



A century of paraphyly: A molecular phylogeny of katydids (Orthoptera: Tettigoniidae) supports multiple origins of leaf-like wings



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ABSTRACT

The phylogenetic relationships of Tettigoniidae (katydids and bush-crickets) were inferred using molecular sequence data. Six genes (18S rDNA, 28S rDNA, Cytochrome Oxidase II, Histone 3, Tubulin Alpha I, and Wingless) were sequenced for 135 ingroup taxa representing 16 of the 19 extant katydid subfamilies. Five subfamilies (Tettigoniinae, Pseudophyllinae, Mecopodinae, Meconematinae, and Listroscelidinae) were found to be paraphyletic under various tree reconstruction methods (Maximum Likelihood, Bayesian Inference and Maximum Parsimony). Seven subfamilies – Conocephalinae, Hetrodinae, Hexacentrinae, Saginae, Phaneropterinae, Phyllophorinae, and Lipotactinae – were each recovered as well-supported monophyletic groups. We mapped the small and exposed thoracic auditory spiracle (a defining character of the subfamily Pseudophyllinae) and found it to be homoplasious. We also found the leaf-like wings of katydids have been derived independently in at least six lineages.

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1. Introduction

The katydid family Tettigoniidae (Orthoptera: Ensifera) contains more than 6500 species in 19 extant subfamilies, 74 tribes, and 1193 genera (Eades and Otte, 2009). Katydid have a nearly cosmopolitan distribution and are common throughout tropical and temperate regions. Fossil representatives from the extant tettigoniid subfamilies are known from the Paleogene (Gorochoy, 2010; Nel et al., 2008; Théobald, 1937).

Formal investigations into katydid phylogenetic relationships have never been published so it is unclear whether current taxonomy reflects monophyletic groups. This lack of a published phylogeny has made it difficult to decipher the evolutionary patterns in katydid morphology.

Katydid were first classified as Locustariae (Latreille, 1802) until Burmeister (1838) reclassified tettigoniids along with species now placed in Stenopelmatidae and Gryllacrididae into the Locustina. This classification was questioned by Gerstaecker (1863) but went largely unnoticed for more than 10 years (Stål, 1876). Many of the subfamilies currently recognized were first described as families under the order Locustodea (Brunner von Wattenwyl, 1878). Locustodean families were renamed as subfamilies within Tettigoniidae by Krauss (1902).

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Previous workers inferred katydid subfamilial relationships based on intuitive analyses of morphology. Zeuner (1936) erected Brachycephalia and Dolichocephalia (Table 1) based on the shape of the head, insertion of antennae, and protrusions from the katydid forehead (fastigium of vertex). Zeuner considered Brachycephalia to be primitive katydids, with globose heads, antennae that insert below the margins of the eyes, and the absence of any developed or protruding fastigium of the vertex, presumed plesiomorphic characters held in common with other ensiferan families. In contrast, the presumably more derived katydids, Dolichocephalia, have heads with a pronounced vertex, antennae that insert above the lower margin of the eyes, and a well developed, and in many cases protruding, fastigium of vertex. Zeuner also divided katydids into five groups based on the shape of their thoracic spiracle (tibial foramina), and the internal structure of the auditory trachea. Zeuner's classification was not largely accepted (Ander, 1939), but a similar distinction of "primitive" and "advanced" subfamilies was again proposed by Rentz (1979).

Most of the current katydid subfamilies were originally described over 100 years ago (Brunner von Wattenwyl, 1878) with additional subfamilies, Microtettigoniinae (Rentz, 1979), Austrosaginae (Rentz, 1993), and Lipotactinae (Ingrisch, 1995), added more recently. Cataloging the diversity found in Tettigoniidae using these subfamilies has become difficult due to the lack of distinct morphological characters delineating each subfamily. Evidence for the ambiguity in subfamily descriptions is seen in the difficulty of placing numerous taxa (e.g., *Megatympanopon*, *Terpandroides*,

and *Megalotheca*) within any of the described subfamilies. In addition, the predatory Listroscelidinae is thought to represent a taxonomic “sump” (Naskrecki, 2000b) for new taxa that do not currently fit well within the described subfamilies.

Previous studies using morphological (Desutter-Grandcolas, 2003; Gwynne, 1995) and molecular (Jost and Shaw, 2006) evidence support the monophyly of Tettigoniidae. Diagnostic characters include antennae with more than 30 segments, a sword-like ovipositor, the large edible nuptial gift the male produces and offers to the female when mating (spermatophylax), and simple foregut (proventricular) teeth relative to other ensiferan families (Gwynne, 2001). Relationships between katydids and the remaining families within the orthopteran suborder Ensifera are still unresolved (Legendre et al., 2010). Some have suggested Prophalangopsidae is sister to katydids (Ander, 1939; Ragge, 1955; Zeuner, 1939), although Sharov (1968) found fossil evidence to support Tettigoniidae as sister to all the remaining ensiferan families. More recently, cladistic analyses based on morphology have resulted in various hypotheses for the sister family to tettigoniids including Prophalangopsidae (Gwynne, 1995) or Gryllacrididae (Desutter-Grandcolas, 2003). Molecular phylogenies of Ensifera have found various families as the sister to katydids including Grylloidea + Gryllotalpidae + Schizodactylidae + Rhaphidophoridae when using ribosomal data (Jost and Shaw, 2006), Stenopelmatidae based on mitochondrial data (Fenn et al., 2008), and Gryllacrididae + Anostostomatidae + Prophalangopsidae + Stenopelmatidae when reanalyzing ribosomal data from earlier studies (Legendre et al., 2010).

Our current understanding of tettigoniid phylogenetic relationships comes from Gorochoff (1988) who produced a tree based on an intuitive analysis of wing venation. Since its publication, Gorochoff's tree (Fig. 1) has been appended to account for changes in taxonomy (Gwynne and Morris, 2002). Naskrecki produced a phylogenetic tree based upon morphology as part of a graduate dissertation, but the results remain unpublished (Naskrecki, 2000b). Phylogenetic analyses have been conducted for smaller subsets of tettigoniids including a phylogeny of the genera *Neoconocephalus* (Snyder et al., 2009), *Banza* (Shapiro et al., 2006), *Saga* of Europe (Giannulis et al., 2011), and *Anterastes* (Çıplak, 2004) with taxon sampling for these analyses being designed to address the monophyly of the particular genera and not the higher level relationships within Tettigoniidae.

An understanding of the phylogenetic relationships of tettigoniids is essential to understanding the diverse morphology found in katydids. Many species of katydids have large, thick forewings (tegmina) resembling leaves. Katydids are primarily active at night

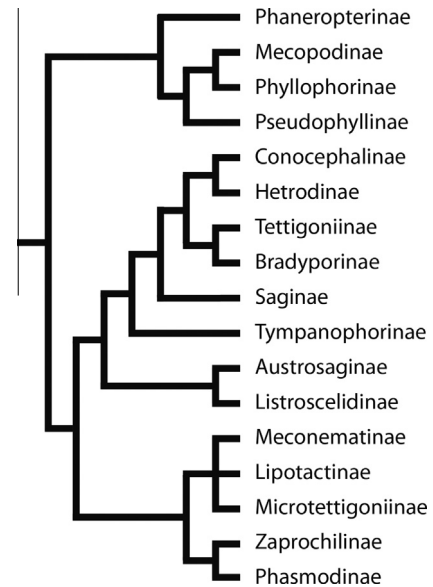


Fig. 1. Gorochoff's (1988) evolutionary tree with changes to account for recently described subfamilies (Ingrisch, 1995; Rentz, 1993). Not included in this tree are Hexacentrinae and Acridoxeninae which were considered tribes within Listroscelidinae (Rentz, 2001) and Mecopodinae (Gorochoff, 1988) respectively.

and many spend their days hidden on leaves. The leaf-like appearance provides protection via crypsis from diurnal predators (Nickle and Castner, 1995). Other katydid defenses include aposematic coloration, mimicry, or protective spines (Castner, 1995; Castner and Nickle, 1995b; Nickle and Castner, 1995) (Fig. 2). Leaf-like wings are known from 11 katydid subfamilies (Gwynne, 2001) but it is unclear whether the wide-spread occurrence represents a single derivation or if there were multiple derivations of leaf-like tegmina.

Katydid have one of the most complex forms of acoustic signaling within insects (Bailey, 1990; Bailey and Stephen, 1978; Bush et al., 2009; Hoy and Robert, 1996; Korsunovskaya, 2008). For tettigoniids, acoustic communication plays an integral role in sexual selection, territorial displays, and in at least one species, attracting prey (Marshall and Hill, 2009). These acoustic signals are received by a complex system involving tympanal membranes on the forelegs (ears), auditory spiracles on the thorax, a tracheal system connecting the two, and sound reception cells (*cristae acoustica*) which detect the sound vibrations and send the signals to the insect's ganglia (Bailey, 1990, 1993; Zeuner, 1936). The shape and size of the organs associated with katydid hearing are one of the characters used to delineate tettigoniid subfamilies (Brunner von Wattenwyl, 1878; Rentz, 1979). Pseudophyllinae are recognized as having a relatively small exposed thoracic spiracle whereas the other subfamilies have thoracic spiracles that are larger and at least partially concealed by the pronotum (Fig. 3). Several studies investigating sexual selection (Simmons and Bailey, 1990; Simmons and Gwynne, 1993; Wedell, 1993), acoustic signaling (Marshall and Hill, 2009; Montealegre-Z, 2009; Naskrecki, 2000a), and morphology (Montealegre-Z, 2009; Nickle and Castner, 1995; Rentz, 1995), have focused on katydids, but without a robust phylogenetic hypothesis in place, it is difficult to decipher the evolution of these characters.

In this work we address the phylogenetic relationships of Tettigoniidae by utilizing six molecular markers from nuclear and mitochondrial genes to (1) test subfamilial monophyly, (2) determine the relationships among the subfamilies and identify the basal tettigoniid lineages, and (3) decipher the evolutionary patterns in leaf-like tegmina and thoracic spiracle morphology.

Table 1

Zeuner's 1939 revision of Tettigoniidae based on antennal insertion in relation to the eyes with Brachycephalia having antennal margins noticeably lower than the ventral margin of the eyes and Dolichocephalia with antennal margins noticeably higher than the ventral margin of the eyes.

Brachycephalia "Primitive"	Bradyporoids	Ephippigerinae Bradyporinae Hetrodinae Acridoxeninae
Dolichocephalia "Advanced"	Pseudophylloids	Pseudophyllinae
	Tettigonioids	Meconematinae Mecopodinae Phyllophorinae Tettigoniinae Saginae
	Conocephaloids	Conocephalinae Tympanophorinae
	Phaneropteroids	Phaneropterinae

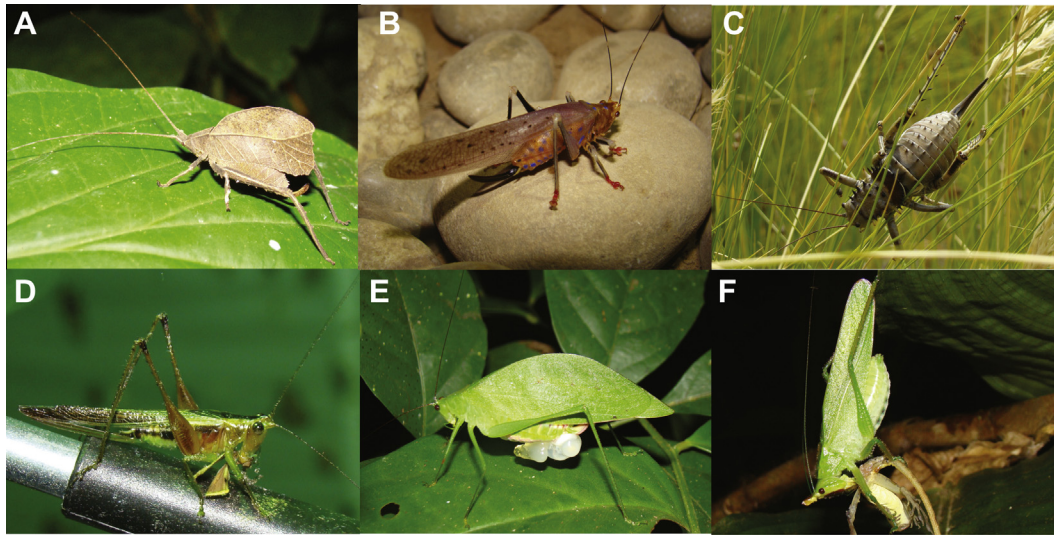


Fig. 2. A sample of katydid diversity showing variation in body form and behaviors: (A) the leaf-like tegmina (*Typophyllum* sp.), (B) aposematic coloration (*Vestria* sp.), (C) dense protective spines (*Hetrodes* sp.), (d) fusiform *Conocephalus* sp. feeding on another insect (Lepidoptera), (E) a female phaneropterine with the spermatophylax, and (F) a *Copiphora rhinoceros* feeding on an anole (*Anolis* sp.). Photos by J. Mugleston.

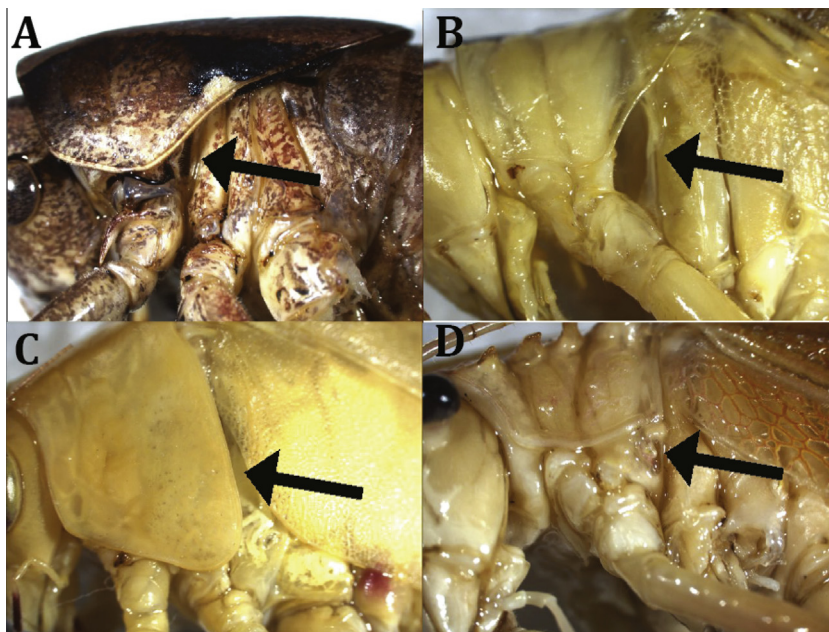


Fig. 3. Variation in the thoracic auditory spiracle: (A and B) large partially covered spiracle, (C) completely covered spiracle, (D) small, uncovered spiracle. Photos A, B, and C by Rebecca S. Buckman; Photo D by J. Mugleston.

2. Materials and methods

2.1. Taxon sampling

Taxon sampling was designed to include the phylogenetic and geographic diversity of Tettigoniidae. Ingroup sampling included 135 tettigoniid exemplars representing 16 of the 19 extant subfamilies (~85%), 43 of 75 tribes (57%), and 122 (10%) of the described genera (Table 2). The majority of tettigoniid diversity (84% described species) is concentrated within five large subfamilies: Phaneropterinae, Conocephalinae, Pseudophyllinae, Tettigoniinae, and Meconematinae. These large subfamilies were sampled in proportion to the number of species described within (e.g., Phaneropterinae contains ~34% of the named tettigoniid species and represents roughly 30% of the taxon sampling). Sampling

was increased to incorporate geographic diversity with subfamilies or tribes without contiguous ranges and for genera that span multiple continents (e.g., *Conocephalus* spp. were sampled from throughout their global range). Three subfamilies, Acridoxeninae, Bradyporinae, and Microtettigoniinae, were not included, as suitable vouchers for DNA extraction could not be acquired. The sister family to Tettigoniidae is still unresolved (Desutter-Grandcolas, 2003; Gwynne, 1995; Legendre et al., 2010) leaving no obvious choice for outgroup taxa. Instead six outgroup taxa were selected from five ensiferan families (Table 3). All specimen vouchers are deposited in the Insect Genomics Collection, M. L. Bean Museum, Brigham Young University.

PhyRe (Plazzi et al., 2010) was used to evaluate the representativeness of the taxon sampling and determine if particular subfamilies were unevenly represented. The reference taxonomy was

Table 2
Ingroup sampling by subfamily.

Subfamily	Tribes sampled	Genera Sampled	Total exemplars
Austrosaginae	NA	2 of 6	2
Conocephalinae	4 of 6	18 of 181	26
Hetrodinae	4 of 5	4 of 14	4
Hexacentrinae	0 of 1	4 of 12	4
Lipotactinae	NA	2 of 2	2
Listrosclidinae	1 of 5	3 of 37	3
Meconematinae	2 of 2	6 of 88	6
Mecopodinae	3 of 6	5 of 55	5
Phaneropterinae	11 of 14		
	Genus Groups 7 of 12	38 of 343	41
Phasmodinae	NA	1 of 1	1
Phyllophorinae	NA	2 of 12	2
Pseudophyllinae	11 of 20	21 of 254	23
Saginae	0 of 1	2 of 4	2
Tettigoniinae	6 of 12	12 of 158	12
Tympanophorinae	NA	1 of 2	1
Zaprochilinae	NA	1 of 4	1
Total	43 of 75	122 of 1197	135

adapted from the katydid species listed on the Orthopteran Species File Online. Confidence limits were calculated with 1000 random resampling of equivalent size to the taxon sampling from the tetti-goniid master list.

2.2. Molecular methods

Muscle was excised from the mesothoracic or metathoracic femur and DNA was extracted using the Qiagen DNeasy Blood and Tissue kit following the standard protocol provided by the manufacturer. Six loci (2 ribosomal DNA, 1 mitochondrial, and three nuclear protein-coding) commonly used in insect phylogenetic studies (Colgan et al., 1998; Svenson and Whiting, 2004, 2009; Whiting, 2002; Wild and Maddison, 2008) were used for this analysis. These include the 28S ribosomal subunit (28S rDNA, ~2.2 kb), the 18S ribosomal subunit (18S, ~1.9 kb), Cytochrome *c* Oxidase Subunit II (COII, ~650 bp), Histone 3 (H3, ~375 bp), Wingless (WG, ~450 bp), and Tubulin Alpha I (TUBA, ~350 bp). Genes were sequenced and amplified using oligonucleotide primers from Integrated DNA Technologies (San Diego, CA). PCR protocol was previously described for H3 (Colgan et al., 1998), 28S and 18S (Whiting, 2002), WG (Wild and Maddison, 2008), COII (Svenson and Whiting, 2004) and TUBA (Buckman et al., 2013) and displayed in Table 4. PCR was performed using 25 µl reactions with Platinum taq DNA polymerase (Invitrogen, Carlsbad, CA). For 28S and 18S ribosomal genes, 1.25 µl of water was replaced with DMSO. Gene amplification parameters were as follows: 2 min at 94 °C and 35 cycles of 30 s. at 94 °C, 30 s. at 46–58 °C, and 45–120 s. at 72 °C, with a final extension at 72 °C for 7 min with specific annealing temperature and extension times by gene detailed in Table 4. All reactions were run on GeneAmp® PCR system 9700 (Applied Biosystems, Foster City, CA). PCR product was inspected with 2% agarose gel electrophoresis using ethidium bromide to confirm amplification and test for contamination. Products were cleaned with PrepEase® purification plates (USB Corporation, Cleveland, OH) using the manufacturer's instructions. Products were sequenced with BigDye chain terminating chemistry and fractioned on an AB13730xl (Applied Biosystems Inc.) at the Brigham Young University DNA Sequencing Center (Provo, UT).

2.3. Sequence alignment

Contigs were assembled and edited using Sequencher V.4.9 (GeneCodes 2006), and submitted to GenBank (Table 3).

Protein coding genes were uploaded into MEGA V5 (Kumar et al., 2008). Nucleotide sequences were translated into amino acid

sequences and after the correct reading frame was determined, alignment was conducted using the default parameters in MUSCLE (Edgar, 2004). Sequences were then back translated to nucleotides for the phylogenetic analyses. Amino acid sequences were highly conserved throughout the taxa, making the final protein alignments unambiguous. Ribosomal genes were aligned using the E-INS-I algorithm and default settings in MAFFT V6 (Kato et al., 2005) available through the online server at <http://mafft.cbrc.jp/alignment/server/>. Alignments of the individual ribosomal sequences were also conducted using MUSCLE (Edgar, 2004) to determine the sensitivity of the data to the alignment methods. Gaps placed within the alignments were treated as missing data in the parsimony analysis.

Because ribosomal genes can be difficult to align due to the multiple conserved regions flanked by the variable expansion regions, we tested the sensitivity of the ribosomal alignments via GBLOCKS v0.91b (Castresana, 2000) using server at http://molevol.cmima.csic.es/castresana/Gblocks_server.html. Two parameters were selecting using the online server (1) allow for smaller final blocks and (2) allow gap positions within the final blocks.

2.4. Phylogenetic analyses

Maximum Likelihood (ML), Bayesian analysis (BI), and Maximum Parsimony (MP) methods were used to reconstruct trees. The concatenated dataset was partitioned by gene for the ML and BI searches. JModelTest (Posada, 2008) returned the GTR + I + Γ as the best fit model for sequence evolution for each gene partition. Maximum likelihood searches were conducted using RAXML V7.0.3 (Stamatakis, 2006) implemented on the supercomputer resources available at BYU (<https://marylou.byu.edu>). Searches were conducted using a random starting tree and the GTRGAMMAI model for each partition. Bootstrap support was calculated with 1000 bootstrap replicates.

Bayesian inferences using flat priors were performed with MrBayes V1.3.2 (Ronquist and Huelsenbeck, 2003) on the BYU supercomputing resources. Two independent runs of 20 Markov chain Monte Carlo (MCMC) were run for 200 million generations and sampled every 5000 generations. Tracer V1.5 (Rambaut and Drummond, 2003) was used to view the progress of the Bayesian run and to determine the adequate level to “burn-in”.

The most parsimonious trees for the concatenated dataset were found using TNT (Goloboff et al., 2008) and incorporated the new technology searches (Goloboff, 1999; Nixon, 1999). Search parameters included 1000 random addition replicates including 20 iterations of the parsimony ratchet, 10 cycles of tree drifting, and 15 rounds of tree fusing. Equally parsimonious trees were collapsed into a strict consensus. Nodal support was calculated using bootstrap and partitioned Bremer support (Bremer, 1994). One thousand bootstrap replicates were performed in TNT. Partitioned Bremer support values were calculated using PAUP* (Swofford, 2003) with a script developed in TREEROT V1.3 (Sorenson and Franzosa, 2007).

2.5. Character mapping

Tegmina were coded as a single binary character as being either leaf-like or not leaf-like. Calipers were used to measure the height of the thorax and the width of the wings. Leaf-like tegmina were defined as being oblong with the maximum width of the wing larger than the height of the thorax, or not leaf-like, with narrow forewings that are not wider than the height of the thorax (Fig. 4). The wing characters for juvenile vouchers were determined from the literature. Auditory spiracles were coded as a binary character either being small, round, exposed, and slightly larger than the thoracic respiratory spiracles (Fig. 3D), or large, round or oval, and at

Table 3

Taxon sampling with voucher number (#), locality, and GenBank accession number for each gene. Subfamily abbreviations are as follows: Austrosaginae (Aus), Conocephalinae (Cono), Hetrodinae (Het), Hexacentrinae (Hex), Lipotactinae (Lip), Listrosclidinae (List), Meconematinae (Mecon), Mecopodinae (Mecop), Phaneropterinae (Phan), Phasmodinae (Phas), Phyllophorinae (Phyll), Pseudophyllinae (Psued), Saginae (Sagi), Tettigoniinae (Tett), Tympanophorinae (Tymp), and Zaprochilinae (Zap).

Taxon	Subfamily	Voucher	Locality	18S	28S	COII	H3	TUBA	WG
<i>Conocephalus sp.</i>	Cono	OR030	LA, USA	KF570784	KF570938	KF570959	KF571079	KF571352	KF571214
<i>Microcentrum sp.</i>	Phan	OR033	UT, USA	-----	KF570836	KF570960	KF571080	KF571353	KF571215
<i>Anabrus sp.</i>	Tett	OR034	NV, USA	KF570763	KF570890	KF570961	KF571081	KF571354	KF571216
<i>Acrometopa sp.</i>	Phan	OR043	Slovenia	KF570717	KF570853	KF570962	KF571082	KF571355	KF571217
<i>Leptophyes sp.</i>	Phan	OR044	Germany	KF570751	KF570851	KF570963	KF571083	KF571356	KF571218
<i>Barbitistes sp.</i>	Phan	OR069	Germany	KF570742	KF570859	KF570964	KF571084	KF571357	KF571219
<i>Platycoleis sp.</i>	Tett	OR071	Slovenia	KF570764	KF570891	KF570965	KF571085	KF571358	KF571220
<i>Poecilimon sp.</i>	Phan	OR074	Slovenia	KF570752	KF570852	KF570966	KF571086	-----	KF571221
<i>Tettigonia sp.</i>	Tett	OR075	Germany	KF570765	-----	-----	KF571087	KF571359	KF571222
<i>Phaneroptera sp.</i>	Phan	OR076	Germany	KF570718	KF570864	-----	KF571088	KF571360	KF571223
<i>Pholidoptera sp.</i>	Tett	OR079	Germany	KF570767	KF570893	KF570967	KF571089	KF571361	KF571224
<i>Pachytrachis sp.</i>	Tett	OR081	Slovenia	KF570769	KF570892	KF570968	KF571090	KF571362	KF571225
<i>Conocephalus sp.</i>	Cono	OR082	Germany	-----	-----	KF570969	KF571091	KF571363	KF571226
<i>Aganacris sp.</i>	Phan	OR084	Bolivia	KF570720	KF570839	KF570971	KF571093	KF571365	KF571228
<i>Acanthoproctus sp.</i>	Het	OR091	Zambia	KF570689	KF570870	KF570972	KF571094	KF571366	KF571229
<i>Sasima sp.</i>	Phyll	OR131	PNG	KF570770	KF570910	KF570973	KF571095	KF571367	KF571230
<i>Phyllophora sp.</i>	Phyll	OR132	PNG	KF570816	KF570911	KF570974	KF571096	KF571368	KF571231
<i>Zabalius ophthalmicus</i>	Pseud	OR138		KF570778	KF570884	KF570975	KF571097	KF571369	KF571232
<i>Cymatomera sp.</i>	Pseud	OR139	Africa	KF570779	KF570885	KF570976	KF571098	KF571370	KF571233
<i>Copiphora sp.</i>	Cono	OR142	Peru	KF570790	KF570918	KF570977	KF571099	KF571371	KF571234
<i>Salomona sp.</i>	Cono	OR145	PNG	KF570791	KF570928	KF570978	KF571100	KF571372	KF571235
<i>Acanthoptus sp.</i>	Het	OR176	Namibia	KF570692	KF570873	KF570979	KF571101	-----	KF571236
<i>Enyaliopsis sp.</i>	Het	OR177	Zambia	KF570690	KF570871	KF570980	KF571102	KF571373	KF571237
<i>Typophyllum sp.</i>	Pseud	OR196	Peru	KF570693	KF570946	KF570981	KF571103	KF571374	KF571238
<i>Peringueyella sp.</i>	Sagi	OR199	South Africa	KF570810	KF570905	KF570982	KF571104	KF571375	KF571239
<i>Clonia sp.</i>	Sagi	OR201	South Africa	KF570699	KF570880	KF570983	KF571105	KF571376	KF571240
<i>Panoploscelis sp.</i>	Pseud	OR377	Peru	KF570713	KF570826	KF570984	KF571106	KF571377	KF571241
<i>Trigonocorypha sp.</i>	Phan	OR378	Madagascar	KF570745	KF570844	KF570985	KF571107	KF571378	KF571242
<i>Odontolakis sp.</i>	Cono	OR379	Madagascar	KF570792	KF570932	KF570986	KF571108	KF571379	KF571243
<i>Ruspolia sp.</i>	Cono	OR380	South Africa	KF570793	KF570923	KF570987	KF571109	KF571380	KF571244
<i>Macroxiphus sp.</i>	Cono	OR381	Malaysia	KF570803	KF570930	KF570988	KF571110	KF571381	KF571245
<i>Hexacentrus sp.</i>	Hex	OR382	South Korea	KF570685	-----	KF570989	KF571111	-----	KF571246
<i>Zitsikama tessellata</i>	Mecop	OR384	South Africa	KF570756	KF570881	KF570990	KF571112	-----	KF571247
<i>Eumecopoda sp.</i>	Mecop	OR385	PNG	KF570771	KF570912	-----	KF571113	KF571382	KF571248
<i>Dysonia sp.</i>	Phan	OR386	Peru	KF570722	KF570849	KF570991	KF571114	KF571383	KF571249
<i>Eurycophora sp.</i>	Phan	OR387	Cameroon	KF570743	KF570863	KF570992	KF571115	KF571384	KF571250
<i>Phylloptera sp.</i>	Phan	OR388	Panama	KF570858	-----	KF570993	KF571116	-----	KF571251
<i>Idiarthron sp.</i>	Pseud	OR389	Costa Rica	KF570712	KF570827	KF570994	KF571117	KF571385	KF571252
<i>Teleutias sp.</i>	Pseud	OR391	Peru	KF570815	KF570829	KF570995	KF571119	KF571387	KF571254
<i>Pantecphylus sp.</i>	Pseud	OR392	Ghana	KF570707	KF570823	-----	KF571120	KF571388	KF571255
<i>Phrictaetypus viridis</i>	Mecop	OR393	PNG	KF570772	KF570909	KF570997	KF571121	-----	KF571256
<i>Hemisaga sp.</i>	Aust	OR483	Australia	KF570758	KF570896	KF570999	KF571123	KF571390	KF571257
<i>Pachysaga sp.</i>	Aust	OR484	Australia	KF570757	KF570897	KF571000	KF571124	-----	KF571258
<i>Phasmodes sp.</i>	Phas	OR485	Australia	KF570817	KF570944	KF571001	KF571125	-----	KF571259
<i>Tympanophora sp.</i>	Tymp	OR486	Australia	KF570777	KF570947	KF571002	KF571126	-----	KF571260
<i>Kawanaphila sp.</i>	Zap	OR487	Australia	KF570700	KF570882	KF571003	KF571127	-----	KF571261
<i>Conocephalus sp.</i>	Cono	OR548	Namibia	KF570788	KF570936	KF571004	KF571128	-----	KF571262
<i>Aerotegmina sp.</i>	Hex	OR549	Tanzania	KF570687	KF570904	KF571005	KF571129	-----	KF571263
<i>Agraecia sp.</i>	Cono	OR550	Peru	KF570798	KF570921	KF571006	KF571130	-----	KF571264
<i>Scudderia sp.</i>	Phan	OR551	LA, USA	KF570753	KF570837	KF571007	KF571131	-----	KF571265
<i>Monticolaria sp.</i>	Phan	OR552	Africa	KF570747	KF570854	-----	KF571132	-----	KF571266
<i>Requena sp.</i>	List	OR553	Western Australia	KF570696	KF570901	KF571008	KF571133	-----	KF571267
<i>Hetrodes sp.</i>	Het	OR554	South Africa	KF570691	KF570872	KF571009	KF571134	-----	KF571268
<i>Ruspolia sp.</i>	Cono	OR555	South Africa	KF570804	KF570924	-----	KF571135	-----	KF571269
<i>Conocephalus sp.</i>	Cono	OR556	South Africa	KF570789	KF570937	KF571010	KF571136	-----	KF571270
<i>Pseudorhynchus sp.</i>	Cono	OR557	Zambia	KF570805	KF570925	KF571011	KF571137	-----	KF571271
<i>Nicsara sp.</i>	Cono	OR558	Australia	KF570802	KF570929	KF571012	KF571138	-----	KF571272
<i>Arachnoscelis rehni</i>	List	OR582	Costa Rica	KF570695	KF570900	KF571013	KF571139	-----	KF571273
<i>Phlugis sp.</i>	Mecon	OR583	Costa Rica	KF570754	KF570898	KF571014	KF571140	-----	KF571274
<i>Haemodiasma sp.</i>	Pseud	OR584	Costa Rica	KF570709	KF570830	KF571015	KF571141	KF571391	KF571275
<i>Nannonotus sp.</i>	Pseud	OR585	Costa Rica	KF570710	KF570832	KF571016	KF571142	KF571392	KF571276
<i>Lirometopum coronatum</i>	Cono	OR586	Costa Rica	KF570800	KF570919	KF571017	KF571143	KF571393	KF571277
<i>Sathrophyllia sp.</i>	Pseud	OR587	India	KF570780	KF570887	KF571018	KF571144	KF571394	KF571278
<i>Cocconotus sp.</i>	Pseud	OR588	Costa Rica	KF570775	KF570913	KF571019	KF571145	KF571395	KF571279
<i>Dysmopha sp.</i>	Phan	OR589	Malaysia	KF570739	KF570868	KF571020	KF571146	KF571396	KF571280
<i>Metrioptera sp.</i>	Tett	OR590	Japan	KF570760	KF570895	KF571021	KF571147	KF571397	KF571281
<i>Capnobotes sp.</i>	Tett	OR591	Utah, USA	KF570759	-----	KF571022	KF571148	KF571398	KF571282
<i>Stilpnochlora sp.</i>	Phan	OR592	FL, USA	KF570731	-----	KF571023	KF571149	KF571399	KF571283
<i>Steiroxys sp.</i>	Tett	OR593	WA, USA	KF570762	-----	KF571024	KF571150	KF571400	KF571284
<i>Plagiostira sp.</i>	Tett	OR594	UT, USA	KF570768	KF570894	KF571025	KF571151	KF571401	KF571285
<i>Neococephalus triops</i>	Cono	OR595	FL, USA	KF570796	KF570950	KF571026	KF571152	KF571402	KF571286
<i>Pyrgocorypha sp.</i>	Cono	OR596	SC, USA	KF570797	KF570927	KF571027	KF571153	KF571403	KF571287

Table 3 (continued)

Taxon	Subfamily	Voucher	Locality	18S	28S	COII	H3	TUBA	WG
<i>Amblycorypha</i> sp.	Phan	OR597	USA	KF570727	KF570842	-----	KF571154	KF571404	KF571288
<i>Atlanticus</i> sp.	Tett	OR598	FL, USA	KF570761	-----	KF571028	KF571155	KF571405	KF571289
<i>Conocephalus</i> sp.	Cono	OR599	VA, USA	KF570783	KF570951	KF571029	KF571156	KF571406	KF571290
<i>Anoedopoda erosa</i>	Mecop	OR600	Cameroon	KF570774	KF570952	KF571030	KF571157	KF571407	KF571291
<i>Adapantus pragerorum</i>	Pseud	OR601	Cameroon	KF570704	KF570953	-----	KF571158	-----	KF571292
<i>Stenamypx annulicornis</i>	Pseud	OR602	Cameroon	KF570782	KF570886	KF571031	KF571159	KF571408	KF571293
<i>Arytropteris modesta</i>	Tett	OR603	South Africa	KF570809	KF570874	KF571032	KF571160	-----	-----
<i>Horatosphaga</i> sp.	Phan	OR604	Namibia	KF570733	KF570954	KF571033	KF571161	KF571409	KF571294
<i>Weissenbornia</i> sp.	Phan	OR605	Cameroon	KF570730	-----	-----	KF571162	-----	KF571295
<i>Enochletica affinis</i>	Phan	OR606	Cameroon	KF570716	KF570857	KF571034	-----	KF571411	KF571296
<i>Tylopsis</i> sp.	Phan	OR607	South Africa	KF570738	KF570869	-----	KF571163	KF571412	KF571297
<i>Poecilomerus</i> sp.	Mecon	OR608	Madagascar	KF570811	KF570907	-----	KF571164	KF571413	KF571298
<i>Conocephalus (Megalotheca) vaginalis</i>	Cono	OR609	SouthAfrica	KF570786	KF570915	-----	KF571165	KF571414	KF571299
<i>Sphyrometopa</i> sp.	Cono	OR610	Costa Rica	KF570807	KF570920	-----	KF571166	KF571415	KF571300
<i>Holochlora</i> sp.	Phan	OR611	India	KF570724	KF570860	-----	KF571167	KF571416	KF571301
<i>Mimetica tuberala</i>	Pseud	OR612	Costa Rica	KF570694	KF570945	-----	KF571168	KF571417	KF571302
<i>Nicsara bifasciatum</i>	Cono	OR613	Australia	KF570806	KF570916	KF571035	KF571169	KF571418	KF571303
<i>Ischnomela</i> sp.	Pseud	OR614	Costa Rica	KF570776	KF570914	-----	KF571170	KF571419	KF571304
<i>Championica</i> sp.	Pseud	OR615	Peru	KF570705	KF570831	KF571036	KF571171	KF571420	KF571305
<i>Insara</i> sp.	Phan	OR616	Costa Rica	KF570744	KF570856	KF571037	KF571172	KF571421	KF571306
<i>Chloroscirtus discocercus</i>	Phan	OR617	Costa Rica	KF570729	KF570843	KF571038	KF571173	KF571422	KF571307
<i>Steirodon</i> sp.	Phan	OR618	Costa Rica	KF570732	KF570840	KF571039	KF571174	KF571423	KF571308
<i>Aegimia</i> sp.	Phan	OR619	Costa Rica	KF570749	KF570955	KF571040	KF571175	KF571424	KF571309
<i>Microcentrum</i> sp.	Phan	OR620	Costa Rica	KF570721	KF570835	KF571041	KF571176	KF571425	KF571310
<i>Ceraiaemytra</i> sp.	Phan	OR621	Panama	KF570728	KF570838	KF571042	KF571177	KF571426	KF571311
<i>Acantheremus colwelli</i>	Cono	OR622	Costa Rica	KF570821	KF570917	KF571043	KF571178	KF571427	KF571312
<i>Diyillus</i> sp.	Pseud	OR623	Costa Rica	KF570711	KF570828	KF571044	-----	-----	KF571313
<i>Phlugis irregularis</i>	Mecon	OR624	Bolivia	KF570755	KF570899	KF571045	KF571179	KF571428	KF571314
<i>Dolichocercus</i> sp.	Phan	OR625	Peru	KF570740	KF570855	-----	KF571180	KF571429	-----
<i>Acropsis</i> sp.	Phan	OR626	Peru	KF570741	KF570834	KF571046	KF571181	KF571430	KF571315
<i>Anaulacoma</i> sp.	Phan	OR627	Peru	KF570723	KF570841	KF571047	KF571182	KF571431	KF571316
<i>Xiphophyllum</i> sp.	Pseud	OR628	Bolivia	KF570706	KF570825	KF571048	KF571183	KF571432	KF571317
<i>Parapleminia</i> sp.	Pseud	OR629	Brazil	KF570708	KF570824	-----	KF571184	KF571433	KF571318
<i>Schedocentrus</i> sp.	Pseud	OR630	Peru	KF570714	KF570906	KF571049	KF571185	KF571434	KF571319
<i>Torbia viridissima</i>	Phan	OR631	Australia	KF570750	KF570866	KF571050	KF571186	KF571435	KF571320
<i>Polichne argentata</i>	Phan	OR632	Australia	KF570719	KF570865	KF571051	KF571187	KF571436	KF571321
<i>Mortoniellus ovatus</i>	Lip	OR633	Borneo	KF570697	KF570875	KF571052	KF571188	KF571437	KF571322
<i>Lipotactes maculatus</i>	Lip	OR634	Malaysia	KF570698	KF570876	KF571053	KF571189	KF571438	KF571323
<i>Kuzicus megaterminatus</i>	Mecon	OR635	India	KF570701	KF570877	KF571054	KF571190	KF571439	KF571324
<i>Alloteratura</i> sp.	Mecon	OR636	Malaysia	KF570703	KF570878	KF571055	KF571191	KF571440	KF571325
<i>Xiphidiopsis</i> sp.	Mecon	OR637	Malaysia	KF570702	KF570879	KF571056	KF571192	KF571441	KF571326
<i>Glenophysis</i> sp.	Hex	OR638	Malaysia	KF570686	KF570903	KF571057	KF571193	KF571327	-----
<i>Conocephalus</i> sp.	Cono	OR639	India	KF570787	KF570934	KF571058	KF571194	KF571328	-----
<i>Pyrgocorypha</i> sp.	Cono	OR640	India	KF570801	KF570935	KF571059	KF571195	KF571442	KF571329
<i>Paroxylakis</i> sp.	Cono	OR641	Malaysia	KF570799	KF570931	KF571060	KF571443	KF571330	-----
<i>Pseudorhynchus</i> sp.	Cono	OR642	Malaysia	KF570794	KF570926	KF571061	KF571196	KF571444	KF571331
<i>Phyllozelus</i> sp.	Pseud	OR643	India	KF570781	KF570888	KF571062	KF571197	KF571445	KF571332
<i>Ductetia japonica</i>	Phan	OR644	India	KF570746	KF570862	KF571063	-----	KF571446	KF571333
<i>Phaneroptera</i> sp.	Phan	OR645	Malaysia	KF570734	KF570861	KF571064	KF571198	KF571447	KF571334
<i>Elimaea</i> sp.	Phan	OR646	India	KF570735	KF570845	KF571065	KF571199	KF571448	KF571335
<i>Deflorita integra</i>	Phan	OR647	Malaysia	KF570737	KF570847	KF571200	KF571449	KF571336	-----
<i>Rectimarginalis ensis</i>	Phan	OR648	Malaysia	KF570725	KF570850	KF571201	KF571450	KF571337	-----
<i>Mirollia</i> sp.	Phan	OR649	Malaysia	KF570736	KF570846	KF571066	KF571202	KF571451	KF571338
<i>Letana megastridulata</i>	Phan	OR650	India	KF570748	KF570848	KF571067	KF571203	KF571452	KF571339
<i>Trigonocorypha</i> sp.	Phan	OR651	India	KF570726	KF570867	KF571068	KF571204	KF571453	KF571340
<i>Acauloplacella</i> sp.	Pseud	OR652	PNG	KF570808	KF570883	KF571069	KF571205	KF571454	KF571341
<i>Phrictaeformia insulana</i>	Mecop	OR653	PNG	KF570773	KF570908	KF571070	KF571206	KF571455	KF571342
<i>Conocephalus</i> sp.	Cono	OR654	PNG	KF570785	KF570933	KF571071	KF571207	KF571456	KF571343
<i>Pseudorhynchus cornutum</i>	Cono	OR655	PNG	KF570795	KF570922	KF571072	KF571208	KF571344	-----
<i>Teuthroides mimeticus</i>	Hex	OR656	PNG	KF570688	KF570902	KF571073	KF571209	KF571457	KF571345
<i>Meiophysis micropennis</i>	List	OR657	PNG	KF570812	KF570889	KF571074	KF571458	KF571346	-----
<i>Pterophylla camellifolia</i>	Pseud	OR658	Kentucky	KF570715	KF570833	KF571210	KF571459	KF571347	-----
<i>Pediodes</i> sp.	Tett	OR659	Texas	KF570766	KF570939	KF571075	KF571211	KF571460	KF571348
OUTGROUPS	Family	Voucher	Locality	18S	28S	COII	H3	TUBA	WG
<i>Henicus brevimucronatus</i>	Anostomatidae	OR420	South Africa	KF570822	KF570949	KF570998	KF571122	KF571389	-----
<i>Camptonotus carolinensis</i>	Gryllacrididae	OR024	N.C., USA	KF570818	KF570941	KF570958	KF571078	KF571351	KF571213
<i>Gryllacrididae</i>	Gryllacrididae	OR390	Malaysia	KF570819	KF570942	KF570995	KF571118	KF571386	KF571253
<i>Cyphoderris monstrosa</i>	Prophalangopsidae	OR021	Canada	KF570814	KF570943	KF570957	KF571077	KF571350	KF571212
<i>Troglophilus neglectus</i>	Raphidophoridae	OR083	Slovenia	KF570820	KF570948	KF570970	KF571092	KF571364	KF571227
<i>Stenopelmatus fuscus</i>	Stenopelmatidae	OR014	Utah, USA	KF570813	KF570940	KF570956	KF571076	KF571349	-----

Table 4
Primer pairs and PCR protocol used for sequence amplification. Primer and protocol source cited in text (2.2).

Primers	Sequence 5' → 3'	Annealing (°C)	Elongation (s)
<i>18SrDNA</i>			
18S 1F	TACCTGGTTGATCCTGCCAGTAG	52°	105 s
18S bi	GAGTCTCGTTTCGTTATCGGA		
18S b5.0 ^a	TAACCGCAACAACCTTAAT		
18S a0.7	ATTAAAGTTGTTCGGTT	46°	105 s
18S 9R	GATCCTTCCGAGGTTACCTAC		
18S a2.0 ^a	ATGGTTGCAAAGCTGAAAC		
<i>28SrDNA</i>			
28S Tetrd1a ^b	CGAGCGAACAGGGAAGAGCC	54°	120 s
28S rD5B	CCACAGCGCCAGTTCTGATTA		
28S 3b ^a	CCYTGAACGGTTTCACGTA		
28S 3a ^a	AGTACGTGAAACCGTTCAGG		
28S B ^a	TCGGAAGGAACAGCTAC		
28S A	GACCCGTCTTGAAAGCAGC	54°	120 s
28S Tet7b1 ^b	CTCTCCCGGATTTTCAAGGTC		
28S Tet4.7 ^{ab}	CCGGTCAAGCGAATGATTAGA		
<i>COII</i>			
COII Flue	TCTAATATGGCAGATTAGTGC	52°	75 s
COII R-lys	GAGACCAGTACTTGCTTCAGTCATC		
COII 2a ¹	ATAGAKCWTCYCCHTTAATAGAACA	52°	75 s
CPOO 9b ¹	GTACTTGCTTTCAGTCATCTWATG		
<i>Histone 3</i>			
H3 AF	ATGGCTCGTACCAAGCAGACV	50°	45 s
H3 AR	ATATCCTTRGGCATRATRGTG		
<i>Tubulin Alpha</i>			
294F1	GAAACCRGTKGGRACCAGTC	50°	50 s
294R1	GARCCCTACAAYTCYATTCT		
TH_Tub294F ²	CGGTACARGAKRCAGCAVGCAT	58°	45 s
TH_Tub294R ²	ACAYTCVGYAYTGYCCCTTCATGG		
<i>Wingless</i>			
WG 550F	ATGCGTCAGGARTGYAARTGY	50°	45 s
WG ABRZ	CACTTNACYTCRCARCACCAR		
WG 578F ²	TGCACNGTGAARACYTCGTGG	50°	45 s
WG ABR ²	ACYTCGCAGCACCARTGGAA		

¹ Second primer set used if the previous set was unsuccessful in amplifying desired sequence.

² Nested PCR with only the nested primers used for sequencing.

^a Internal primers used for sequencing only.

^b Primers designed for this study.

least partially covered by the pronotum (Fig. 3A–C). Characters were mapped onto the ML and MP topology in Mesquite V2.74 (Maddison and Maddison, 2009). Ancestral states were reconstructed with the Markov *k*-stat 1 parameter model (Pagel, 1994) on the ML topology and with unambiguous optimization under parsimony on the MP topology. We used the Approximately Unbiased (AU) test (Shimodaira, 2002) as implemented in Consel (Shimodaira and Hasegawa, 2001) to test for the monophyly of the following: (1) each subfamily that was not recovered as monophyletic; (2) the sister group relationship between the predatory Listroscolidinae + Austrosaginae; and (3) the sister group relationship between Zaprochilinae and Phasmodinae (Rentz, 1993). One hundred independent ML searches in RAxML V7.0.4 (Stamatakis, 2006) were performed to find the best scoring tree that kept these groups monophyletic, and then this score was compared to the unconstrained tree using the AU test.

3. Results

3.1. Taxon sampling

The Average Taxonomic Distinctiveness (AvTD) and Variation in Taxonomic Distinctiveness (VarTD) results calculated by the PhyRe script can be seen in Supplementary Fig. 1. The AvTD and VarTD for the taxon sampling are above the highest AvTD and below lowest

VarTD respectively across all samples. These results are indicative of a highly representative taxon sampling (Plazzi et al., 2010).

3.2. Alignment

No amino acid indels were found in the TUBA, H3 and COII alignments. Our alignment of WG resulted in an insertion from position 109 to 111 in the outgroup Raphidophoridae. In two taxa (*Phlugis* sp.) this gap extended an additional three base pairs (positions 112–114) for a total gap spanning six base pair positions. Alignments of 18S and 28S were unambiguous with few expansion regions. No significant difference (AU test *p*-value < 0.05) was found between trees resulting from Muscle, MAFFT, and GBlocks alignments, demonstrating that our results were not sensitive to alignment. The MAFFT alignment including the expansion regions of 18S and 28S was used for the final analyses.

3.3. Phylogenetic analyses

A six-parameter model, Gamma distribution, and proportion of invariable sites (GTR + I + G) was the best fit model of sequence evolution for each partition using the Akaike's Information Criterion (AIC) in JModeltest (Posada, 2008). The best ML tree found had a score of −95309.766555 (Fig. 5). The Bayesian tree (Supplementary Fig. 2) recovered had an average score of −95127.115 over

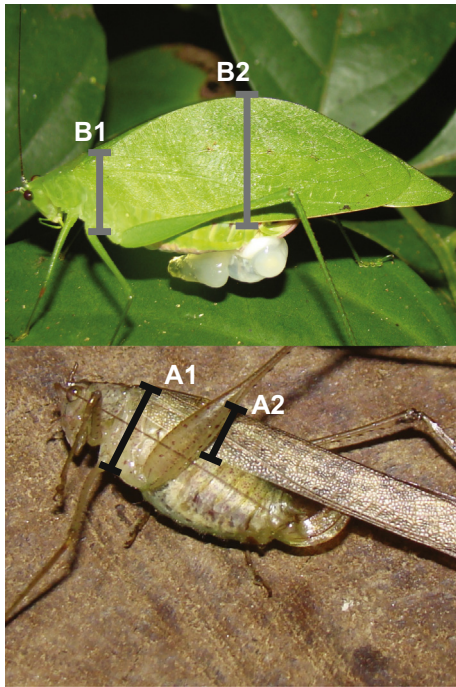


Fig. 4. Examples of katydids without leaf-like tegmina (A) having a wing width (A2) shorter than the height of the thorax (A1), and with leaf-like tegmina (B) having the width of the wing (B2) greater than the height of the thorax (B1). Photos by J. Mugleston.

the two runs. Both analyses recovered largely similar topologies. Minor differences are noted in the Tettigoniinae clade with the position of the sister groups *Platycleis affinis* + *Eobiana japonica* and *Pholidoptera* sp. + *Pachytrachis gracilis* varying in either analysis. The position of two Phaneropterinae species (*Aganacris* sp. and *Dysomorpha* sp.), and one small Phaneropterinae clade (*Ducetia japonica* + (*Tylopsis* sp. + *Letania megastridulata*) differed between the ML and BI analyses. The trees did not differ significantly (AU test p -value > 0.05) and despite the minor differences at weakly supported nodes, the overall topology in regards to subfamilial relationships was identical.

The MP search found three most parsimonious trees with a length of 20847 (CI 0.181, RI 0.542). The majority of positive Bremer Support for the MP topology came from WG (80.5%) with additional support coming from H3 (54.7%) and 28S (31.8%). 18S (−4.6%), COII (−20.1%) and TUBA (−54.7%) provided conflicting signal to the overall MP topology. The strict consensus (Fig. 6) resulted in a single polytomy within the apical Phaneropterinae clade. The MP topology did differ significantly from the ML tree (AU test p -value < 0.05). These differences are most notable at the poorly supported deeper nodes that lacked support in all analyses; these are discussed further below.

3.4. Monophyly of katydid subfamilies

Tettigoniidae was recovered as a monophyletic family in all analyses. The sister family to Tettigoniidae varied by analysis with ML and BI recovering Raphidophoridae as the sister lineage (Fig. 5 and Supplementary Fig. 2) while MP recovered Prophalangopsidae as the sister family (Fig. 6). The tettigoniid lineage sister to all other katydids is comprised of the pseudophylline tribe Pterochrozini. The endemic Australian subfamilies Tympanophorinae, Phasmodinae, and Zaprochilinae are monophyletic and sister to the remainder of the katydids in the ML and BI trees. A striking difference is seen in the placement of Saginae in the MP tree. ML and BI analyses

recovered the sagines as diverging from a more apical node sister to the Phaneropterinae + Pseudophyllinae (*sans* Pterochrozini) + Phyllophorinae + Mecopodinae clade. MP recovered Saginae diverging at an earlier node and sister to the Zaprochilinae.

Two large clades divide the remaining of Tettigoniidae with one (Clade A) comprised of the subfamilies Tettigoniinae, Conocephalinae, Meconematinae, Listrosclidinae, Hexacentrinae, Austrosaginae, Lipotactinae, and Hetrodinae. A second large clade (Clade B) is made up of Pseudophyllinae (except Pterochrozini) Mecopodinae, Phyllophorinae, and Phaneropterinae. The predatory katydids (Saginae) were found to be monophyletic, but the relationship of this subfamily to the other katydids is not well supported in any of the analyses.

Subfamilial relationships within Clade A were identical in the BI and ML topologies but differed at the weakly supported nodes from that of the MP topology. In each analysis, Clade A contained the well-supported monophyletic subfamilies Conocephalinae, Lipotactinae, Hexacentrinae, Hetrodinae, and Austrosaginae. Meconematinae was rendered paraphyletic with three distinct lineages: a clade of the New World species in the tribe Phlugidini, a clade of the Old World tribe Meconematini, and lineage represented by the Malagasy Phlugidini *Poecilomerus*. The positions of these Meconematinae clades are still unclear as they were not well supported in any analysis and constrained trees forcing Meconematinae to be monophyletic did not differ significantly (AU test p -value < 0.05) from the ML topology. Listrosclidinae was also rendered paraphyletic with three distinct lineages. One listrosclidine (*Meiophis*) from was recovered in a well-supported sister relationship with the Madagascan meconematine *Poecilomerus*. A second listrosclidine, *Arachnoscelis*, was recovered as sister to the New World Meconematinae tribe Phlugidini, but this relationship was not well supported in the ML or BI analysis. A third Listrosclidinae was recovered in each analysis as a well-supported sister to the monophyletic Hexacentrinae. The small predatory katydid subfamily Lipotactinae is monophyletic, but its relationship to the other tettigoniid subfamilies is unclear. The ML and BI trees have a poorly supported sister relationship between the Lipotactinae and the Austrosaginae + Tettigoniinae clade, but this relationship was not recovered with the MP analysis. Tettigoniinae was found to be monophyletic and sister to the Austrosaginae with the exception of the African tribe Arytropteridini which renders Tettigoniinae paraphyletic.

In Clade B, Phaneropterinae and Phyllophorinae were found to be monophyletic. Mecopodinae is paraphyletic with Phyllophorinae nested within and the tribe Aprophylini sister to the remaining katydids in Clade B. Pseudophyllinae is also paraphyletic with the tribe Pterochrozini as sister to the remaining Tettigoniidae, and two genera *Ischnomela* and *Cocnotus* being sister to the mecopodine clade.

Regardless of the method of tree reconstruction, Meconematinae, Tettigoniinae, Listrosclidinae, Mecopodinae, and Pseudophyllinae were recovered as paraphyletic. The results of the AU test showed the constrained topologies forcing a monophyletic Tettigoniinae, Pseudophyllinae, or Mecopodinae scored significantly worse than the unconstrained ML topology (AU test p -value < 0.05). The position of the Meconematinae and Listrosclidinae is not well resolved in any analysis, and the constrained topologies forcing either of those subfamilies were not significantly different than the unconstrained topology (AU test p -value > 0.05).

3.5. Character optimization

Characters mapped using the Mk-1 model on the ML topology (Fig. 5) and unambiguous optimization across the MP topology (Fig. 6) produced largely similar results with differences due to dis-

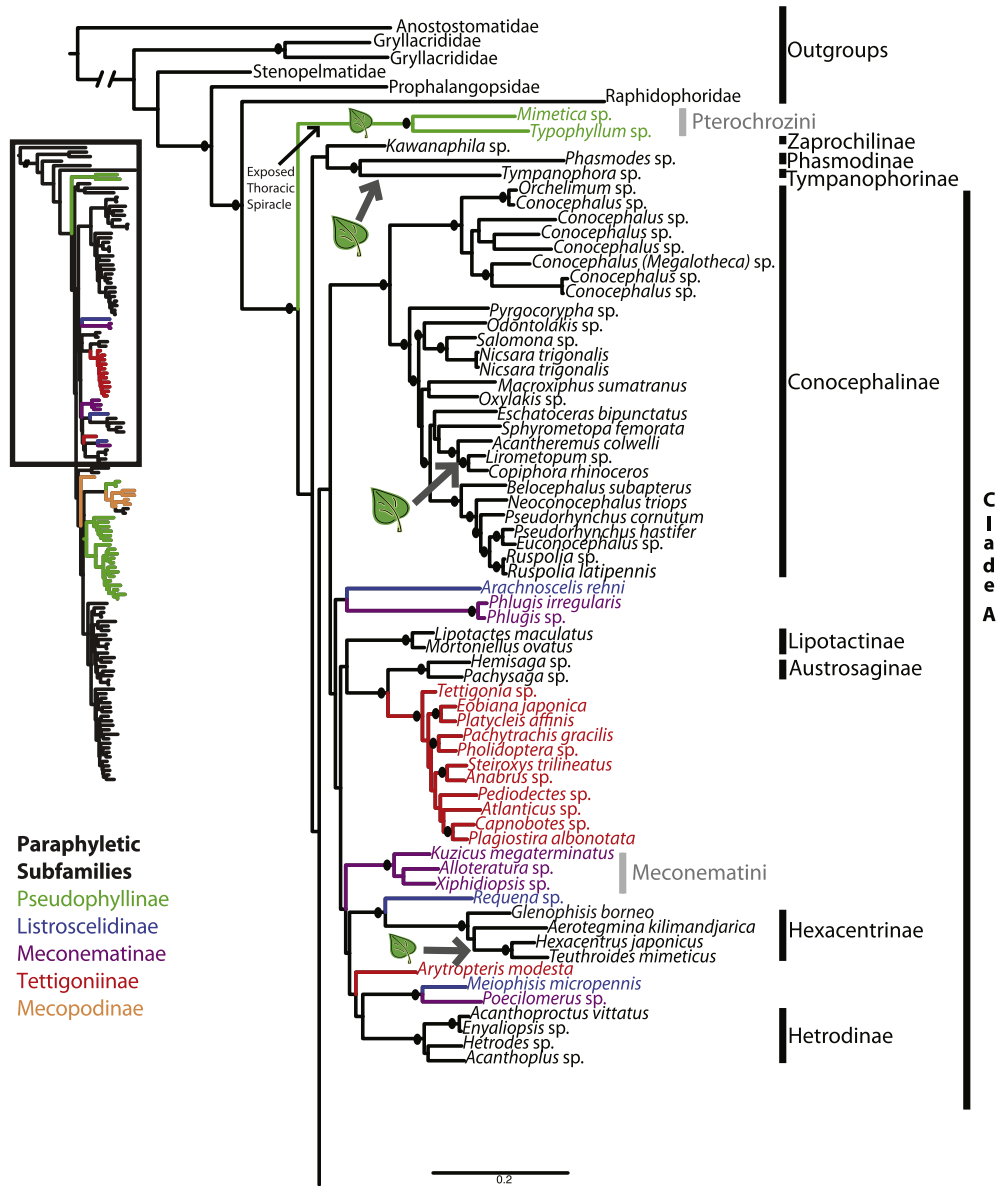


Fig. 5. Maximum Likelihood tree. Bootstrap values over 75 are marked by a black spot at the node. Colored branches indicate Paraphyletic subfamilies. Vertical bars denote monophyletic subfamilies. Leaf icons show the development of leaf-like tegmina while the leaf icon with a diagonal line show a loss of leaf-like tegmina. Character state changes (exposed or open) in thoracic auditory spiracle are displayed at the respective nodes.

agreements at the weakly supported nodes. The exposed thoracic auditory spiracle was derived at least twice within the Tettigoniidae: once after the basal divergence giving rise to Pterochrozini and at least once with the remaining Pseudophyllinae clade in B. There was at least a single transition from the exposed auditory spiracle to the larger and partially covered spiracle in the Phyllophorinae + *Anoedopoda erosa* + *Eumecopoda cyrtoscelis* clade.

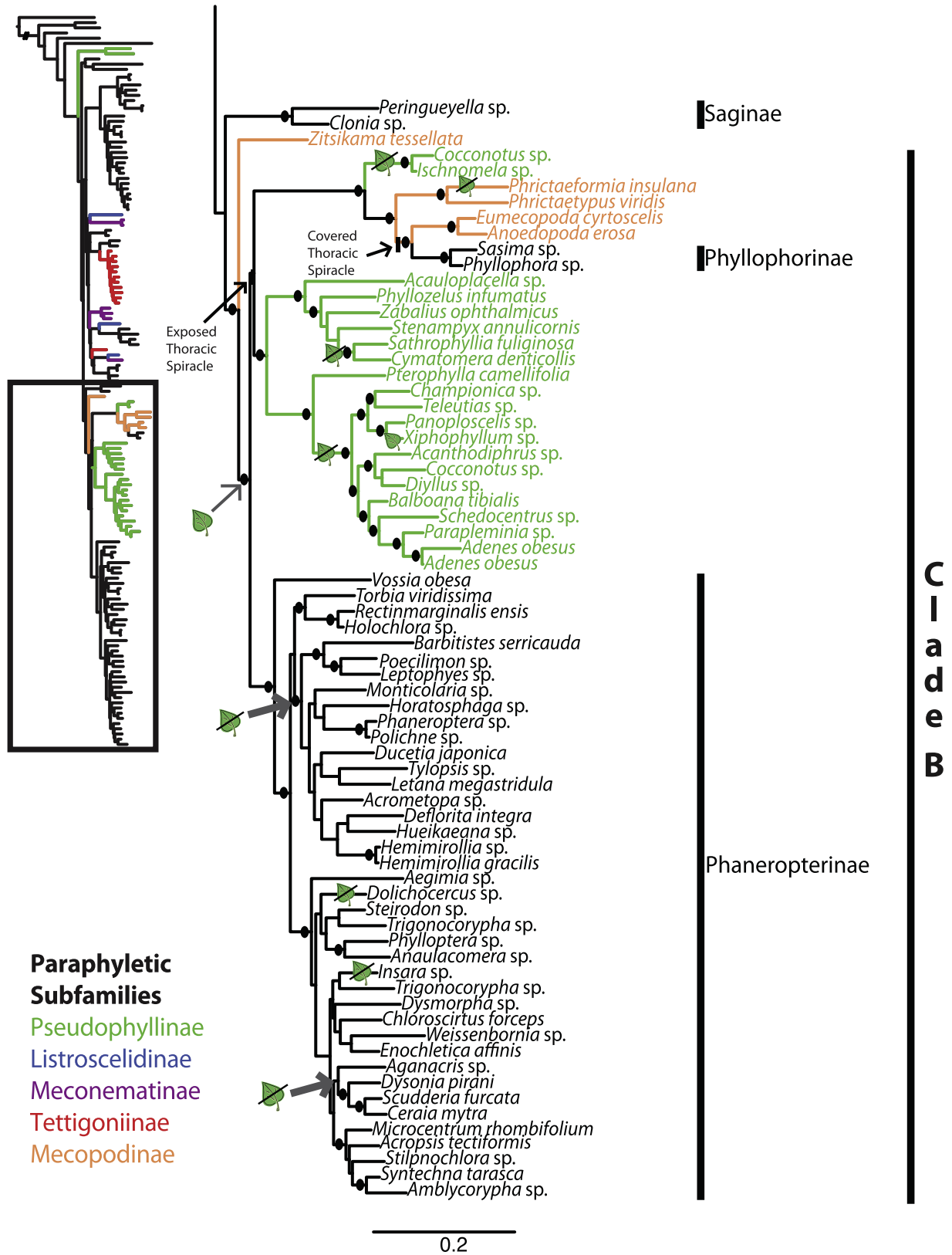
Eight of the 16 subfamilies in this analysis include taxa with leaf-like tegmina. The ancestral condition for katydids is lacking the leaf-like wings, but two lineages derived from basal nodes Pterochrozini and Tympanophorinae developed the specialized tegmina independently. Two subfamilies, Conocephalinae and Hexacentrinae have tropical species with leaf-like tegmina whereas most others within those subfamilies lack the specialized wings. In contrast to Clade A, leaf-like wings were derived at a basal node within Clade B but have since been subsequently lost in multiple lineages as detailed below (4.2).

4. Discussion

4.1. Katydid subfamilies

Our results represent the first formal phylogenetic analysis of Tettigoniidae. Previously classifications that divided the family into “primitive” and “advanced” groups were not supported in our analyses, as the “primitive” subfamily Hetrodinae is nested within a more “advanced” clade. Instead we found two large clades that contain the vast majority of katydid species (Clade A and B). Gorochov predicted a clade containing Pseudophyllinae, Phaneropterinae, Phyllophorinae, and Mecopodinae. A similar relationship was recovered in Clade B in our study.

The sister family to Tettigoniidae still remains unresolved. Rhaphidophoridae was recovered as sister to Tettigoniidae in the ML and BI analyses. Prophalangopsidae was found to be sister in the MP tree. Further work is needed to elucidate the relationship of Tettigoniidae to the other families within Ensifera.



Pterochrozini, a tribe currently placed within the subfamily Pseudophyllinae, was recovered as the sister lineage to the rest of Tettigoniidae. Pseudophyllinae is currently characterized by having a small thoracic spiracle not covered by the pronotum

and antennae that are strongly margined (Rentz, 1979). Our results show the small exposed auditory spiracle is homoplasious and is not a useful character for delineating this subfamily (Figs. 5 and 6). In addition, two subfamilies, Phyllophorinae and Mecopodinae

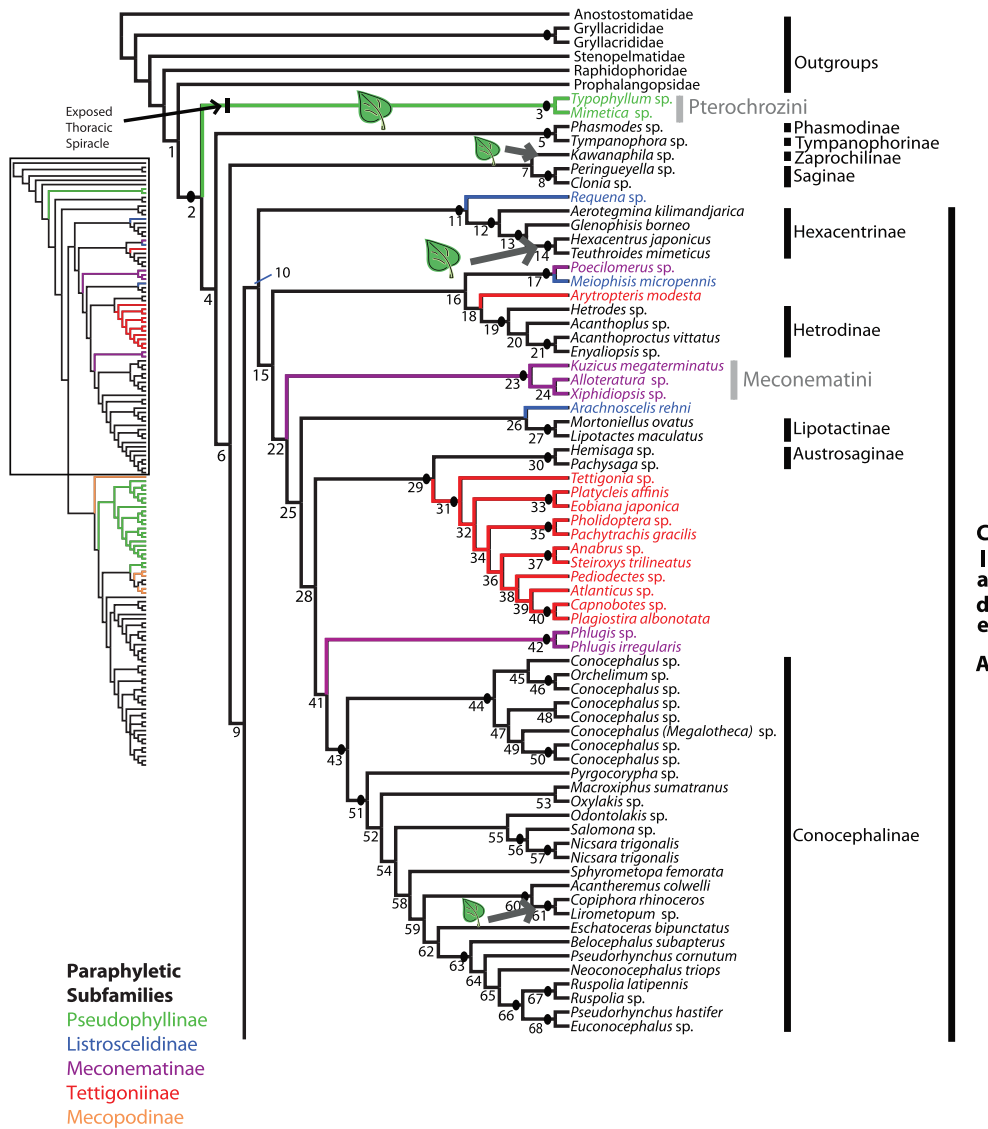


Fig. 6. Maximum Parsimony tree. Bootstrap values over 75 are marked by a black spot at the node. Node labels correspond to partitioned and total Bremer support values listed in [Supplementary Table 1](#). Leaf icons show the development of leaf-like tegmina while the leaf icon with a diagonal line show a loss of leaf-like tegmina. Character state changes (exposed or open) in thoracic auditory spiracle are displayed at the respective nodes.

(except Aprospylini) are nested within the Pseudophyllinae making further revisions necessary to split these subfamilies into monophyletic groups or to expand the synapomorphies that define this group and incorporate the diverse lineages nested within.

The widespread subfamily Conocephalinae was recovered as monophyletic. The sister relationship between Conocephalinae and the remaining subfamilies in Clade A is not well supported, and its position is sensitive to the methods used for tree reconstruction. Within Conocephalinae, the meadow katydid tribe Conocephalini (represented by *Conocephalus* sp. and *Orchelimum* sp. in this analysis) was recovered as a well-supported monophyletic tribe, but the tribes Agraciini and Copiphorini were found to be paraphyletic.

Within Clade A the smaller subfamilies Lipotactinae, Hexacentrinae, Austrosaginae, and Hetrodinae were all recovered as well-supported monophyletic groups. The Austrosaginae are sister to genera in the subfamily Tettigoniinae and not Listrosclidinae or Saginae as has been previously proposed. The African Hetrodinae were thought to be “primitive” by previous authors, but in each

analysis they were recovered as a derived group within Clade A. Previous authors have proposed Hetrodinae as sister to Tettigoniinae though this relationship was not supported in our study. Lipotactinae have been classified as a tribe in either Listrosclidinae (Hebard, 1922) or Tympanophorinae (Zeuner, 1936) but our results support [Ingrish's \(1995\)](#) elevation of this group to subfamilial rank.

The nominate subfamily Tettigoniinae was monophyletic save for the African tribe, Arytropteridini. Tettigoniinae from the southern hemisphere and the North American *Neduba*, are thought to be a monophyletic clade within Tettigoniinae ([Rentz, 1988](#)) but our results show at least some of this clade (*Arytropteris*) should be removed from the subfamily.

Mecopodinae is found primarily distributed throughout Southeast Asia to Australia with a few species found in South America and Africa. This subfamily includes the largest (>10 cm) and also loudest katydids. Currently Mecopodinae is diagnosed by characters similar to Phaneropterinae with the exception of a pair of prothoracic ventral spines found on Mecopodinae. Our results show Mecopodinae is a paraphyletic group with its posited sister taxon

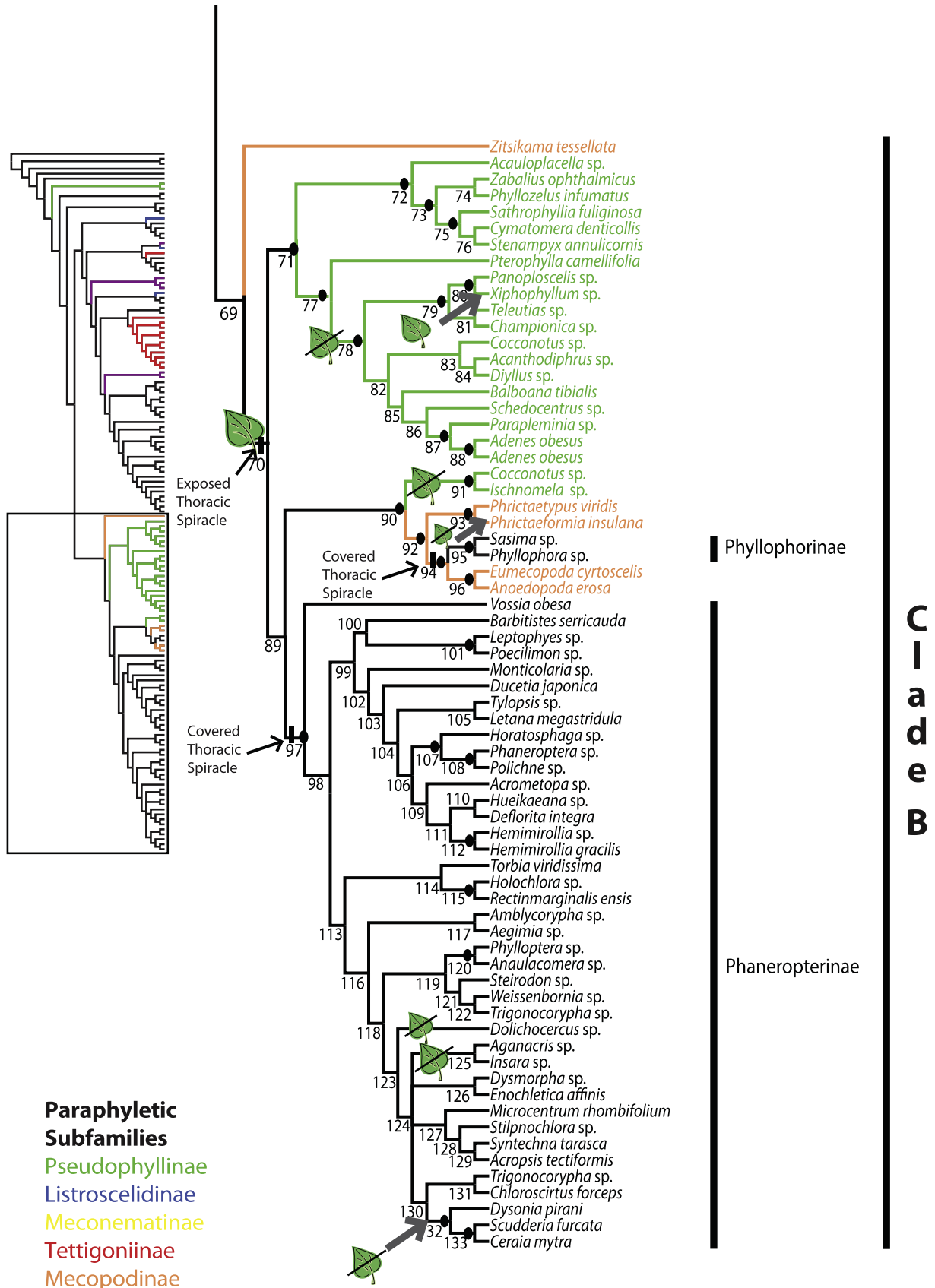


Fig. 6 (continued)

(Phyllophorinae) being nested within Mecopodinae. Additionally, the Mecopodinae tribe Aproshylini was recovered as the sister

lineage to the rest of Clade B. The placement of Aproshylini refutes the validity of Mecopodinae, but it supports previous comments

distinguishing Aprosphyliini as a “relict” relative to the other Mecopodinae (Naskrecki, 1994).

Phyllophorinae was found to be monophyletic, but it was nested within the paraphyletic Mecopodinae. Phyllophorinae have been proposed as sister to the Mecopodinae as seen by both subfamilies inclusion into Zeuner's “tettigonoids” (Table 1) and the sister relationship proposed by Gorochov (Fig. 1). Two characters define Phyllophorinae: a row of spines along the dorsal-lateral margin of the pronotum and a lack of the tegminal stridulation organ. In light of the phylogenetic analyses, Phyllophorinae is a subclade within Mecopodinae. The close relationship of Mecopodinae and Phyllophorinae provide an interesting opportunity to investigate opposing calling strategies with the large and loud Mecopodinae sharing much of their range with the equally large but much less boisterous Phyllophorinae.

Phaneropterinae contains nearly 1/3 of all described katydid species. This large subfamily was found to be monophyletic regardless of reconstruction methods. The relationships within this subfamily are still unclear with some genera (e.g., *Trigonocorypha*) supported as paraphyletic. Further sampling will be necessary to determine the relationships within these larger clades.

4.2. Leaf-like wings and thoracic spiracles

With a better understanding of tettigoniid relationships we can begin to look at the evolution of some aspects of their unique morphology. Our cursory analysis of katydid leaf-like tegmina and external auditory spiracles reveals a number of interesting patterns. At least six independent derivations of leaf-like tegmina have occurred within Tettigoniidae. Leaf-like tegmina are quite pronounced in the basal Pterochrozini lineage as well as within the Tympanophorinae. Most of the Conocephalinae found in the temperate regions have a fusiform body (Fig. 2D) while a few of the tropical Conocephalinae have developed leaf-like tegmina (Fig. 2F). Leaf-like tegmina are some of the most prominent characters of species within Clade B. Pseudophyllinae (false leaf katydids), Phaneropterinae (broad wing katydids), and Phyllophorinae (giant leaf katydids) all take their common names from their typical resemblance to leaves. Leaf-like wings were developed early within this clade, but show multiple losses throughout Clade B. At least four losses occur in the Pseudophyllinae + Mecopodinae + Phyllophorinae clade with a regain of the leaf-like wings in *Xiphophyllum* sp. Additional losses are within the Phaneropterinae including species that look like fungus and lichen (*Dysonia* sp.) as well as wasp mimics (*Aganacris* sp.) showing that the leaf-like wings are not a dead end, but that the level of mimicry and tegmina shape within tettigoniids is quite plastic and variable.

Pseudophyllinae (*sensu lato*) contains some of the most convincing leaf mimicking katydids. Some species have portions of their forewings that resemble fungus on a dead leaf. Others will have what appear to be holes, or chew marks in their wings, which presumably enhances the illusion of being a dead or decaying leaf. Though the leaf-like wings do not unite this group, the open auditory spiracle has long been thought to be a diagnostic character. The small exposed auditory spiracle is also seen in the Mecopodinae Sexavaini but remains a unifying character of Pseudophyllinae. This character is homoplasious with at least two independent derivations within katydids. Further work will need to be done to see if the open spiracle is associated with the leaf-like tegmina and small pronotum found on many Pseudophyllinae (*s.l.*) or if the exposed spiracle is associated with other changes associated with the katydid acoustic reception.

Though not a formal revision of Tettigoniidae we provide the following recommendations for future changes in the current taxonomy:

Arytropteridini should be removed from Tettigoniinae. Rentz (1988) posited that the southern hemisphere shield-backs and the North American *Neduba* form a monophyletic clade sister to the remaining Tettigoniinae. The current taxon sampling included only a single representative of these southern shield-backs making further sampling necessary to determine if additional revisions will be necessary for this subfamily.

Pterochrozini includes some of the most convincing leaf-like katydids. At least three genera within Pterochrozini are known to exhibit intraspecific color polymorphism (Castner and Nickle, 1995a). The legs of these katydids bear lobes and spines that complete the façade and make these insects look even more like an extension to the plant that they are resting on (Belwood, 1993). As mentioned above, this tribe is currently included within the Pseudophyllinae based on the small thoracic spiracle that is relatively similar in size to the respiratory spiracles. Pterochrozini were found to be sister to the remaining katydids and making it necessary to redefine the synapomorphies that define Pseudophyllinae and exclude this clade.

The characters defining Mecopodinae are homoplasious (?) resulting in a paraphyletic relationship for this subfamily. The supposed relict tribe Aprosphyliini should be removed from the Mecopodinae due to its basal position within Clade B. and not being sister to any of the other Mecopodinae.

5. Conclusions

Katydid are well recognized for their complex acoustic signaling and the amazing leaf-like tegmina found in many species. Studies into these two systems have been limited due to a lack of a formal phylogenetic hypothesis necessary to make testable predictions. Our results provide the framework for future studies investigating the evolution of these unique characters. We found that the basal lineage is not the posited “primitive” subfamilies presented in earlier studies, but instead the Pterochrozini, currently described within the paraphyletic Pseudophyllinae. In addition to deciphering relationships critical to understanding the evolution of katydids we show the need for further revisionary work to correct the currently misleading taxonomy. Our current understanding of tettigoniid diversity far surpasses that which was present when many of the subfamilies were first erected. Our results show that further revisions are necessary to better delineate the tettigoniid subfamilies. Revisionary work is most needed for three of the larger subfamilies Pseudophyllinae, Meconematinae, Tettigoniinae as well as the smaller Mecopodinae and Listroscolidinae.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympmv.2013.07.014>.

References

- Ander, K., 1939. Vergleichend-anatomische und phylogenetische Studien über die Ensifera (Saltatoria). *Opuscula Entomologica* (Supplementum II), 306.
- Bailey, W.J., 1990. The Ear of the Bushcricket. Springer-Verlag, New York.
- Bailey, W.J., 1993. The tettigoniid (Orthoptera, Tettigoniidae) ear – multiple functions and structural diversity. *International Journal of Insect Morphology and Embryology* 22, 185–205.
- Bailey, W.J., Stephen, R.O., 1978. Directionality and auditory slit function: a theory of hearing in bushcrickets. *Science* 201, 633–634.
- Belwood, J.J., 1993. Anti-predator defences and ecology of neotropical forest katydids, especially the pseudophyllinae. In: Bailey, W.J., Rentz, D.C.F. (Eds.), *The Tettigoniidae: Biology, Systematics, and Evolution*. Springer-Verlag, pp. 8–26.
- Bremer, K.D., 1994. Branch support and tree stability. *Cladistics* 10, 295–304.
- Brunner von Wattenwyl, C., 1878. *Monographie der Phaneropteriden*.
- Buckman, R.S., Mound, L.A., Whiting, M.F., 2013. Phylogeny of thrips (Insecta: Thysanoptera) based on five molecular loci. *Systematic Entomology* 38, 133.
- Burmeister, H., 1838. *Kaukerfe, Gymnognatha* (Erste Hälfte: Vulgo Orthoptera). *Handbuch der Entomologie* 22 (I–VIII), 397–756.
- Bush, S.L., Beckers, O.M., Schul, J., 2009. A complex mechanism of call recognition in the katydid *Neoconocephalus affinis* (Orthoptera: Tettigoniidae). *Journal of Experimental Biology* 212, 648–655.
- Castner, J.L., 1995. Defensive behavior and display of the leaf-mimicking katydid *Pterochroza ocellata* (L.) (Orthoptera: Tettigoniidae: Pseudophyllinae: Pterochrozini). *Journal of Orthoptera Research*, 89–92.
- Castner, J.L., Nickle, D.A., 1995a. Intraspecific color polymorphism in leaf-mimicking katydids (Orthoptera: Tettigoniidae: Pseudophyllinae: Pterochrozini). *Journal of Orthoptera Research*, 99–103.
- Castner, J.L., Nickle, D.A., 1995b. Observations on the behavior and biology of leaf-mimicking katydids (Orthoptera: Tettigoniidae: Pseudophyllinae: Pterochrozini). *Journal of Orthoptera Research*, 93–97.
- Castresana, J., 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* 17, 540–552.
- Çiplak, B., 2004. Systematics, phylogeny and biogeography of anterastes (Orthoptera, Tettigoniidae, Tettigoniinae): evolution within a refugium. *Zoologica Scripta* 33, 19–44.
- Colgan, D.J., McLauchlan, A., Wilson, G.D.F., Livingston, S.P., Edgecombe, G.D., Macaranas, J., Cassis, G., Gray, M.R., 1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Australian Journal of Zoology* 46, 419–437.
- Desutter-Grandcolas, L., 2003. Phylogeny and the evolution of acoustic communication in extant Ensifera (Insecta, Orthoptera). *Zoologica Scripta* 32, 525–561.
- Eades, D.C., Otte, D., 2009. *Orthoptera Species File Online*.
- Edgar, R.C., 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 5, 1–19.
- Fenn, J.D., Song, H., Cameron, S.L., Whiting, M.F., 2008. A preliminary mitochondrial genome phylogeny of Orthoptera (Insecta) and approaches to maximizing phylogenetic signal found within mitochondrial genome data. *Molecular Phylogenetics and Evolution* 49, 59–68.
- Gerstaecker, 1863. *Scepastus* und *Phylloscyrtus*, zwei käferähnliche Grylloden-Gattungen. *Stettiner Entomologische Zeitung* 29, 408–436.
- Giannuli, T., Dutrillaux, A.M., Lemonnier-Darcemont, M., Darcemont, C., Myrthianou, E., Stamatidis, C., Dutrillaux, B., Mamuris, Z., 2011. Molecular phylogeny of European saga: comparison with chromosomal data. *Bulletin of Insectology* 64, 263–267.
- Goloboff, P.A., 1999. Analyzing large data sets in reasonable times: solutions for composite optima. *Cladistics* 15, 415–428.
- Goloboff, P.A., Farris, J.S., Nixon, K.C., 2008. TNT, a free program for phylogenetic analysis. *Cladistics* 24, 774–786.
- Gorochoff, 2010. New and little-known orthopteroiid insects (Polyneoptera) from fossil resins. *Paleontological Journal* 44, 657–671.
- Gorochoff, A.V., 1988. The classification and phylogeny of grasshoppers (Gryllida-Orthoptera, Tettigoniidae). (In Russian). In: *The Cretaceous Biocenotic Crisis and the Evolution of Insects*.
- Gwynne, D.T., 1995. Phylogeny of the Ensifera (Orthoptera): a hypothesis supporting multiple origins of acoustical signalling, complex spermatophores and maternal care in crickets, katydids, and weta. *Journal of Orthoptera Research*, 203–218.
- Gwynne, D.T., 2001. *Katydid and Bush-Crickets*. Comstock Publishing Associates.
- Gwynne, D.T., Morris, G.K., 2002. *Tettigoniidae: Katydid, Long-Horn Grasshoppers, and Bushcrickets*. Version 26 November 2002. <http://tolweb.org/Tettigoniidae/13298/2002.11.26> in *The Tree of Life Web Project*, <http://tolweb.org/>.
- Hebard, 1922. Studies in Malayan, Melanesian and Australian Tettigoniidae (Orthoptera). *Proceeding of the Academy of Natural Sciences, Philadelphia* 74, 121–299.
- Hoy, R.R., Robert, D., 1996. Tympanal hearing in insects. *Annual Review of Entomology* 41, 433–450.
- Ingrisch, S., 1995. Revision of the Lipotactinae, a new subfamily of Tettigoniidae (Ensifera). *Insect Systematics & Evolution* 26, 273–320.
- Jost, M.C., Shaw, K.L., 2006. Phylogeny of Ensifera (Hexapoda: Orthoptera) using three ribosomal loci, with implications for the evolution of acoustic communication. *Molecular Phylogenetics and Evolution* 38, 510–530.
- Katoh, K., Kuma, K.-i., Toh, H., Miyata, T., 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Research* 33, 511–518.
- Korsunovskaya, O., 2008. Acoustic signals in katydids (Orthoptera, Tettigoniidae). *Communication I. Entomological Review* 88, 1032–1050.
- Krauss, 1902. Die Namen der ältesten Dermapteren- (Orthopteren-) Gattungen und ihre Verwendung für Familien- und Unterfamilien-Benennungen auf Grund der jetzigen Nomenclaturregeln. *Zoologischer Anzeiger* 25, 530–543.
- Kumar, S., Nei, M., Dudley, J., Tamura, K., 2008. MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. *Briefings in Bioinformatics* 9, 299–306.
- Latreille, 1802. *Histoire Naturelle. Générale et particulière, des Crustacés et des Insectes* 3, 267–284.
- Legendre, F., Robillard, T., Song, H., Whiting, M.F., Desutter-Grandcolas, L., 2010. One hundred years of instability in ensiferan relationships. *Systematic Entomology* 35, 475–488.
- Maddison, W.P., Maddison, D.R., 2009. *Mesquite: a modular system for evolutionary analysis*. Version 2.74 <http://mesquiteproject.org>
- Marshall, D.C., Hill, K.B.R., 2009. Versatile aggressive mimicry of cicadas by an Australian predatory katydid. *PLoS ONE* 4, e4185.
- Montealegre-Z, F., 2009. Scale effects and constraints for sound production in katydids (Orthoptera: Tettigoniidae): correlated evolution between morphology and signal parameters. *Journal of Evolutionary Biology* 22, 355–366.
- Naskrecki, P., 1994. The Mecopodinae of southern Africa (Orthoptera: Tettigoniidae: Tettigoniinae). *Journal of African Zoology* 108, 279–320.
- Naskrecki, P., 2000a. *Katydid of Costa Rica Volume 1. Systematics and Bioacoustics of the Cone-head Katydid*. The Orthopterists' Society at the Academy of Natural Sciences of Philadelphia.
- Naskrecki, P., 2000b. *The phylogeny of katydids (Insecta: Orthoptera: Tettigoniidae) and the evolution of their acoustic behavior*. University of Connecticut.
- Nel, A., Prokop, J., Ross, A.J., 2008. New genus of leaf-mimicking katydids (Orthoptera: Tettigoniidae) from the late Eocene–early Oligocene of France and England. *Systematic Palaeontology* 7, 211–216.
- Nickle, D.A., Castner, J.L., 1995. Strategies utilized by katydids (Orthoptera: Tettigoniidae) against diurnal predators in rainforests of northeastern Peru. *Journal of Orthoptera Research*, 75–88.
- Nixon, K.C., 1999. The parsimony ratchet, a new method for rapid parsimony analysis. *Cladistics* 15, 407–414.
- Pagel, M., 1994. Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. *Proceedings: Biological Sciences* 255, 37–45.
- Plazzi, F., Ferrucci, R.R., Passamonti, M., 2010. Phylogenetic representativeness: a new method for evaluating taxon sampling in evolutionary studies. *BMC Bioinformatics* 11.
- Posada, D., 2008. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* 25, 1253–1256.
- Ragge, D.R., 1955. *Wing-Venation of the Orthoptera Saltatoria with notes on Dictyopteran Wing-Venation*. British Museum, London.
- Rambaut, A., Drummond, A.J., 2003. *Tracer v1.3*. Oxford, UK.
- Rentz, D.C.F., 1979. Comments on the classification of the Orthoptera family Tettigoniidae, with a key to subfamilies and description of 2 new subfamilies. *Australian Journal of Zoology* 27, 991–1013.
- Rentz, D.C.F., 1988. The shield-backed katydids of southern Africa: their taxonomy, ecology and relationships to the faunas of Australia and South America (Orthoptera: Tettigoniidae: Tettigoniinae). *Invertebrate Systematics* 2, 223–335.
- Rentz, D.C.F., 1993. *A Monograph of the Tettigoniidae of Australia: Volume 2: The Austrosaginae, Phasmodinae and Zaprochilinae*. CSIRO, Melbourne.
- Rentz, D.C.F., 1995. Do the spines on the legs of katydids have a role in predation? (Orthoptera: Tettigoniidae: Listrosclidinae). *Journal of Orthoptera Research*, 199–200.
- Rentz, D.C.F., 2001. *Tettigoniidae of Australia Volume 3: Listrosclidinae, Tympanophorinae, Meconematinae, and Microtettigoniinae*. CSIRO.
- Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19, 1572–1574.
- Shapiro, L.H., Strazanac, J.S., Roderick, G.K., 2006. Molecular phylogeny of Banza (Orthoptera: Tettigoniidae), the endemic katydids of the Hawaiian archipelago. *Molecular Phylogenetics and Evolution* 41, 53–63.
- Sharov, A.G., 1968. *Phylogeny of orthopteroidea*. Paleontological Institute Academy of Science USSR 118, 1–216.
- Shimodaira, H., 2002. An approximately unbiased test of phylogenetic tree selection. *Systematic Biology* 51, 492–508.
- Shimodaira, H., Hasegawa, M., 2001. CONSEL: for assessing the confidence of phylogenetic tree selection. *Bioinformatics* 17, 1246–1247.
- Simmons, L.W., Bailey, W.J., 1990. Resource influenced sex-roles of zaprochiline tettigoniids (Orthoptera, Tettigoniidae). *Evolution* 44, 1853–1868.
- Simmons, L.W., Gwynne, D.T., 1993. Reproductive investment in bush-crickets – the allocation of male and female nutrients to offspring. *Proceedings of the Royal Society of London Series B – Biological Sciences* 252, 1–5.
- Snyder, R.L., Frederick-Hudson, K.H., Schul, J., 2009. Molecular phylogenetics of the genus *Neoconocephalus* (Orthoptera, Tettigoniidae) and the evolution of temperate life histories. *PLoS ONE* 4 (article no.: e7203).
- Sorenson, M.D., Franzosa, E.A., 2007. *Treeroot*. Boston University, Boston, MA.
- Stål, 1876. *Observations orthoptérologiques 2. Les genres des acridioïdes de la faune européenne*. *Bihang till Kungliga Svenska Vetenskaps-Akademiens Handlingar* 4, 1–58.

- Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22, 2688–2690.
- Svenson, G.J., Whiting, M.F., 2004. Phylogeny of Mantodea based on molecular data: evolution of a charismatic predator. *Systematic Entomology* 29, 359–370.
- Svenson, G.J., Whiting, M.F., 2009. Reconstructing the origins of praying mantises (Dictyoptera, Mantodea): the roles of Gondwanan vicariance and morphological convergence. *Cladistics* 25, 468–514.
- Swofford, D.L., 2003. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Sinauer Associates, Sunderland, Massachusetts.
- Théobald, 1937. Les Insectes Fossiles des terrains oligocenes de France. *Bulletin de la Société des Sciences de Nancy*. Nancy, 1–473.
- Wedell, N., 1993. Spermatophore size in bush-crickets – comparative evidence for nuptial gifts as a sperm protection device. *Evolution* 47, 1203–1212.
- Whiting, M.F., 2002. Mecoptera is paraphyletic: multiple genes and phylogeny of Mecoptera and Siphonaptera. *Zoologica Scripta* 31, 93–104.
- Wild, A.L., Maddison, D.R., 2008. Evaluating nuclear protein-coding genes for phylogenetic utility in beetles. *Molecular Phylogenetics and Evolution* 48, 877–891.
- Zeuner, F.E., 1936. The subfamilies of Tettigoniidae (Orthoptera). *Proceedings of the Royal Entomological Society of London. Series B, Taxonomy* 5, 103–109.
- Zeuner, F.E., 1939. Fossil Orthoptera, Ensifera. *British Museum Natural History*, London.