

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

Fran Rebrina<sup>a,\*,\*\*</sup>, Andreja Brigić<sup>a</sup>, Niko Kasalo<sup>b</sup>, and Josip Skejo<sup>a,c,\*,\*\*</sup>

<sup>a</sup>University of Zagreb, Faculty of Science, Department of Biology, Division of Zoology, Horvatovac 102a, HR-10000 Zagreb, Croatia

<sup>b</sup>Ruder Bošković Institute, Division of Molecular Biology, Laboratory of Evolutionary Genetics, Bijenička cesta 54, HR-10000 Zagreb, Croatia

<sup>c</sup>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](mailto:fran.rebrina@biol.pmf.hr); Josip Skejo. E-mail: [josip.skejo@biol.pmf.hr](mailto:josip.skejo@biol.pmf.hr)

\*\*These authors contributed equally to this work.

Handling editor: Zhi-Yun Jia and Zu-Shi Huang

## 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

Received 2 November 2023; accepted 17 May 2024

© The Author(s) 2024. Published by Oxford University Press on behalf of Editorial Office, Current Zoology.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (<https://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact [reprints@oup.com](mailto:reprints@oup.com) for reprints and translation rights for reprints. All other permissions can be obtained through our RightsLink service via the Permissions link on the article page on our site—for further information please contact [journals.permissions@oup.com](mailto:journals.permissions@oup.com).

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

Table 1. Continued

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

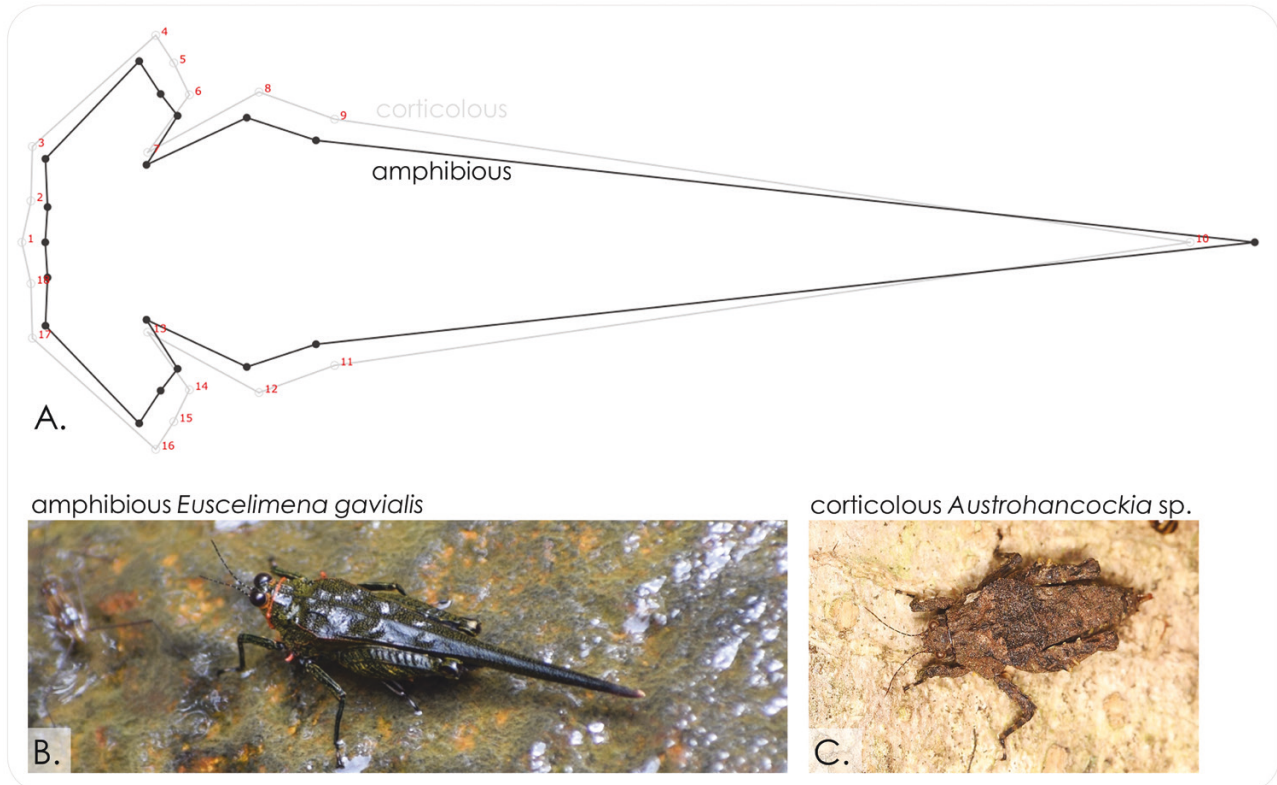
## Results

### 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*.

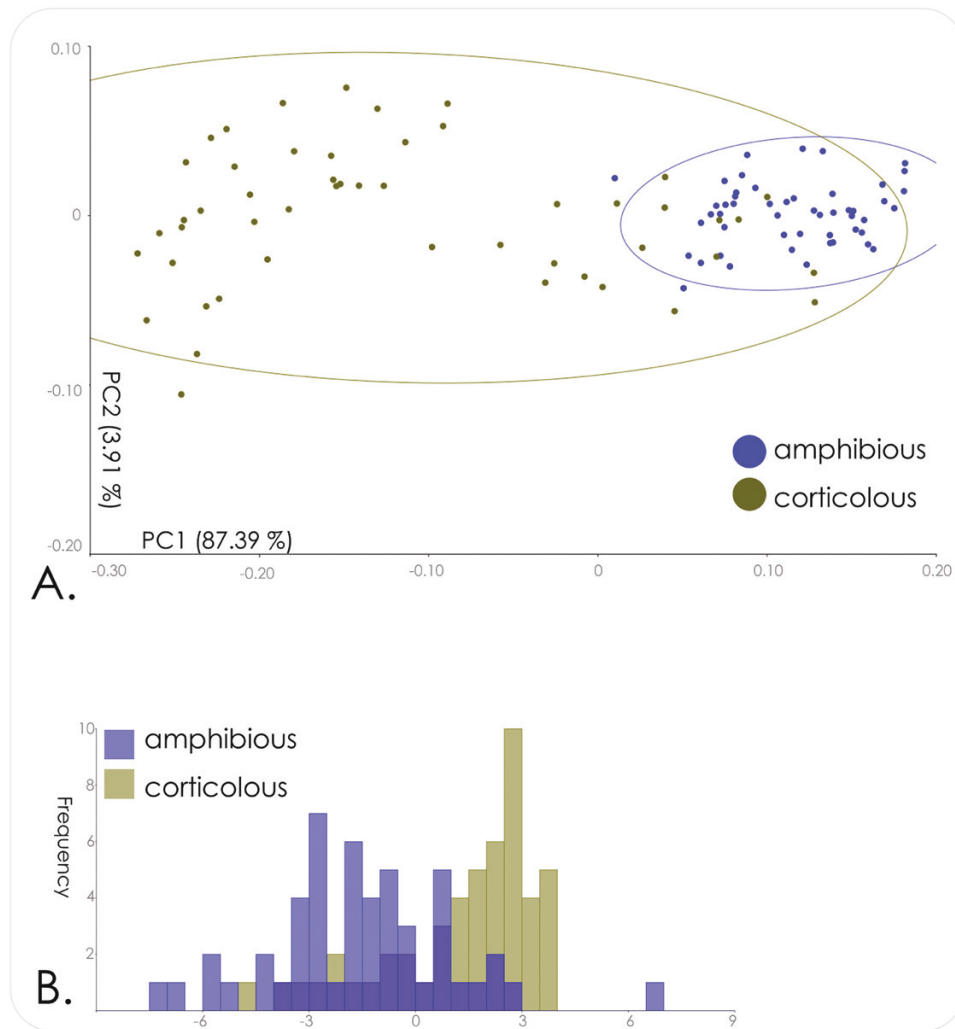
### Phylogenetic signal

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



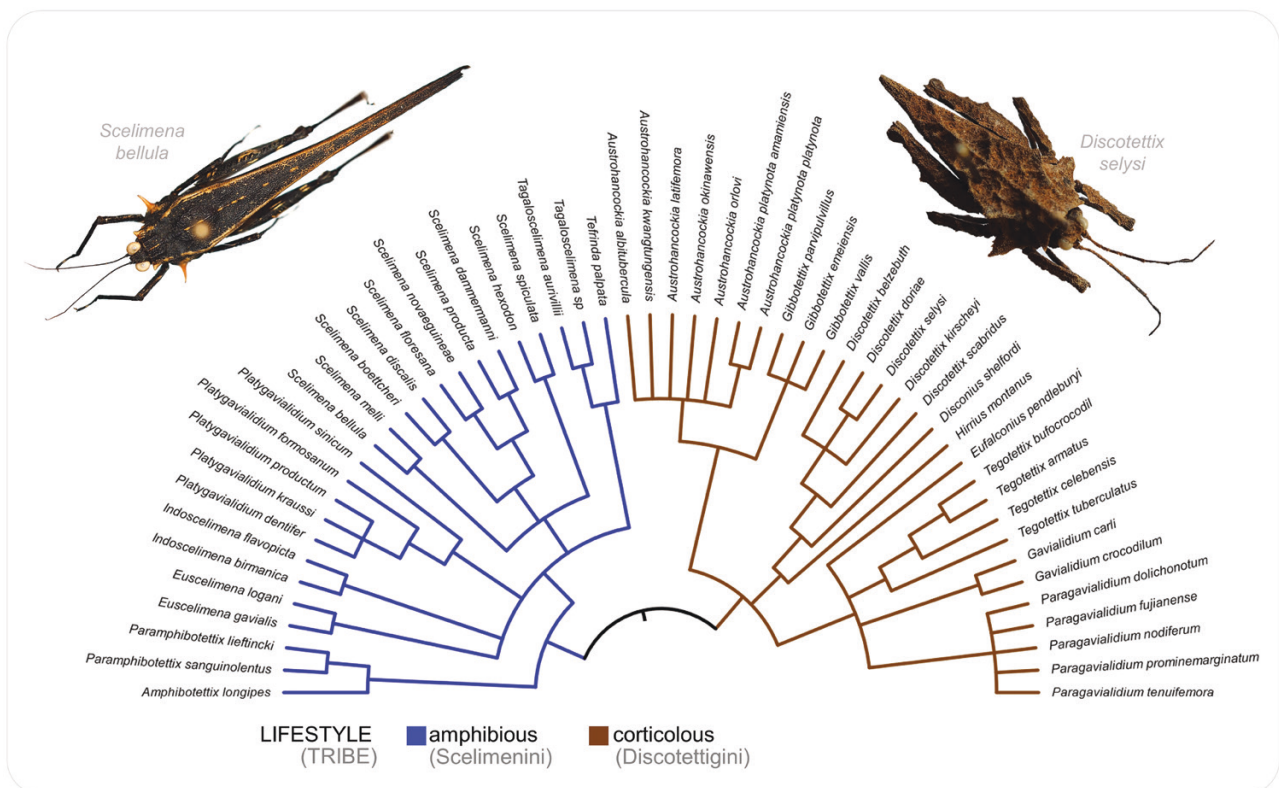
**Figure 3.** A–B. Principal component analysis (PCA) and discriminant function analysis (DFA). A. PCA scatterplot showing the differences in body shape between amphibious (blue) and corticolous Scelimeninae (brown). Confidence ellipses account for 95% probability that a new observation will fall within 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 Bhalariao 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 epizotic 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 scelimene 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.

## Acknowledgments

Many thanks to Josef Tumbrinck who kindly sent us many high-resolution photographs of Scelimeninae species as well as some older literature. We would like to thank the anonymous reviewers for their valuable comments.

## Funding

This study has been in part kindly supported by the Croatian Science Foundation (DinDRY project, UIP-2020-02-5385, grant to A.B.).

## 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>.

## References

- Adams DC, Rohlf FJ, Slice DE, 2004. Geometric morphometrics: Ten years of progress following the 'revolution.'. *Ital J Zool* 71(1):5–16.
- Adžić K, 2021. *Pygmy Grasshoppers (Orthoptera: Tetrigidae) of Peninsular Malaysia*. Master Thesis thesis. University of Zagreb, Faculty of Science.
- Adžić K, Deranja M, Franjević D, Skejo J, 2020. Are Scelimeninae (Orthoptera: Tetrigidae) monophyletic and why it remains a question? *Entomol News* 129(2):128.
- Adžić K, Deranja M, Pavlović M, Tumbrinck J, Skejo J, 2022. Endangered Pygmy Grasshoppers (Tetrigidae). In: DellaSala DA, Goldstein ML, editor. *Imperiled: The Encyclopedia of Conservation*. Amsterdam, Netherlands: Elsevier, 606–14.
- Bhalerao AM, Paranjape SY, 1986. Studies on the bioecology of a grouse locust *Euscelimena harpago* Serv. (Orthoptera: Tetrigidae). *Geobios* 13:145–150.
- Bolnick DI, Svanbäck R, Araújo MS, Persson L, 2007. Comparative support for the niche variation hypothesis that more generalized populations also are more heterogeneous. *Proc Natl Acad Sci USA* 104(24):10075–10079.
- Caro T, Izzo A, Reiner RC, Walker H, Stankowich T, 2014. The function of zebra stripes. *Nat Commun* 5(1):3535.
- Chen YZ, Deng WA, Wang JM, Lin LL, Zhou SY, 2018. Phylogenetic relationships of Scelimeninae genera (Orthoptera: Tetrigoidea) based on COI, 16S rRNA and 18S rRNA gene sequences. *Zootaxa* 4482(2):392–400.
- Cigliano MM, Braun H, Eades DC, Otte D, 2022. *Orthoptera Species File. Version 5.0/5.0*. <http://Orthoptera.SpeciesFile.org>
- Clemente CJ, 2014. The evolution of bipedal running in lizards suggests a consequential origin may be exploited in later lineages. *Evol Int J Org Evol* 68(8):2171–2183.
- Cortesi F, Feeney WE, Ferrari MCO, Waldie PA, Phillips GAC et al., 2015. Phenotypic plasticity confers multiple fitness benefits to a mimic. *Curr Biol* 25(7):949–954.



- Day EH, Hua X, Bromham L, 2016. Is specialization an evolutionary dead end? Testing for differences in speciation, extinction and trait transition rates across diverse phylogenies of specialists and generalists. *J Evol Biol* 29:1257–1267.
- Ditsche P, Hoffmann F, Kaehler S, Kesel A, Gorb S, 2023. Hydrofoil-like legs help stream mayfly larvae to stay on the ground. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 209(2):325–336.
- Foster K, Bower L, Piller K, 2015. Getting in shape: Habitat-based morphological divergence for two sympatric fishes. *Biol J Linn Soc Lond* 114:152–162.
- Fruciano C, Pappalardo AM, Tigano C, Ferrito V, 2014. Phylogeographical relationships of Sicilian brown trout and the effects of genetic introgression on morphospace occupation. *Biol J Linn Soc Lond* 112:387–398.
- Gearty W, Carrillo E, Payne JL, 2021. Ecological filtering and exaptation in the evolution of marine snakes. *Am Nat* 198(4):506–521.
- Gillespie RG, Bennett GM, De Meester L, Feder JL, Fleischer RC et al., 2020. Comparing adaptive radiations across space, time, and taxa. *J Hered* 111(1):1–20.
- Gould SJ, Vrba ES, 1982. Exaptation—a missing term in the science of form. *Paleobiology* 8(1):4–15.
- Grimaldi D, Engel MS, 2005. *Evolution of the Insects*. Cambridge, UK: Cambridge University Press.
- Guan DL, Huang CM, Deng WA, 2024. Reassessment of the phylogenetics of two pygmy grasshopper generic groups *Tetrix* and *Systolederus* through mitochondrial phylogenomics using four new mitochondrial genome assemblies. *Insects* 15(3):174.
- Günther K, 1938. Revision der Acrydiinae, I. Sectiones Tripetalocerae, Discotettigiae, Lophotettigiae, Cleostratae, Bufonidae, Cladonotae, Scelimenae verae. *Mitt Zool Mus Berl* 23:299–437.
- Günther K, 1955. Über die Dornschröcken (Orth. Acrid. Tetrigidae) von Sumba und Flores mit faunenhistorischen Anmerkungen zur Verbreitung einiger Gattungsgruppen der Tetrigidae im südostasiatischen Inselbereich. *Verh Naturforsch Ges Basel* 66(2):147–175.
- Gutiérrez-Valencia J, Gutiérrez Y, Dias LG, 2017. Species delimitation in the crypsis-defended and polymorphic stick insects of the genus *Libethra* (Phasmatodea, Diapheromeridae). *Zool Scr* 46(6):693–705.
- Hancock JL, 1904. The Tettigidae of Ceylon. *Spolia zeylan* 2:97–157.
- Hancock JL, 1907. Orthoptera Fam. Acridiidae. Subfam. Tetriginae. *Gen Ins* 48:1–79.
- Holt RD, 2009. Bringing the Hutchinsonian niche into the 21st century: Ecological and evolutionary perspectives. *Proc Natl Acad Sci USA* 106(2):19659–19665.
- Honma A, Oku S, Nishida T, 2006. Adaptive significance of death feigning posture as a specialized inducible defence against gape-limited predators. *Proc Biol Sci* 273(1594):1631–1636.
- Imai S, Kobayashi K, Ohkubo Y, Yagi N, Hasegawa E, 2016. Difference in evolutionary patterns of strongly or weakly selected characters among ant populations. *Sci Rep* 6:39451.
- iNaturalist, 2022. iNaturalist observations of Scelimenini (including genus Falconius). [inaturalist.org/observations?place\\_id=any&taxon\\_id=524051&view=species](https://www.inaturalist.org/observations?place_id=any&taxon_id=524051&view=species)
- Ito G, Azrie A, Maryati M, 2005. Tetrigid pygmy grasshoppers and their associations with altitude and habitats in Mt. Trus Madi, Sabah, Borneo (Insecta: Orthoptera). *J Trop Biol Conserv* 1:21–25.
- Kelley TC, Stewart REA, Yurkowski DJ, Ryan A, Ferguson SH, 2015. Mating ecology of beluga (*Delphinapterus leucas*) and narwhal (*Monodon monoceros*) as estimated by reproductive tract metrics. *Mar Mamm Sci* 31(2):479–500.
- Klingenberg CP, 2011. MorphoJ: An integrated software package for geometric morphometrics. *Mol Ecol Resour* 11(2):353–357.
- Klingenberg CP, 2016. Size, shape, and form: Concepts of allometry in geometric morphometrics. *Dev Genes Evol* 226:113–137.
- Lahti DC, Johnson NA, Ajie BC, Otto SP, Hendry AP et al., 2009. Relaxed selection in the wild. *Trends Ecol Evol* 24(9):487–496.
- Letunic I, Bork P, 2021. Interactive Tree Of Life (iTOL) v5: An online tool for phylogenetic tree display and annotation. *Nucleic Acids Res* 49(W1):W293–W296.
- Liuti T, Dixon PM, 2020. The use of the geometric morphometric method to illustrate shape difference in the skulls of different-aged horses. *Vet Res Commun* 44(3):137–145.
- Lytle DA, Smith RL, 2004. Exaptation and flash flood escape in the giant water bugs. *J Insect Behav* 17(2):169–178.
- Maddison WP, Maddison DR, 2023. Mesquite: A Modular System for Evolutionary Analysis. Version 3.81.
- Matsubayashi KW, Ohshima I, Nosil P, 2010. Ecological speciation in phytophagous insects. *Entomol Exp Appl* 134(1):1–27.
- McGuigan K, Chenoweth SF, Blows MW, 2005. Phenotypic divergence along lines of genetic variance. *Am Nat* 165(1):32–43.
- Meer Mohr JC van der, 1941. Korte zoölogische aantekeningen (VII). *De Tropische Natuur* 30(12):173–179.
- Mérot C, Le Poul Y, Théry M, Joron M, 2016. Refining mimicry: Phenotypic variation tracks the local optimum. *J Anim Ecol* 85(4):1056–1069.
- Merritt RW, Wallace JB, 2009. Aquatic Habitats. In: Resh VH, Cardé RT, editors. *Encyclopedia of Insects*. Amsterdam, Netherlands: Elsevier, pp. 38–48.
- Moraes EM, Manfrin MH, Laus AC, Rosada RS, Bomfin SC et al., 2004. Wing shape heritability and morphological divergence of the sibling species *Drosophila mercatorum* and *Drosophila paranaensis*. *Heredity (Edinb)* 92(5):466–473.
- Muhammad AA, Deranja M, Adžić K, Abdullah NA, 2023. Towards a better understanding of the genus *Scelimenina* (Orthoptera, Tetrigidae, Scelimeninae): New insights and notes on the taxonomy, ecology, and physiology of the genus in Peninsular Malaysia. *J Orthoptera Res* 32(1):55–62.
- Muhammad AA, Tan MK, Abdullah NA, Azirun MS, Bhaskar D et al., 2018. An annotated catalogue of the pygmy grasshoppers of the tribe Scelimenini Bolívar, 1887 (Orthoptera: Tetrigidae) with two new *Scelimenina* species from the Malay Peninsula and Sumatra. *Zootaxa* 4485(1):1–70.
- Musiolek D, Kočárek P, 2017. Effect of substrate on the risk of being washed away by floods for the Groundhoppers *Tetrix subulata* and *Tetrix tenuicornis* (Orthoptera: Tetrigidae). *River Res Appl* 33(7):1071–1078.
- Musiolek D, Kuřavová K, Kočárek P, 2017. Breathing apparatus or just life buoy for a drowning? The function of elongated pronotum in groundhoppers (Orthoptera, Tetrigidae) under water. *Zoologické Dny Brno* 2017.
- Naskrecki P, 2013. Grasshoppers and their relatives. In Scheiner S, editor. *Encyclopedia of Biodiversity*. Amsterdam, Netherlands: Elsevier, 722–736.
- O'Connor TK, Sandoval MC, Wang J, Hans JC, Takenaka R et al., 2021. Ecological basis and genetic architecture of crypsis polymorphism in the desert clicker grasshopper (*Ligurotettix coquilletti*). *Evolution (N Y)* 75(10): 2441–1459.
- Orlofske JM, Baird DJ, 2014. A geometric morphometric approach to establish body-shape trait criteria for aquatic insects. *Freshwater Sci* 33(3):978–994.
- Paranjape SY, Bhalerao AM, Bhalerao AM, 1994. Distribution and etho-ecology of grouse locusts of certain localities in Maharashtra with a note on the status of tetrigid taxonomy. *Rec Zool Surv India* 94(2–4):351–366.
- Pearman PB, Guisan A, Broennimann O, Randin CF, 2008. Niche dynamics in space and time. *Trends Ecol Evol* 23(3):149–158.
- Pyron RA, Costa GC, Patten MA, Burbrink FT, 2015. Phylogenetic niche conservatism and the evolutionary basis of ecological speciation. *Biol Rev Camb Philos Soc* 90(4):1248–1262.
- Qi D, Zhang C, He J, Yue Y, Wang J et al., 2021. Observation and analysis of diving beetle movements while swimming. *Sci Rep* 11(1):11658.
- Qin Y, Liu J, Li X, Chen Y, Deng W, 2023. On the specific status of *Scelimenina spicupennis* and a new record of *S. discalis* from

- China with mitochondrial genome characterization (Orthoptera, Tetrigidae). *Zookeys* 1185:83–104.
- Regul J, 2022. *Systematics of Pygmy rowerhoppers (Insecta: Orthoptera: Scelimeninae) Based on Morphological Characters*. Master thesis. University of Zagreb, Faculty of Science.
- Rodríguez-González AG, Sarabeev V, Balbuena JA, 2017. Evolutionary morphology in shape and size of haptoral anchors in 14 *Ligophorus* spp. (Monogenea: Dactylogyridae). *PLoS One* 12(5):e0178367.
- Rohlf FJ, 2019. tpsRelw, version 1.70.
- Rohlf FJ, 2021. tpsDig, version 2.31.
- Rohlf FJ, Slice D, 1990. Extensions of the procrustes method for the optimal superimposition of landmarks. *Syst Zool* 39(1):40–59.
- Sexton JP, Montiel J, Shay JE, Stephens MR, Slatyer RA, 2017. Evolution of ecological niche breadth. *Annu Rev Ecol Evol Syst* 48:183–206.
- Silos RA, Hernando BJ, Juario J, Patiño S, Casas PA et al., 2015. Sexual dimorphism of flathead mullet (*Mugil cephalus*) from Northern Mindanao rivers using geometric morphometric analysis. *Int Lett Nat Sci* 45:34–48.
- Silva DSM, Cadena-Castañeda OJ, Pereira MR, De Domenico FC, Sperber CF, 2019. New tribes, overview and checklist of Neotropical Cladonotinae (Orthoptera: Caelifera: Tetrigidae). *Insecta Mundi* 0723:1–38.
- Skejo J, Pushkar TI, Kasalo N, Pavlović M, Deranja M et al., 2022. . Spiky pygmy devils: revision of the genus *Discotettix* (Orthoptera: Tetrigidae) and synonymy of Discotettiginae with Scelimeninae. *Zootaxa* 5217(1):1–64.
- Song H, Amédégnato C, Cigliano MM, Desutter-Grandcolas L, Heads SW et al., 2015. 300 million years of diversification: Elucidating the patterns of orthopteran evolution based on comprehensive taxon and gene sampling. *Cladistics* 31(6):621–651.
- Storozhenko SY, Dawwrueng P, 2015. New and little-known pygmy grasshoppers (Orthoptera: Tetrigidae) from Thailand. *Zootaxa* 4052(5):527–554.
- Svanbäck R, Schluter D, 2012. Niche specialization influences adaptive phenotypic plasticity in the three spine stickleback. *Am Nat* 180(1):50–59.
- Tatsuta H, Takahashi KH, Sakamaki Y, 2018. Geometric morphometrics in entomology: Basics and applications. *Entomol Sci* 21(1):164–184.
- Triques ML, Christoffersen ML, 2009. Exaptations in the conquest of land by Tetrapoda. *Gaia Scientia* 3(2):69–74.
- Tumbrinck J, 2019. Taxonomic and biogeographic revision of the genus *Lamellitettigodes* (Orthoptera: Tetrigidae) with description of two new species and additional notes on *Lamellitettix*, *Probolotettix*, and *Scelimena*. *J Orthoptera Res* 28(2):167–180.
- Xu Z, Lenaghan SC, Reese BE, Jia X, Zhang M, 2012. Experimental studies and dynamics modeling analysis of the swimming and diving of whirligig beetles (Coleoptera: Gyrinidae). *PLoS Comput Biol* 8(11):e1002792.
- Zeffer A, Johansson LC, Marmebro A, 2003. Functional correlation between habitat use and leg morphology in birds (Aves). *Biol J Linn Soc* 79(3):461–484.
- Zha L, Wen T, Pan Z, Hyde KD, 2016. Taxonomy of *Paragavialidium* (Orthoptera: Tetrigidae: Scelimeninae) with description of one new species and notes on ecology and habits. *Entomol News* 126(1):43–51.
- Zha L, Yu F, Boonmee S, Eungwanichayapant PD, Wen T, 2017. A new species of the genus *Scelimena* (Orthoptera: Tetrigidae) in Thailand and notes on its biology and ecology. *Entomol News* 126(5):372–382.