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Original Article

The pronotum shape of scelimenine grasshoppers (Orthoptera: Tetrigidae) likely represents an exaptation for heterogeneous niche colonization

Fran Rebrina[a,*,], Andreja Brigić[a], Niko Kasalo[b], and Josip Skejo[a,c,*,**]**

[a]University of Zagreb, Faculty of Science, Department of Biology, Division of Zoology, Horvatovac 102a, HR-10000 Zagreb, Croatia
[b]Ruđer Bošković Institute, Division of Molecular Biology, Laboratory of Evolutionary Genetics, Bijenička cesta 54, HR-10000 Zagreb, Croatia
[c]Leibniz-Institut zur Analyse des Biodiversitätswandels, Standort Hamburg, Martin-Luther-King-Platz 3, 20146 Hamburg, Germany

[*]Address correspondence to Fran Rebrina. E-mail: fran.rebrina@biol.pmf.hr; Josip Skejo. E-mail: josip.skejo@biol.pmf.hr
[**]These authors contributed equally to this work.
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Abstract

Insects are suitable model organisms for functional morphology research, especially in the context of exaptation, when the same morphological trait represents an advantage in disparate niches. Phylogenetically distant groups of pygmy grasshoppers (Orthoptera: Tetrigidae) have various pronotal projections defining their general appearance and body shape. However, body shape has never been related to niche occupation in these insects, thus the aim of this study is to investigate the relationship between pronotum shape and macrohabitat adaptation in Scelimeninae, a group of Asian and Papuan tetrigids encompassing amphibious and corticolous (bark-dwelling) representatives. With the use of geometric morphometrics and phylogenetic comparative methods, two morphological and functional groups were distinguished, with the body shape exhibiting a significant phylogenetic signal. The first group consists of elongated amphibious taxa (Scelimenini tribe) with highly uniform pronotum morphology, likely due to a strong selection for streamlined body shape. Stouter corticolous taxa (Discotettigini tribe) exhibit more conspicuous body shape variability, possibly increasing camouflage efficiency in tree bark habitats. Ecological divergence associated with macrohabitat adaptation may thus have been the primary driver of speciation in this insect group, but the evolutionary constraints leading to this divergence are still to be identified.

Key words: amphibious, corticolous, functional morphology, geometric morphometrics, insect, phylogeny.

Niche divergence is among the most important drivers of speciation and ecological diversification, playing an important role in both sympatric and allopatric events (Pearman et al. 2008). Namely, adaptive evolution occurs within the environmental framework approximated by the ecological niche of a species (Grimaldi and Engel 2005; Holt 2009), which can show high uniformity across related taxa and/or through evolutionary history (niche conservatism), but may also exhibit shifts over evolutionary time (Pearman et al. 2008; Holt 2009). From the perspective of niche conservatism, ecological speciation occurs along niche gradients, with closely related lineages occupying the closest available analogs of the ancestral niche, gradually leading to niche divergence in heterogeneous or rapidly changing habitats (Pyron et al. 2015). The adaptive potential is thereby primarily associated with trait variability, which is, in turn, closely related to the breadth of the ecological niche (Svanbäck and Schluter 2012). A narrower niche generally implies stronger evolutionary constraints and thus a more uniform phenotype, whereas a broader niche allows for higher phenotypic variation (Bolnick et al. 2007; Svanbäck and Schluter 2012). Nevertheless, although previously considered evolutionary dead-ends, specialized lineages can also exhibit directional niche shifts, either expanding or contracting over evolutionary time (Day et al. 2016; Sexton et al. 2017).

Colonization of disparate niches may sometimes be predisposed by the same phenotypic trait. In such cases, speciation occurs through exaptation, that is, a phenotypic trait provides a selective advantage in a novel context, distinct from its original function (Gould and Vrba 1982; Clemente 2014). The often-cited example of the role of feathers in bird flight (with a likely original role in thermoregulation; Gould and Vrba, 1982) suggests that exaptation can bring about key innovations (Clemente 2014), introducing new ways of interacting with the environment and thus often leading to adaptive radiation (Gillespie et al. 2020). The current literature provides a number of examples of exaptation across animal taxa and evolutionary contexts, including the colonization of terrestrial environments by tetrapods (Triques and Christoffersen 2009) and marine environments by snakes (Gearty et al. 2021), the evolution of bipedal running in lizards (Clemente 2014) and escape behavior from flash floods in water bugs (Lytle and Smith 2004). From the perspective of functional morphology, as the same trait becomes subject to different evolutionary constraints, its relationships with other morphological traits changes, driving morphological divergence

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as a result of adaptation (Moraes et al. 2004; McGuigan et al. 2005). Such changes are often better reflected by overall body shape than by individual morphological traits (McGuigan et al. 2005; Foster et al. 2015). Accordingly, geometric morphometrics has become a powerful and widely adopted tool in recent years for obtaining body shape data that can be used to detect phylogenetic signal associated with phenotypic variation (Adams et al. 2004; Rodríguez-González et al. 2017). In particular, finding a correlation between a phenotypic trait (such as body shape) and a function in an evolutionary context can provide interesting insights into natural history, sometimes offering answers to long-standing questions in biology (Caro et al. 2014; Kelley et al. 2015).

Encompassing numerous fascinating examples of diversification, insects represent a suitable model group for evolutionary research (Grimaldi and Engel 2005) looking into the patterns of morphological divergence in closely related taxa (Moraes et al. 2004). However, the vast majority of publications focus on several best-studied insect groups including *Drosophila* flies and mosquitos (Diptera), various groups of beetles (Coleoptera), bees, and ants (Hymenoptera), while other diverse and functionally important groups such as grasshoppers and crickets (Orthoptera) remain largely unexplored from this aspect. According to a review by Tatsuta et al. (2018), less than 3% of the studies using geometric morphometrics have included this insect order (with only one out of 472 articles looking into thorax shape), despite grasshoppers being useful model organisms for examining links between selection and structural variation (O'Connor et al. 2021).

This is particularly true of the pygmy grasshoppers (Tetrigidae), a family more than 200 million years old (Song et al. 2015), encompassing numerous morphologically distinct groups, including twig-like, leaf-like and stone-like taxa (Silva et al. 2019). A unique feature of this grasshopper family is an elongated pronotum that covers most of the body, including hind wings (Musiolek and Kočárek 2017; Tumbrinck 2019), and is, therefore, a good proxy for body shape. Its original function may have been defence against predators (Honma et al. 2006) coupled with providing advantages in wet environments close to waterbodies, possibly reducing the susceptibility to being washed away by floods (Musiolek and Kočárek 2017), increasing buoyancy and/or allowing underwater respiration (Musiolek et al. 2017). In the current study, we focus on Asian and Papuan subfamily Scelimeninae, which contains representatives with either amphibious or corticolous (bark-dwelling) lifestyles (Skejo et al. 2022). Freshwater and tree bark represent two substantially different environments, each with its own particular evolutionary constraints; a combination of biotic and abiotic pressures has likely resulted in consistent morphological differences between amphibious and corticolous taxa that should be reflected in their body shape. However, the relationship between body shape and macrohabitat adaptation has not been studied yet in a phylogenetic context, neither in this group nor in Tetrigidae in general.

Therefore, the first aim of this study was to investigate whether and how the pronotum shape (as a proxy for body shape) differs between amphibious and corticolous representatives of Scelimeninae, using geometric morphometrics. Because ecological specialization is commonly associated with adaptive changes in morphology, we expected to find substantial differences in body shape between the two groups. Secondly, we aimed to map the pronotum shape determined by geometric morphometrics onto the reference phylogenetic tree of Scelimeninae and test whether body shape exhibits a phylogenetic signal in this group. We expected to find a significant phylogenetic signal, with pronotum morphology reflecting phylogeny as a result of particular evolutionary constraints on body shape associated with lifestyle divergence.

Materials and Methods

Taxa and traits

Dataset

The current study includes the representatives of all the major genera within the subfamily Scelimeninae (Orthoptera: Tetrigidae), altogether 54 taxa (53 species, one of which included 2 subspecies; Table 1), amounting to about half of the total number of species in the subfamily (Skejo et al. 2022). A male and a female belonging to the genus *Tagaloscelimena*, incorrectly identified as *Tefrinda palpata* (Stål, 1877) in the Orthoptera Species File (Cigliano et al. 2022), could not be identified at the species level due to the lack of photographs in lateral view. Each species was annotated with amphibious 1) or corticolous 2) lifestyle, based on literature data (Hancock 1904, 1907; Günther 1938, 1955; Meer Mohr 1941; Bhalerao and Paranjape 1986; Paranjape and Bhalerao 1994; Ito et al. 2005; Storozhenko and Dawwrueng 2015; Zha et al. 2016, 2017; Muhammad et al. 2018; Adžić 2021; Adžić et al. 2022; Regul 2022), iNaturalist and Flickr observations, and expert knowledge. Photographs of dry-mounted individuals from the dorsal aspect were obtained from the Orthoptera Species File Online (Cigliano et al. 2022), encompassing mostly type material from museum collections (listed under specimen metadata in Cigliano et al. 2022). The number of individuals per taxon varied from one to five (depending on availability), but for most species, two to three individuals were included in the analyses. Data were obtained from 100 individuals in total (50 amphibious, 50 corticolous; Table 1).

Landmarks

Landmarking was performed in tpsDig ver. 2.31 (Rohlf 2021). Altogether 18 fixed points (landmarks) on the dorsal portion of the pronotum were selected in order to describe the pronotal shape, as a proxy for body shape (Figure 1): **1**—the most prominent part of the median carina of the pronotum at the anterior margin; **2/18**—anterior tips of prozonal carinae, that is, first frontolateral projection (FL1); **3/17**—anterior tips of the extralateral carinae, that is, second frontolateral projection (FL2); **4/16**—anterior most prominent tip of the lateral spines, that is, tip of the ventrolateral projection (VL); **5/15**—anterior ventrolateral sinus, that is, the one before the posterior ventrolateral plate; **6/14**—posterior ventrolateral sinus, that is, the one after the posterior ventrolateral plate; **7/13**—the beginning of the humero-apical carina, at the end of prozona and the beginning of metazona; **8/12**—the widest point of the shoulders (humeral region), that is, the most prominent part of the mediolateral projection (ML); **9/11**—lateral carina of the pronotum on the base of the hind femora; **10**—posterior tip of the pronotum.

Geometric morphometrics

Principal component analysis

After landmarking, generalized Procrustes analysis (GPA) was performed in tpsRelw ver. 1.70 (Rohlf 2019) to achieve optimal alignment among the corresponding landmarks, regardless of size, location, and orientation (Rohlf and Slice 1990). Centroid size estimates were computed for each specimen. Landmark configuration and average shape change between amphibious and corticolous Scelimeninae were visualized using a wireframe graph in MorphoJ (Klingenberg 2011). The variation in body shape within the dataset was then visualized using principal component analysis (PCA) based on a covariance matrix in MorphoJ (Klingenberg 2011), by plotting the scores along the first two principal components (Fruciano et al. 2014).

Group comparison

Multivariate regression of the symmetric shape component on centroid size with 10,000 randomizations was performed to account for the variation due to allometry (effect of size on body shape; Klingenberg 2016). It was followed by discriminant function analysis (DFA) with leave-one-out cross-validation, widely adopted in geometric morphometrics research (Silos et al. 2015; Liuti and Dixon 2020), to compare regression residuals between the groups (amphibious *vs.* corticolous) using a Procrustes distance-based permutation test with 1,000 permutation runs. In this way, it was possible to test for differences in body shape while controlling for the effects of body size. All of the above-mentioned analyses were done in MorphoJ (Klingenberg 2011), with a significance level set to 0.05.

Phylogenetic analysis

Cladistic analysis

To independently test phylogenetic relationships within Scelimeninae, a matrix containing 26 morphological traits of the head and legs in 57 taxa: 25 Scelimenini and 29 Discotettigini (see *Dataset* above) and three outgroup taxa (*Falconius deceptor* Günther, 1938, *F. inaequalis* (Brunner von Wattenwyl, 1893) and *Saussurella decurva* Brunner von Wattenwyl, 1893) were created (Supplementary Tables S1, S2). Unlike pronotum morphology, head and leg characters are not subject to strong selection pressure and are thus likely to reflect time-dependent changes (Imai et al. 2016). Because pronotum shape was used as a proxy for body shape (see above), pronotum traits were not coded in order to avoid a circular analysis. Certain head and leg traits were coded as binary (0, 1), whereas others were coded as multi-state traits (0, 1, 2 . . . ; Supplementary Table S1). Traits with ambiguities were coded as intermediate (e.g., 1/2; Supplementary Table S1). Cladogram was inferred using cluster analysis, both through Single Linkage and UPGMA, based on the distances from the character matrix (uncorrected or general distance), with 2,000 replicates per analysis. Clade frequencies were then calculated from these replicates. Retention and consistency indices were calculated for each tree and are shown next to its Newick format (Supplementary Table S3). Furthermore, a heuristic search for the most parsimonious tree was performed using

Table 1. List of Scelimeninae taxa included in the analysis. Lifestyle annotation (amphibious vs. corticolous) and the number of examined individuals per gender are shown for each taxon

		Taxon	Individuals
Amphibious (tribe Scelimenini)	1	*Amphibotettix longipes* Hancock, 1906	2 ♂, 1 ♀
	2	*Euscelimena gavialis* (Saussure, 1862)	3 ♀
	3	*E. logani* (Hancock, 1904)	1 ♂
	4	*Indoscelimena birmanica* (Brunner von Wattenwyl, 1893)	1 ♂, 2 ♀
	5	*I. flavopicta* (Bolívar, 1909)	1 ♂, 1 ♀
	6	*Paramphibotettix lieftincki* Günther, 1938	1 ♂, 1 ♀
	7	*P. sanguinolentus* (Bolívar, 1887)	1 ♀
	8	*Platygavialidium dentifer* (Stål, 1877)	1 ♂, 1 ♀
	9	*P. formosanum* (Tinkham, 1936)	1 ♂, 1 ♀
	10	*P. kraussi* (Bolívar, 1887)	2 ♀
	11	*P. productum* (Walker, 1871)	1♀
	12	*P. sinicum* Günther, 1939	1♀
	13	*Scelimena bellula* Storozhenko and Dawwrueng, 2015	1♂, 1♀
	14	*S. boettcheri* Günther, 1938	1♂, 2♀
	15	*S. dammermanni* Günther, 1938	1♂
	16	*S. discalis* (Hancock, 1915)	1♂, 1♀
	17	*S. floresana* Günther, 1955	1♂
	18	*S. hexodon* (Haan, 1843)	2♂, 1♀
	19	*S. melli* Günther, 1938	1♂, 1♀
	20	*S. novaeguineae* (Bolívar, 1898)	1♂, 2♀
	21	*S. producta* (Serville, 1838)	1♂, 2♀
	22	*S. spiculata* (Stål, 1877)	2♀
	23	*Tagaloscelimena aurivillii* (Bolívar, 1887)	1♂, 1♀
	24	*Tagaloscelimena* sp.	1♂, 1♀
	25	*Tefrinda palpata* (Stål, 1877)	1♀

Table 1. Continued

		Taxon	Individuals
Corticolous (tribe Discotettigini)	26	*Austrohancockia albitubercula* Deng, 2019	1♀
	27	*A. kwangtungensis* (Tinkham, 1936)	2♀
	28	*A. latifemora* Deng, 2019	1♀
	29	*A. okinawensis* Yamasaki, 1994	1♂
	30	*A. orlovi* Storozhenko, 2016	2♀
	31	*A. platynota amamiensis* Yamasaki, 1994	1♀
	32	*A. platynota platynota* (Karny, 1915)	1♂, 1♀
	33	*Disconius shelfordi* (Hancock, 1907)	1♂, 2♀
	34	*Discotettix belzebuth* (Serville, 1838)	2♂, 2♀
	35	*D. doriae* Bolívar, 1898	1♀
	36	*D. kirscheyi* Skejo, Pushkar, Tumbrinck and Tan, 2022	1♂, 1♀
	37	*D. scabridus* (Stål, 1877)	1♂, 4♀
	38	*D. selysi* Bolívar, 1887	2♂, 1♀
	39	*Eufalconius pendleburyi* Günther, 1938	2♀
	40	*Gavialidium carli* Hebard, 1930	2♂,1♀
	41	*G. crocodilum* (Saussure, 1862)	2♀
	42	*Gibbotettix emeiensis* Zheng, 1992	1♀
	43	*G. parvipulvillus* Deng, Zheng and Wei, 2016	1♀
	44	*G. vallis* Zha and Wen, 2016	1♀
	45	*Hirrius montanus* Günther, 1937	1♂, 1♀
	46	*Paragavialidium dolichonotum* Deng, 2019	1♂
	47	*P. fujianense* Deng, 2019	2♀
	48	*P. nodiferum* (Walker, 1871)	1♀
	49	*P. prominemarginatum* Zha and Ding, 2017	1♀
	50	*P. tenuifemora* Deng, 2019	1♀
	51	*Tegotettix armatus* Hancock, 1913	1♀
	52	*T. bufocrocodil* (Storozhenko and Dawwrueng, 2015)	1♀
	53	*T. celebensis* Günther, 1937	1♀
	54	*T. tuberculatus* (Bolívar, 1887)	1♀

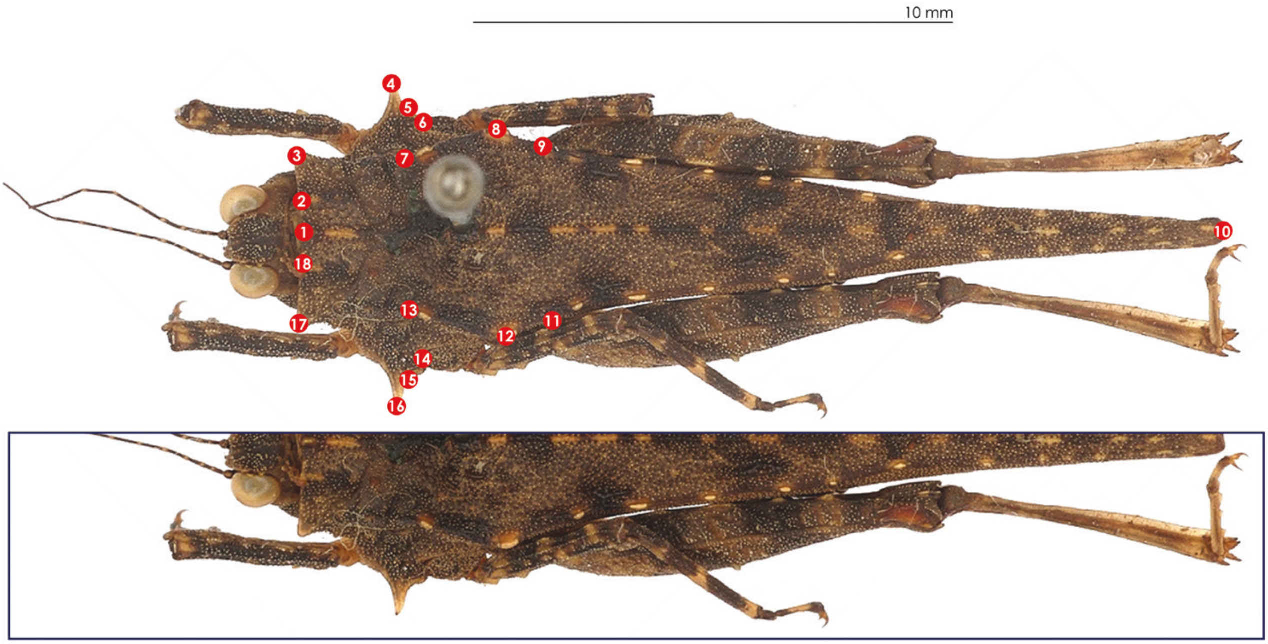

Figure 1. Scelimeninae pronotum landmarks. Landmarks (1-18) used for geometric morphometrics, shown in the example of *Indoscelimena birmanica* (above). Detail of the original photograph without landmarks is shown in the dark blue rectangle (below) to make the landmarked structures visible. Photograph by Josef Tumbrinck.

SPR, subtree pruning, and regrafting model. Cladistic analysis was performed in Mesquite ver. 3.81 (Maddison and Maddison 2023).

Reference tree

A reference phylogenetic tree follows hitherto published data on Scelimeninae evolution (Günther 1955; Chen et al. 2018; Muhammad et al. 2018; Adžić et al. 2020; Regul 2022), in combination with our cladistic analysis (see above). Published molecular phylogenies were used to check whether or not our results were in accordance with the molecular data (Qin et al. 2023). Only highly supported nodes are shown, thus polytomies are present in certain places. Newick format of the reference tree (Supplementary Table S3) was edited in iTol (Letunic and Bork 2021) software.

Body shape and phylogeny

Body shape data obtained by geometric morphometrics were mapped onto the reference tree and tested for the presence of a phylogenetic signal of body shape using a permutation test in MorphoJ (Klingenberg 2011), employing the unweighted squared-change parsimony method with 10,000 randomization rounds. In this analysis, the sum of squared shape changes along the branches of the tree is minimized over the entire phylogeny (Rodríguez-González et al. 2017). Prior to mapping, body shape was averaged across individuals of each species and a multivariate regression of the symmetric shape component on centroid size was performed, to account for the effect of body size (see *Group comparison*, above). Regression residuals were then used in the analysis.

Results

Geometric morphometrics

Wireframe graph shows that amphibious Scelimeninae species tend to have on average slenderer, more elongated body shapes than corticolous species, which are characterized by on average broader, shorter bodies (Figure 2).

The first principal component of the PCA explained 87.39 % of the variance (eigenvalue = 0.021) in body shape, whereas the second principal component explained 3.91 % of the variance (eigenvalue = 0.001). The first two components thus accounted for altogether 91.30 % of the explained variance in Scelimeninae body shape. The PCA plot showed that amphibious taxa are grouped closely together on the right side of the diagram, whereas corticolous taxa are more scattered along the PC1 (Figure 3A).

Multivariate regression of the symmetric shape component on centroid size detected a statistically significant dependence of body shape on body size, which predicted altogether 45.74 % of shape variation (permutation test, $P < 0.001$). Nevertheless, the results of the DFA showed a statistically significant difference in mean body shape between amphibious and corticolous Scelimeninae even after the correction for body size (Procrustes distance permutation test, $P < 0.001$).

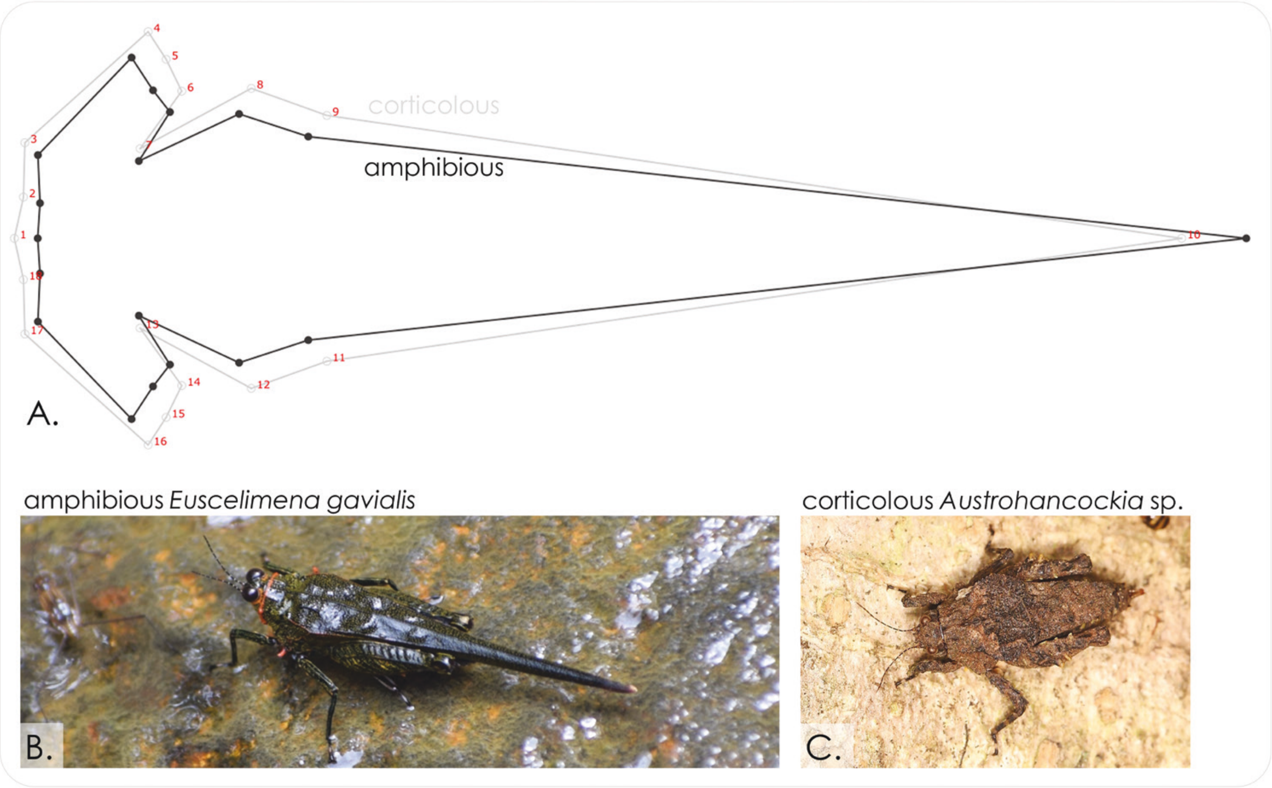

Figure 2. A–C. Corticolous versus amphibious body shape. A. Wireframe graph showing average body shape divergence between amphibious (black) and corticolous lifestyle (gray) in Scelimeninae. Landmarks are shown in red. B. Amphibious *Euscelimena gavialis* in its natural habitat (Photo: thilinahettiarachchi, iNaturalist, https://www.inaturalist.org/observations/31747090). C. Corticolous *Austrohancockia* sp. in its natural habitat (Photo: carol1970, iNaturalist, https://www.inaturalist.org/observations/76166938).

Clear separation between the groups was evident from the cross-validation scores (Figure 3B), with only 12 (of 50) amphibious taxa allocated to the corticolous group and *vice versa*.

Phylogenetic signal

When lifestyle was mapped onto the reference tree, the amphibious and the corticolous groups separated exceptionally well (Figure 4). The shortest tree found by the heuristic search for the most parsimonious tree was topologically similar to the one presented in Figure 4, with the same internal nodes and tree length of 76 steps. Multivariate regression of the symmetric shape component averaged across individuals of each species on centroid size detected a statistically significant dependence of body shape on body size, the latter predicting 61.98 % of shape variation (permutation test, $P < 0.001$). Mapping of the size-corrected shape onto Scelimeninae phylogeny yielded a tree length of 0.287, with body shape exhibiting a statistically significant phylogenetic signal (permutation test, $P = 0.002$).

Discussion

Adopting geometric morphometrics approach, the current study provides the first quantitative data on pronotum morphology of the pygmy grasshopper subfamily Scelimeninae, with potential functional implications. Our results reveal a clear difference in body shape between amphibious and corticolous representatives (Figures 2 and 3), suggesting that pronotum morphology is highly indicative of lifestyle and habitat use (Zeffer et al. 2003), its potential for evolutionary innovation through exaptation likely enabling two closely related scelimenine lineages to occupy widely different ecological niches.

In amphibious Scelimeninae, the pronotum is on average more elongated and slenderer compared with the pronotum of their corticolous relatives (Figure 2). Furthermore, according to the PCA, amphibious taxa are grouped more closely together than corticolous taxa based on pronotum shape variation (Figure 3A), likely suggesting conserved morphology owing to strong selection pressure for streamlined body shape (Xu et al. 2012; Qi et al. 2021). Hydraulic forces are known to play a key role in shaping the morphology of aquatic and

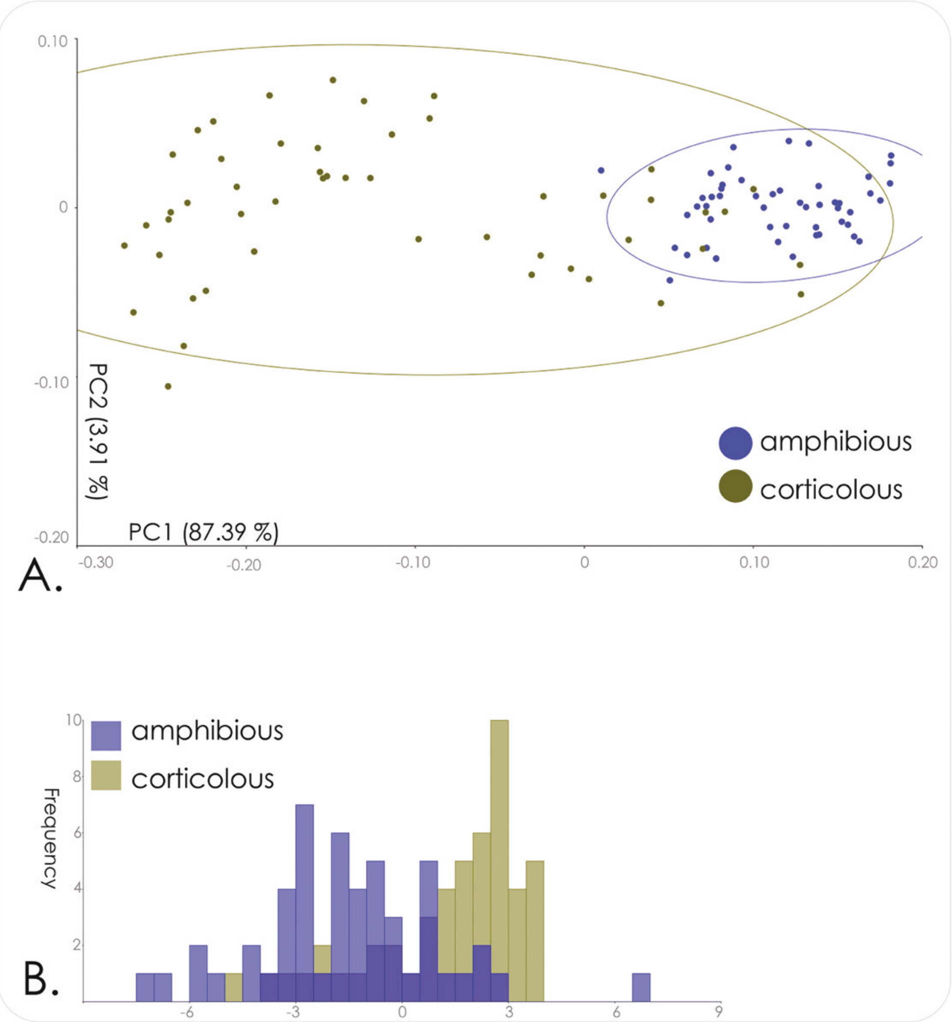

Figure 3. A–B. Principal component analysis (PCA) and discriminant function analysis (DFA). A. PCA scatterplot showing the differences in body shape between amphibious (blue) and corticolous Scelimeninae (brown). Confidence ellipses account for 95% probability that a new observation will fall within the amphibious or corticolous group, respectively. B. DFA leave-one-out cross-validation scores for body shape between amphibious (blue) and corticolous Scelimeninae (brown).

amphibious invertebrates (Orlofske and Baird 2014). In combination with dorsoventrally flattened pronotum characteristic of most Tetrigidae, streamlined body shape possibly enables amphibious Scelimeninae to achieve optimal drag-to-lift ratio (akin to e.g., mayfly or stonefly nymphs; Orlofske and Baird 2014; Ditsche et al. 2023), as a prerequisite for dwelling in the lotic environment of forest streams (Muhammad et al. 2023). Additionally, elongated pronotum with strong lateral spines could provide protection from underwater predators such as fishes or amphibians, as demonstrated in *Criotettix japonius* (Honma et al. 2006). Streamlined body shape may also play a role in reducing fluid resistance while swimming underwater (Musiolek and Kočárek 2017), as in *Baetis*, *Centroptilum* or *Isonychia* mayflies and numerous aquatic beetle larvae (Merritt and Wallace 2009). Further indication of aquatic locomotion in amphibious Scelimeninae is provided by leg morphology, that is, dorsoventral (not lateral, as written in Muhammad et al. 2023) compression of hind tibiae and the first segment of hind tarsi (Paranjape and Bhalerao 1994). Nevertheless, in-depth studies on their habitat preferences and behavioral patterns are needed to put these morphological adaptations in the appropriate ecological and behavioral context.

On the other hand, the comparatively stouter and shorter pronotum of the corticolous representatives (Figure 2) is also substantially more variable in overall shape than in amphibious taxa, as shown by the PCA (Figure 3A). Such conspicuous morphological variability allows corticolous Scelimeninae to occupy a wider morphospace, possibly increasing deception efficiency (Cortesi et al. 2015) by enabling individuals to be inconspicuous on variable substrate (O'Connor et al. 2021) and/or by impairing the ability of predators to generalize shapes (Mérot et al. 2016). Camouflage efficiency may be further promoted by rough pronotum surface that, in addition to its textural similarity to tree bark, seems to provide a suitable substrate for epizoic organisms (e.g., mosses, algae, fungi, and lichens; Skejo et al. 2022). Pronounced body shape variation in corticolous taxa could either be the result of relaxed selection (Lahti et al. 2009) in tree bark habitat or it could be maintained *via* polymorphisms often arising through apostatic selection, that is, selection against common prey morphs mediated by visual predators in mimetic taxa (Gutiérrez-Valencia et al. 2017; O'Connor et al. 2021). Nevertheless, no conclusion can be drawn on this point without reconstructing the ancestral state and the associated selection pressures.

Ecological divergence associated with macrohabitat adaptation may indeed have been the primary driver of speciation in Scelimeninae grasshoppers; according to our results, body shape exhibits a strong phylogenetic signal in this group (Figure 4). Naturally, this finding should be considered within the context of the currently accepted Scelimeninae phylogeny, which suggests deep divergence also supported by molecular data, albeit of a small number of genes (Chen et al. 2018; Adžić et al. 2020). According to a recent study by Guan et al. (2024), divergence between Scelimenini and Discotettigini has been dated to 93 million years ago, whereas the ancestor of the genus *Scelimena* has been dated to 75 million years ago. Assuming this phylogeny is correct, it follows that neither corticolous nor amphibious morphology represents the ancestral state (Figure 4). Rather, the common ancestor of the two lineages was possibly characterized by an intermediate phenotype with a lower degree of ecological specialization, that is,

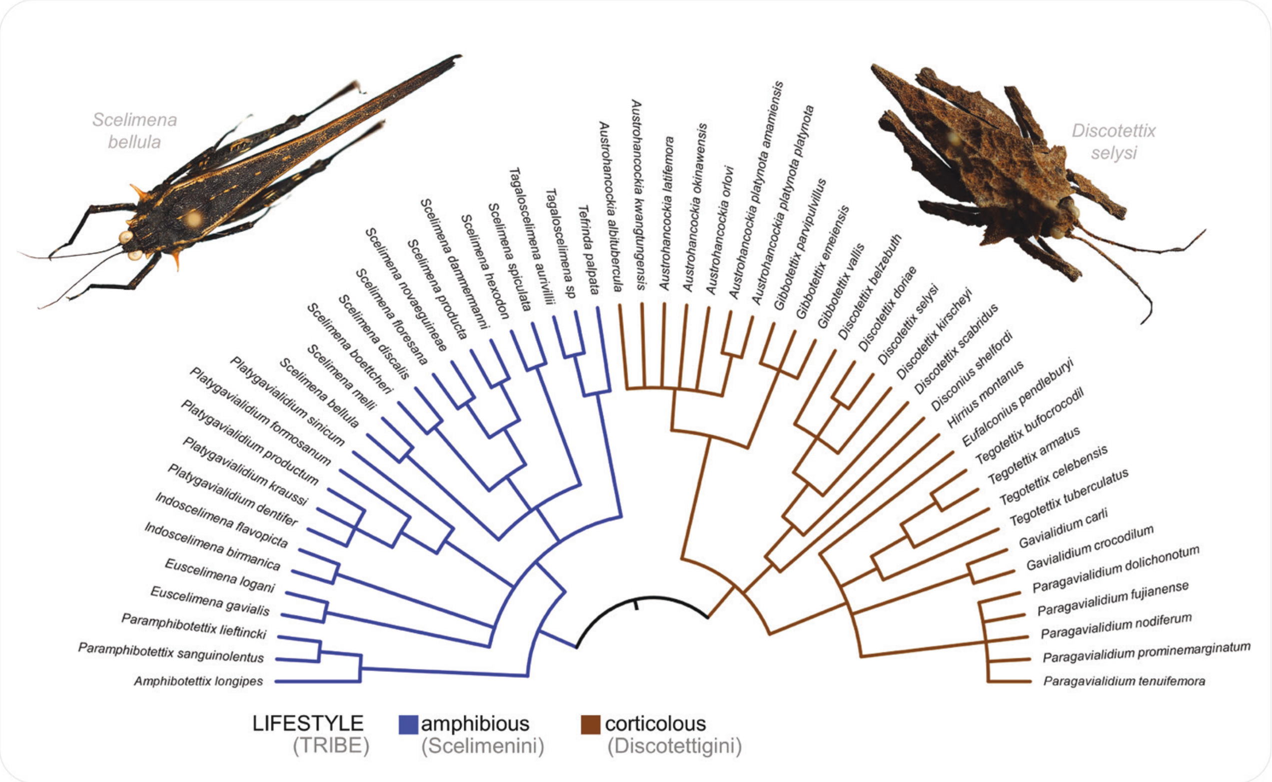

Figure 4. Reference tree showing the proposed phylogeny of Scelimeninae with lifestyle annotated. Amphibious taxa are marked in blue and corticolous taxa in brown. A representative of amphibious species is *Scelimena bellula* from Thailand, whereas *Discotettix selysi* from peninsular Malaysia and Sumatra represents corticolous species. The cladogram was constructed and annotated in iTol. Photographs by Josef Tumbrinck.

occupying a wider ecological niche that encompassed (at least partially) both lifestyles, one likely predominant and the other facultative. This supposed ancestral state is best illustrated by extant representatives such as *Gavialidium crocodilum*, a corticolous species often associated with streams, or *Scelimena hexodon*, an amphibious species that is often found on tree bark (iNaturalist 2022). According to this evolutionary scenario, it seems likely that the primary driver of lineage separation was indeed ecological specialization (Matsubayashi et al. 2010), but the true causes of the supposed deep divergence are beyond the scope of the current paper.

However, with the current level of knowledge, there are no grounds to exclude other possible evolutionary scenarios. Considering its comparatively high morphological variability (see above; Figure 3A), the corticolous phenotype might represent the ancestral state, that is, the amphibious lineage could lie within the corticolous one. In this case, one or more times during evolutionary history, a population of corticolous individuals living in proximity to water and possessing amphibious exaptations inhabited a freshwater habitat and obtained phenotypic traits associated with amphibious lifestyle. Alternatively, the corticolous lineage could lie within the amphibious one, that is, the corticolous lineage possibly arose from an amphibious ancestral population that colonized tree bark in proximity to water. Considering that the amphibious morphology is highly specialized and rather uniform (see above; Figure 3A), and that it represents an anomaly in Tetrigidae as a hygrophilous but predominantly terrestrial insect group (Naskrecki 2013; Adžić et al. 2022), amphibious phenotype as the ancestral state is highly doubtful. Nevertheless, the definitive answer regarding the evolutionary history of Scelimeninae can only be obtained using multigene phylogeny or phylogenomics.

Finally, potential constraints of a restricted dataset used in the current study need to be considered. Namely, when selecting the material to be included in the study, we were confronted with several difficulties: 1) for most Scelimeninae taxa, only a few individuals are available in the museum collections, 2) only good quality photographs taken from the same angle (dorsal aspect from above) can be used in the analyses, and 3) to avoid bias, the dataset should be balanced, that is, the number of individuals should not vary greatly among the taxa. To meet these requirements, we were able to include only a few individuals per taxon, which provided us with sufficient statistical power to compare the groups (50 amphibious *vs.* 50 corticolous individuals), but a larger dataset is needed to assess intraspecific variability and clarify the relationships within each group. Future systematic research on sceliminine ecology, particularly looking into assemblage composition and interspecific interactions (for instance, potential competitive exclusion between taxa occurring in the same habitat), is necessary to elucidate environmental pressures that may have led to ecological specialization in the past.

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Conflict of Interest

There is no conflict of interest to declare.

Author Contributions

F.R. and J.S. contributed to study conceptualization, data collection and visualization, with F.R. performing geometric morphometrics and statistical analyses, and J.S. performing phylogenetic analyses. F.R. and J.S. drafted the original manuscript, which was written mostly by F.R., while A.B. and N.K. both contributed to the writing process. All authors read, commented on and approved the final version. A.B. provided the resources needed to perform this research.

Data Availability

The data that support the findings of this study are available on request from the corresponding authors, F.R. and J.S.

Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

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