*. While we did not observe autotomy in every individual for the other four species, our criterion of two individuals

Fig. 2. Coreids can autotomize limbs to escape fouled molts. An adult *Narnia femorata* with its missing leg in its terminal exuviae.
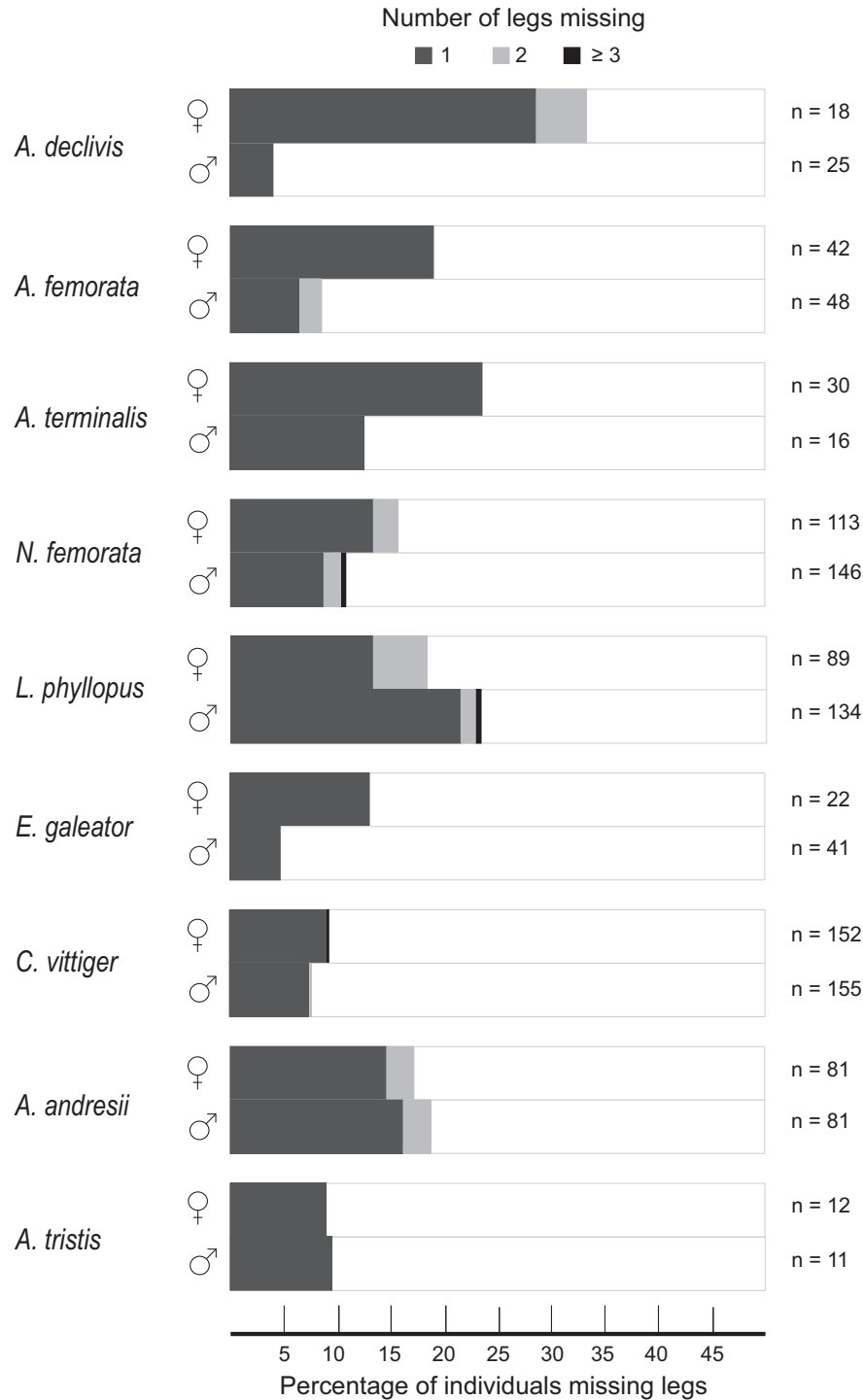
Fig. 3. Percentage of wild-caught coreid adults observed missing legs. Exact numbers, proportions, and analyses performed can be found in the supplementary material (Supp. Table 1 [online only]).

autotomizing per species was well exceeded (Supp. Table 2 [online only]). Interestingly, autotomy was not observed for males (but it was for females) in *A. terminalis* and *A. declivis* during our "one-hour escape from entrapment" scenario. Additionally, we also observed a significant sex difference in the number of individuals that experimentally autotomized for all of the *Acanthocephala* spp. (Supp. Table 2 [online only]). Furthermore, two or more individuals of each species autotomized a limb at the trochanter–femur joint within 60 s (Supp. Table 3 [online only]). However, in this scenario,

we did not observe any sex differences in the number of individuals that experimentally autotomized (Supp. Table 3 [online only]).

Discussion

We found that all nine species of coreids autotomized, and these results were consistent whether we looked at autotomy within 60 s or within an hour. While the ability to autotomize was found within

Table 1. Percentage of missing limbs by limb location

Species	n	Missing leg position (%)			P
		Hind leg	Middle leg	Front leg	
Elaborated hind legs					
A. declivis	8	87.5	12.5	0.0	0.084
A. femorata	13	69.2	30.8	0.0	0.077
A. terminalis	9	77.8	22.2	0.0	0.088
N. femorata	41	75.6	19.5	4.9	**<0.001**
L. phyllopus	56	26.8	42.9	30.4	0.565
E. galeator	5	60.0	20.0	20.0	1.000
C. vittiger	29	69.0	20.7	10.3	**0.019**
Total	161	57.1	29.8	13.0	**<0.001**
Nonelaborated hind legs					
A. andresii	33	45.5	42.4	12.1	0.119
A. tristis	2	50.0	0.0	50.0	1.000
Total	35	45.7	40.0	14.3	0.169

We compared our observed wild-caught data against the null hypothesis that leg absences should be evenly distributed amongst leg positions (hind, middle, and front). In general, missing legs were not evenly distributed, as some species with elaborated hind legs were disproportionally missing hind legs.

each species, we also observed sex differences in the propensity to autotomize for *A. terminalis*, *A. femorata*, and *A. declivis*. In fact, we did not experimentally observe a single male autotomizing for *A. terminalis* nor *A. declivis*. However, wild-caught specimens revealed missing limbs for both males and females for every species investigated. Additionally, in wild coreid populations, the frequency of missing limbs varied across species (Fig. 3), within species (e.g., sex difference in *A. declivis*; Fig. 3), and at the individual level (i.e., by limb location; Table 1). These results set the stage for further study of autotomy in this clade and for comparative work with other clades.

Autotomy and Sexually Selected Weaponry

Some coreids (e.g., *N. femorata*, *A. femorata*, and *A. declivis*), with sexually dimorphic hind femurs, have been observed using their legs in intraspecific competitions over territories and females (Mitchell 1980, Fujisaki 1981, Miyatake 1993, Eberhard 1998, Procter et al. 2012), suggesting that their hind legs are sexually selected weapons. Thus, by demonstrating that coreids can autotomize their legs, we have also demonstrated that at least some coreids can drop their sexually selected weapons. The ability to drop a sexually selected weapon is not uncommon: cervids shed their antlers (Goss 1983), antilocaprids drop their horns (Kitchen 1974), and crustaceans can release their claws (Smith 1992). However, the previously mentioned taxa will regenerate their weapons if given another breeding season and/or have molts remaining in their life cycle. Adult coreids, like all hemimetabolous insects, have completed development and cannot regrow lost structures. Therefore, when a coreid adult autotomizes its weapon, the weapon is permanently lost. Sexually selected weapons are usually important to an individual's reproductive success (Anderson 1994), making a permanent loss of a weapon counterintuitive and particularly costly to overall fitness. Thus, while the animal may use autotomy to survive an otherwise fatal event, it may not be able to successfully secure any future reproductive fitness.

If these weapons are indeed important for reproductive success, then selection should act on individuals to retain them. One way weapons could be retained is through differential autotomy.

Previously, in the field cricket *Gryllus bimaculatus* De Geer, a female's mating history was shown to affect the willingness to autotomize a limb that helps with locating mates, suggesting that the reproductive cost of losing a limb can affect the propensity to autotomize (Bateman and Fleming 2006). In principle, a similar difference in reproductive cost might explain why we did not observe any males in *A. terminalis* or *A. declivis* autotomizing. However, it is also possible that our observational period was not long enough to detect their ability to autotomize (e.g., males might autotomize after an hour). Still, our observations demonstrate that there is at least a sex difference in the propensity to autotomize in all three *Acanthocephala* spp. Future research should investigate the degree to which the ability and timing of autotomy correlates with sexual dimorphism and the strength of sexual selection for weaponry across the coreid clade and across other groups of animals.

Autotomy and Natural Selection

The elaborate hind legs found in many coreid species are not exclusively used as weapons in male–male competitions. Certainly, they have a locomotory function. In addition, they may reduce predation in some species and in some situations. For example, the passiflora bug, *Anisoscelis flavolineata* Blanchard, has brightly colored legs that extend in response to disturbances (C.W.M., personal observation). These large legs may play a role in survival by directing predators to attack their legs, as opposed to their body. This risky-decoy hypothesis (Bateman et al. 2014) becomes even more plausible now that we have confirmed that coreids autotomize. Alternatively, these elaborate hind legs could play a role in conspecific signaling. Nonetheless, since elaborate hind legs in coreids are disproportionally absent from wild populations, relative to other legs (Table 1), our data suggest that elaborate hind legs may be more costly to maintain. Thus, it is also possible that autotomy evolved as a cost-reducing mechanism (Møller 1996). Future research should explore the role autotomy has had in driving the form of coreid hind legs, as well as more broadly, identifying how selection differentially acts on autotomizable limbs. For example, to what degree does having the ability to autotomize a limb change the limbs form? Does having the ability to drop these limbs allow them to become more elaborate and conspicuous, or less? Additionally, future scientific gains are likely to be made by investigating autotomy during juvenile development, where species may posses the ability to regenerate their lost limb or weapon or reallocate the energy elsewhere.

Acknowledgments

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