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Reinventing the leaf: multiple origins of leaf-like wings in katydids (Orthoptera : Tettigoniidae)

Joseph Mugleston[A,C], *Michael Naegle*[A], *Hojun Song*[B], *Seth M. Bybee*[A], *Spencer Ingley*[A], *Anton Suvorov*[A] *and Michael F. Whiting*[A]

[A]Department of Biology and M.L. Bean Life Science Museum, Brigham Young University, 4102 Life Science Building, Provo, UT 84602, USA.
[B]Department of Entomology, Texas A&M University, 2475 TAMU, College Station, TX 77843-2475, USA.
[C]Corresponding author. Email: joseph.mugleston@gmail.com

Abstract. Insects have developed incredible means to avoid detection by predators. At least five insect orders have species that resemble leaves. Katydids (Orthoptera : Tettigoniidae) are the most diverse and wide-ranging of the leaf-like insects. At least 14 of the 20 extant katydid subfamilies contain species with leaf-like wings. Although it is undisputed that many katydids resemble leaves, methods for delineating the leaf-like from non-leaf-like forms have varied by author and in many cases are not explicitly stated. We provide a simple ratio method that can be used to differentiate the leaf-like and non-leaf-like forms. Geometric morphometrics were used to validate the ratio method. Leaf-like wings have been independently derived in at least 15 katydid lineages. Relative rates of speciation were found to be greater in the non-leaf-like forms, suggesting that leaf-like wings within Tettigoniidae are not a driver of diversification. Likewise, throughout Tettigoniidae, selection seems to be favouring the transition away from leaf-like wings. However, within the large Phaneropterinae subclade, relative speciation and transition rates between the leaf-like and non-leaf-like forms do not differ significantly.

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Introduction

Selective pressures exerted by visual predators have resulted in an incredible array of animal defences including camouflage and mimicry (Johannesson and Ekendahl 2002; Ruxton *et al*. 2004; Svanbäck and Eklöv 2011). Species that are better able to conceal their identity from a predator through more elaborate forms of crypsis, or more convincing disguises, have a selective advantage over species whose crypsis is less convincing (Johannesson and Ekendahl 2002). How well an organism resembles its surroundings (crypsis), resembles an aposematic organism (mimicry), or resembles an uninteresting object (masquerade or mimesis) can all affect rates of predation (Johannesson and Ekendahl 2002; Ruxton *et al*. 2004; Skelhorn *et al*. 2010*a*; Skelhorn *et al*. 2010*b*; Svanbäck and Eklöv 2011). Studies on the evolution of camouflage from a phylogenetic standpoint are just beginning to elucidate the evolution of crypsis in animals (Vane-Wright and Boppre 1993; Crespi and Sandoval 2000; Hultgren and Stachowicz 2009; Marshall and Gluckman 2015; Rajabizadeh *et al*. 2015).

Insects exhibit a wide range of camouflage including many species that closely resemble leaves (Nentwig 1985; Schmidt 1990; Svenson and Whiting 2009; Conner 2014; Fabricant and Herberstein 2014; Pinheiro and Freitas 2014). At least five insect orders contain species with leaf-like mimesis, including Lepidoptera (butterflies and moths), Neuroptera (lacewings), Mantodea (mantises), Phasmatodea (walking sticks and leaf insects) and Orthoptera (grasshoppers, katydids and crickets) (Fig. 1). In each of these orders, the specialised mimetic morphology, colouration or behaviour is thought to aid in avoiding detection by predators or prey (Robinson 1969; Edmunds 1974; Castner 1995; Castner and Nickle 1995*b*).

Among insects, the most frequently encountered and most diverse range of leaf-like disguises is found in the orthopteran family Tettigoniidae (katydids and bush crickets). Katydids are a cosmopolitan family with more than 7000 described species (Eades *et al*. 2015). The similarity between katydid forewings and leaves has long been recognised and forms the basis for some of their taxonomic names (e.g. Pseudophyllinae: 'false leaf' and *Phylloptera*: 'leaf-winged'). The leaf-like appearance of katydid wings is achieved by modifications to the forewing (tegmina) shape, wing positioning, colour, venation and in some species additional modifications to the legs enhance the overall leaf-like appearance (Fig. 2). The diversity of leaf-like disguises makes Tettigoniidae an ideal system to study the evolution of leaf-like wings and the influence of these modifications on katydid diversity. What remains unclear is what specific morphological

Fig. 1. Examples of insects that resemble leaves from multiple insect orders including (*A*) Orthoptera: *Celidophylla albimacula*, (*B*) Neuroptera: *Drepanepteryx phalaenoides*, (*C*) Phasmatodea: *Phyllium philippinicum* and (*D*) Mantodea: *Choeradodis rhombicollis*. Photo credits are as follows: (*A* and *D*) Nathan Lord, (*B*) Dragiša Savić and (*C*) Joshua Snyder.

modifications make an insect a mimic of leaves and how often 'leaf-like' disguises have evolved throughout the diversification of Tettigoniidae.

Within Tettigoniidae, katydids that resemble leaves occur in 14 of the 20 extant subfamilies (Gwynne 2001; accounting for the elevation of Pterochrozinae). Distantly related katydids have independently derived the leaf-like disguise multiple times (Mugleston *et al*. 2013). Katydid tegmina encompass a range of sizes and shapes including enlarged, rounded or lobed wings, broad rectangular wings, or irregular-shaped wings with tattered edges (Fig. 3). The leaf-like disguises afford katydids some protection via crypsis by allowing them to blend in among leaves (Castner 1995; Castner and Nickle 1995*a*, 1995*b*; Nickle and Castner 1995).

An objective method to diagnose the presence of leaf-like wings is critical to properly study the evolution of leaf-like mimesis across Tettigoniidae. Many katydids possess wings that are clearly similar in appearance to leaves with tattered edges (resembling damage from fungus or herbivores), exaggerated veins resembling the midline vein on a leaf, and modified venation

Fig. 2. Variation in katydids with leaf-like wings. (*A*) *Steirodon* sp., (*B*) *Microcentrum* sp. and (*C*) *Mimeteca* sp.

relative to other katydids. In contrast, some katydids have narrow wings that, while cryptically coloured (green or brown), do not resemble the shape of leaves (Fig. 3). Between these two extremes are numerous species with broadened green or brown wings that at least superficially resemble leaves, but a clear cut-off between the two wing types has not yet been presented. Consequently, there is no consensus among katydid researchers of what constitutes a leaf-like wing and individual definitions of leaf-like wings vary by author.

The purpose of this study is to better understand the evolution of crypsis via leaf-like wings in katydids. To do this, a clear definition of leaf-like wings is provided using geometric morphometrics along with a ratio method developed in this study. The gains and losses of leaf-like wings within Tettigoniidae are investigated using a robust phylogenetic hypothesis. Additionally, we compared the relative rate of speciation of the leaf-like with non-leaf-like katydids to determine whether the leaf-like wings are a driving force in the diversification of katydids.

Materials and methods

Morphometric analysis

Two methods were used in an attempt to objectively identify what constitutes a leaf-like wing in katydids. The first method was a linear morphometric technique that compared the ratio between the width of the forewing and the height of the thorax. The width of the tegmen was measured from leading to trailing edge at its widest portion. The height of the thorax was measured as the distance from the sternites to tergites of the prothorax. Katydids with wing width greater than thorax height were considered to be leaf-like (Fig. 4). Wing data for voucher specimens with damaged wings or vouchers for which we only had immature specimens were taken from the literature or photos of adults available on the Orthoptera Species File Online respectively (http://orthoptera.speciesfile.org/HomePage/Orthoptera/HomePage.aspx). By using the relative measurement of the wing width to the thorax height, the broadened leaf-like wings of the mimetic katydids can be separated from those with the narrow wings typical of most orthopteran insects including the non-leaf-like tettigoniids.

The second method used was a landmark-based geometric morphometric (GM) technique to quantify wing shape with greater detail. With GM methods, morphological landmarks are compared using a multivariate statistical framework, allowing comparisons of shape while controlling for variations in size. We developed a landmarking scheme that included 14 morphological landmarks representing major characteristics of wing shape (Fig. 5). All 14 morphological landmarks were digitised using tpsDig (Rohlf 2005) on a scanned image of the wing for each species. Shape variation from digital landmarks was summarised into relative warps (i.e. principal components) using tpsRelw (Rohlf 2003). All non-shape variation due to position, orientation and scale of the specimens for each image was removed using generalised Procrustes analysis (Rohlf and Slice 1990) and the minimise d^2 option in tpsRelw was used for sliding semi-landmarks. Relative warps are defined as linear combinations of affine and non-affine shape components that

Fig. 3. Diversity in katydid wings showing examples of (*A–Y*) leaf-like wings (green box), (*Z* and *AA*) wings of broad-winged bark mimics (brown box), (*BB–DD*) wings of mimetic species (blue box) and (*EE–II*) non-leaf-like wings (red box). Wings are not to scale. The yellow colouration of the wings is an artefact of preservation; wings were green in life. Species are as follows: (*A*) *Mimeteca tuberata*, (*B*) *Hexacentrus* sp., (*C*) *Lirometopum coronatum*, (*D*) *Copiphora* sp., (*E*) *Copiphora hastata*, (*F*) *Phyllozelus* sp., (*G*) *Dysmorpha* sp., (*H*) *Acropsis* sp., (*I*) *Amblycorypha* sp., (*J*) *Sasima* sp., (*K*) *Microcentrum* sp., (*L*) *Mirrolia* sp., (*M*) *Hyperphrona irregularis*, (*N*) *Euceria rufovariegata*, (*O*) *Enochletica affinis*, (*P*) *Pterophylla camelifolia*, (*Q*) *Ceraia mytra*, (*R*) *Holochlora* sp., (*S*) *Rectimarginalis ensis*, (*T*) *Eurycorypha* sp., (*U*) *Eurycophora* sp., (*V*) *Eumecopoa* sp., (*W*) *Cnemidophyllum eximium*, (*X*) *Aegimia* sp., (*Y*) *Phylloptera* sp., (*Z*) *Cymatomera* sp., (*AA*) *Sathrophylla* sp., (*BB*) *Dysonia* sp., (*CC*) *Quiva* sp., (*DD*) *Aganacris* sp., (*EE*) *Conocephalus* sp., (*FF*) *Ruspolia* sp., (*GG*) *Conocephalus* sp., (*HH*) *Insara* sp. and (*II*) *Platycleis* sp.

describe some portion of the variation observed in the specimens (Rohlf 2003).

To compare wing shape between groups, each specimen was designated as either leaf-like or non-leaf-like based on the ratio method described above. A multivariate analysis of variance (MANOVA) framework was used to compare wing shape between leaf-like and non-leaf-like groups (wing group as a main effect), using wing size (measured in terms of centroid size) as a covariate. The response variables consisted of the first twelve relative warps scores, which collectively explained over 98% of the variation. An interaction term between size and wing group to determine if size affected wing shape differently according to wing group was included. Given the results of the MANOVA (see 'Results' below), we conducted a linear discriminant analysis (LDA) to determine with what accuracy these data would correctly predict which wing group a species belonged to based on our ratio method. This method attempts to build a linear model based on the input data that will maximise the explanatory power of the categorical grouping variables assigned. We used all 24 relative warps as predictor variables in the LDA in order to maximise the explanatory power of the model. Once the model had been created, we conducted

Fig. 4. Leaf-like wings were determined by comparing the wing width at the widest portion measured from the anterior to the posterior edges (yellow bars) with the height of the thorax (white bars) measured at the top of the pronotum to the sternites of the mesothorax. (*A*) Non-leaf-like katydids have a wing width that is less than the height of the thorax. (*B*) Katydids with a wing width greater than the thorax height are leaf-like.

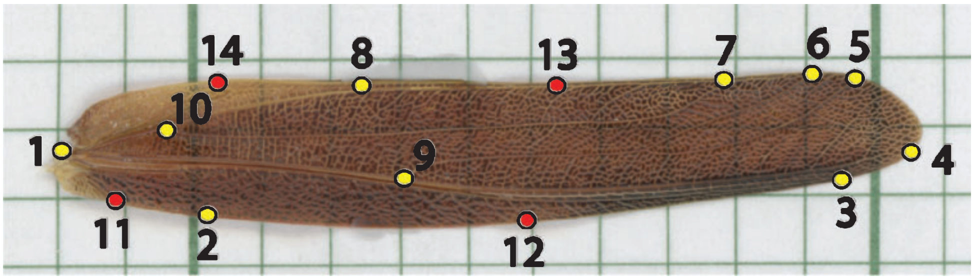

Fig. 5. Landmarks for the geometric morphometric analysis. Numbers 1–10 are placed on fixed landmarks based on the origin or terminus of homologous wing veins (depicted as yellow dots). Positions were as follows: (1) origin of subcosta, (2) anterior terminus of costa, (3) anterior terminus of the subcosta, (4) anterior terminus of the radius, (5) anterior terminus of the radial sector, (6) posterior terminus of radial sector, (7) anterior terminus of the medial anterior, (8) terminus of medial posterior (MP), where MP becomes parallel to the posterior edge, or MP merges with the posterior edge. (9) The location of the split between the radius and radial sector. (10) The point where the medial vein splits to form the medial anterior and medial posterior veins. Numbers 11–14 were positioned as sliding landmarks (represented by red dots) between the fixed landmarks.

a leave-one-out cross-validation procedure to test the predictive power of the LDA model. All statistical analyses were performed in program R (R Core Team 2013).

Taxon sampling

Taxon sampling for the phylogenetic analysis was designed to best represent the taxonomic and geographic diversity in Tettigoniidae. We sampled the larger katydid subfamilies relative to the number of species described within (e.g. Phaneropterinae contains nearly 35% of all described taxa so roughly 35% of the species in our analysis were from this subfamily). Additional sampling was conducted for widely dispersed taxonomic groups (e.g. Conocephalini) and taxa with noncontiguous distributions or large gaps between species ranges (e.g. Listroscelidinae). Further taxa were sampled from subfamilies found to be paraphyletic in earlier studies (Mugleston *et al.* 2013). Total ingroup sampling consisted of 185 katydid species from 17 of the 20 extant subfamilies (90%), 59 of 89 tribes (66%) and 156 of 1247 genera (13%) (Table 1). We were unable to sample two small subfamilies: Acridoxeneninae (1 sp.) and Microtettigoniinae (7 spp.) due to the lack of available vouchers for DNA work. Song *et al.* (2015) found three superfamilies – Stenopelmatoidea, Hagloidea and Rhaphidophoroidea – to be closely related to the Tettigoniidae. Outgroup taxa were selected from four families within these three superfamilies. Specimen vouchers are deposited in the Insect Genomics Collection, M.L. Bean Museum, Brigham Young University.

DNA extraction and sequencing

Muscle tissue (~25 mg) was extracted from the mesothoracic (larger specimens) or metathoracic (smaller specimens) femora of voucher katydids. DNA extractions were conducted using a Qiagen DNeasy Blood and Tissue kit (Qiagen, Valencia, CA, USA) following the protocol supplied by the manufacturer. Five loci (two rRNA, one mitochondrial and two nuclear protein-coding) commonly used in insect phylogenetic studies were used for this analysis (Colgan *et al.* 1998; Whiting 2002; Svenson and Whiting 2004; Svenson and Whiting 2009; Mugleston *et al.* 2013): 28S ribosomal subunit (28S rRNA, 2.2 kb), 18S ribosomal subunit (18S, 1.9 kb), cytochrome *c* oxidase subunit II (COII, 650 bp), histone 3 (H3, 375 bp) and wingless (WG, 450 bp). Genes were sequenced and amplified using oligonucleotide primers from Integrated DNA Technologies (San Diego, CA). Polymerase chain reaction (PCR) protocols were previously developed for H3 (Colgan *et al.* 1998), 28S and 18S (Whiting 2002), WG (Wild and Maddison 2008) and COII (Svenson and Whiting 2004, 2009) (Table 2). Polymerase chain reaction was performed using 25-µL reactions with Platinum taq DNA polymerase (Invitrogen, Carlsbad, CA). For the 28S and 18S ribosomal genes, 1.25 µL of water was replaced with DMSO. All reactions were run on a GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA). Polymerase chain reaction 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) following the manufacturer's instructions. Products were sequenced with BigDye chain terminating chemistry and fractioned on an AB13730xl (Applied Biosystems) at the Brigham Young University DNA Sequencing Center (Provo, UT).

Table 1. Taxonomic distribution of exemplars used in this analysis

Subfamily	Tribes sampled	Genera sampled	Total exemplars
Austrosaginae	NA	2 of 5	2
Bradyporinae	1 of 3	1 of 26	1
Conocephalinae	4 of 6	20 of 193	34
Hetrodinae	4 of 5	4 of 14	4
Hexacentrinae	NA	4 of 12	4
Lipotactinae	NA	2 of 2	2
Listroscelidinae	2 of 5	4 of 21	4
Meconematinae	3 of 3	7 of 123	9
Mecopodinae	3 of 6	6 of 54	7
Phaneropterinae	18 of 28		61
Genus groups	7 of 20	56 of 351	
Phasmodinae	NA	1 of 1	1
Phyllophorinae	NA	2 of 12	2
Pseudophyllinae	16 of 19	22 of 240	25
Pterochrozinae	NA	2 of 14	2
Saginae	NA	2 of 4	2
Tettigoniinae	8 of 12	19 of 159	23
Tympanophorinae	NA	1 of 2	1
Zaprochilinae	NA	1 of 4	1
Total	59 of 89	156 of 1247	185

Alignment

Contigs were concatenated, proofed and edited using Geneious v6.1.5 (Kearse *et al.* 2012). Primer regions were trimmed from the ends of the concatenated sequences. Edited sequences were submitted to GenBank (Table 3). Protein coding sequences were translated to amino acid sequences using Mega V5 (Kumar *et al.* 2008). Once the proper reading frame was established, the sequences were aligned using the MUSLCE plug-in under default parameters found in MEGA. Aligned amino acid sequences were then back-translated into nucleotide sequences and these were exported for further analyses. In some insect groups, ribosomal genes can be troublesome with numerous conserved and non-conserved regions of varied lengths that are difficult to align. However, in Tettigoniidae this has not been a problem (Mugleston *et al.* 2013). The 28S rDNA and 18S rDNA were aligned using MAFFT V6 (Katoh *et al.* 2005) under the E-INS-I algorithm under the default settings. E-INS-I was developed (Katoh *et al.* 2005) to handle data with intermixed conserved and non-conserved regions.

Phylogenetic analyses

Phylogenetic analyses were conducted using BEAST v1.8 (Drummond *et al.* 2012). Data were partitioned by gene for the BEAST analysis. When data were partitioned via PartitionFinder v1.1.1 (Lanfear *et al.* 2012), BEAST did not reach stationarity. To determine whether our results were sensitive to these partitions, we also ran this tree in RAxML using partitions determined by PartitionFinder, which resulted in a topology largely congruent with the BEAST tree (see supplementary information). BEAUti v1.8 (Drummond

Table 2. Primers and polymerase chain reaction protocols used in this study
Primer sources referenced in the text

Primers	Sequence 5→3	Annealing	Elongation
18SrDNA			
18S 1F	TACCTGGTTGATCCTGCCAGTAG	52°C	105 s
18S bi	GAGTCTCGTTCGTTATCGGA		
18S b5.0*	TAACCGCAACAACTTTAAT		
18S a0.7	ATTAAAGTTGTTGCGGTT	46°C	105 s
18S 9R	GATCCTTCCGCAGGTTCACCTAC		
18S a2.0*	ATGGTTGCAAAGCTGAAAC		
28SrDNA			
28S Tetrd1a	CGAGCGAACAGGGAAGAGCC	54°C	120 s
28S rD5B	CCACAGCGCCAGTTCTGATTA		
28S 3b*	CCYTGAACGGTTTCACGTACT		
28S 3a*	AGTACGTGAAACCGTTCAGG		
28S B*	TCGGAAGGAACCAGCTAC		
28S A	GACCCGTCTTGAAGCACG	54°C	120 s
28S Tet7b1	CTCTCCCGGATTTTCAAGGTC		
28S Tet4.7*	CCGGTCAAGCGAATGATTAGA		
COII			
COII Flue	TCTAATATGGCAGATTAGTGC	52°C	75 s
COII R-lys	GAGACCAGTACTTGCTTTCAGTCATC		
COII 2a1	ATAGAKCWTCYCCHTTAATAGAACA	52°C	75 s
CPOO 9b1	GTACTTGCTTTCAGTCATCTWATG		
Histone 3			
H3 AF	ATGGCTCGTACCAAGCAGACV	50°C	45 s
H3 AR	ATATCCTTRGGCATRATRGTG		
Wingless			
WG 550F	ATGCGTCAGGARTGYAARTGY	50°C	45 s
WG ABRZ	CACTTNACYTCRCARCACCAR		
WG 578F2	TGCACNGTGAARACYTCGTGG	50°C	45 s
WG ABR2	ACYTCGCAGCACCARTGGAA		

*Internal primers used for sequencing only.
[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.

et al. 2012) was used to build the .xml files for the BEAST analysis. Parameters of the run included a lognormal relaxed clock with the tree prior set to Yule Process. A starting tree was provided from a RAxML (Stamatakis 2006) analysis partitioning the data by gene. Four separate BEAST runs at 20×10^7 sampling every 10 000 generations were conduced using the BYU super computing resources (https://marylou.byu.edu/). Log files were inspected in Tracer (Rambaut and Drummond 2003) to determine whether the length of the runs was sufficient to reach stationarity, determine the log-likelihood score for the runs, and determine whether the estimated sample sizes (ESS) were sufficient for the analysis. Tree files from the four independent runs were combined using LogCombiner v1.8 and 10% of each tree file was removed as burn-in with a resampling frequency of every 70 000 generations to obtain a single tree file with ~10 000 data points as recommended by the developers. TreeAnnotator was used to find the best tree within the sampled trees. Forewings (tegmina) were coded as a single binary character (either leaf-like or not leaf-like) based on the ratio method described above. Characters were mapped onto the Bayesian topology and ancestral states were reconstructed with the Markov *k-stat* 1 parameter model using Mesquite v2.75 (Maddison and Maddison 2015).

A binary trait speciation and extinction (BiSSE) (Maddison *et al*. 2007) model of discrete character evolution was used to estimate birth (λ) and transition (q) rates for leaf-like and non-leaf-like forms along the Tettigoniidae tree and Phaneropterinae subtree as implemented in the Diversitree V0.9–2 R package (FitzJohn 2012). Likelihood ratio tests (LRT) were performed to test whether the full model was preferred over the null model with constrained birth and transition rates. For Bayesian analyses, posterior parameter (rates) draws were performed with the proper exponential prior using slice sampling Markov chain Monte Carlo (MCMC) of 105 steps, discarding the first 1000 draws as burn-in. We initiated three independent MCMC runs for leaf-like and non-leaf-like forms to verify that chains were not trapped in local optima. Relative speciation rates between the leaf-like and non-leaf-like katydids were estimated as λ_leaf-like/λ_non-leaf-like, where values greater than 1 are expected if leaf-like wings are a driver of speciation relative to non-leaf-like wings. Likewise, transition rates were estimated as q_leaf-like/q_non-leaf-like, where values greater than 1 indicate where selection favours the leaf-like form over the non-leaf-like form.

Results

The MANOVA results (Table 4) found overall wing shape significantly differed between the designated leaf-like and non-leaf-like wings (P-value < 0.001). Leaf-like and non-leaf-like

Table 3. Taxon sampling with subfamily, voucher number, locality and GenBank accession number by gene

Subfamilies are abbreviated as follows: Aust, Austrosaginae; Brad, Bradyporinae; Cono, Conocephalinae; Het, Hetrodinae; Hex, Hexacentrinae; Lip, Lipotactinae; List, Listroscelidinae; Mecon, Meconematinae; Mecop, Mecopodinae; Phan, Phaneropterinae; Phas, Phasmodinae; Phyll, Phyllophorinae; Pseu, Pseudophyllinae; Pter, Pterochrozinae; Sagi, Saginae; Tett, Tettigoniinae; Tymp, Tympanophorinae; Zap, Zaprochilinae; dashes (–) indicate missing sequence data

Taxon	Sub	Voucher	Locality	18S	28S	COII	H3	WG
Hemisaga sp.	Aust	OR483	Australia	KF570758	KF570896	KF570999	KF571123	KF571257
Pachysaga sp.	Aust	OR484	Australia	KF570757	KF570897	KF571000	KF571124	KF571258
Deracantha sp.	Brad	OR667	Mongolia	–	–	–	KX429887	KX429937
Acantheremus colwelli	Cono	OR622	Costa Rica	KF570821	KF570917	KF571043	KF571178	KF571312
Belocephalus subapterus	Cono	OR596	SC, USA	KF570797	KF570927	KF571027	KF571153	KF571287
Conocephalus sp.	Cono	OR609	South Africa	KF570786	KF570915	KX429847	KF571165	KF571299
Conocephalus sp.	Cono	OR147	South Africa	KX429753	KX429797	KX429848	KX429888	KX429938
Conocephalus sp.	Cono	OR149	Australia	KX429754	KX429798	KX429849	KX429889	KX429939
Conocephalus sp.	Cono	OR548	Namibia	KF570788	KF570936	KF571004	KF571128	KF571262
Conocephalus sp.	Cono	OR556	South Africa	KF570789	KF570937	KF571010	KF571136	KF571270
Conocephalus sp.	Cono	OR599	VA, USA	KF570783	KF570951	KF571029	KF571156	KF571290
Conocephalus sp.	Cono	OR639	India	KF570787	KF570934	KF571058	KF571194	–
Conocephalus sp.	Cono	OR654	PNG	KF570785	KF570933	KF571071	KF571207	KF571343
Copiphora hastata	Cono	OR141	Costa Rica	KX429755	KX429799	KX429850	KX429890	KX429940
Copiphora rhinoceros	Cono	OR142	Peru	KF570790	KF570918	KF570977	KF571099	KF571234
Eschatoceras bipunctatus	Cono	OR550	Peru	KF570798	KF570921	KF571006	KF571130	KF571264
Euconocephalus sp.	Cono	OR642	Malaysia	KF570794	KF570926	KF571061	KF571196	KF571331
Euconocephalus sp.	Cono	OR687	PNG	KX431992	KX431993	–	KX431994	KX431995
Lirometopum coronatum	Cono	OR586	Costa Rica	KF570800	KF570919	KF571017	KF571143	KF571277
Macroxiphus sumatranus	Cono	OR381	Malaysia	KF570803	KF570930	KF570988	KF571110	KF571245
Neoconocephalus triops	Cono	OR595	FL, USA	KF570796	KF570950	KF571026	KF571152	KF571286
Nicsara bifasciatum	Cono	OR613	Australia	KF570806	KF570916	KF571035	KF571169	KF571303
Nicsara sp.	Cono	OR558	Australia	KF570802	KF570929	KF571012	KF571138	KF571272
Odontolakis virescens	Cono	OR379	Madagascar	KF570792	KF570932	KF570986	KF571108	KF571243
Orchelimum sp.	Cono	OR030	LA, USA	KF570784	KF570938	KF570959	KF571079	KF571214
Oxylakis sp.	Cono	OR641	Malaysia	KF570799	KF570931	KF571060	KF571443	KX429941
Pseudorhynchus cornutum	Cono	OR655	PNG	KF570795	KF570922	KF571072	KF571208	KX429942
Pseudorhynchus sp.	Cono	OR557	Zambia	KF570805	KF570925	KF571011	KF571137	KF571271
Pyrgocorypha sp.	Cono	OR640	India	KF570801	KF570935	KF571059	KF571195	KF571329
Ruspolia consobrina	Cono	OR198	South Africa	KX429756	KX429800	KX429851	KX429891	KX429943
Ruspolia lineosa	Cono	OR380	South Africa	KF570793	KF570923	KF570987	KF571109	KF571244
Ruspolia sp.	Cono	OR555	South Africa	KF570804	KF570924	KX429852	KF571135	KF571269
Salomona sp.	Cono	OR145	PNG	KF570791	KF570928	KF570978	KF571100	KF571235
Salomona sp.	Cono	OR686	PNG	KX429757	KX429801	KX429853	KX429892	KX429944
Sphyrometopa femorata	Cono	OR610	Costa Rica	KF570807	KF570920	KX429854	KF571166	KF571300
Tabanacris albolineata	Cono	OR680	Malaysia	KX429758	KX429802	KX429855	KX429893	–
Vestria sp.	Cono	OR660	Peru	KX429759	KX429803	–	KX429894	KX429945
Acanthoplus sp.	Het	OR176	Namibia	KF570692	KF570873	KF570979	KF571101	KF571236
Acanthoproctus vittatus	Het	OR091	Zambia	KF570689	KF570870	KF570972	KF571094	KF571229
Enyaliopsis sp.	Het	OR177	Zambia	KF570690	KF570871	KF570980	KF571102	KF571237
Hetrodes sp.	Het	OR554	South Africa	KF570691	KF570872	KF571009	KF571134	KF571268
Aerotegmina kilimandjarica	Hex	OR549	Tanzania	KF570687	KF570904	KF571005	KF571129	KF571263
Glenophisis borneo.	Hex	OR638	Malaysia	KF570686	KF570903	KF571057	KF571193	KX429946
Hexacentrus japonicus	Hex	OR382	South Korea	KF570685	–	KF570989	KF571111	KF571246
Teuthroides mimeticus	Hex	OR656	PNG	KF570688	KF570902	KF571073	KF571209	KF571345
Lipotactes maculatus	Lip	OR634	Malaysia	KF570698	KF570876	KF571053	KF571189	KF571323
Mortoniellus ovatus	Lip	OR633	Borneo	KF570697	KF570875	KF571052	KF571188	KF571322
Arachnoscelis rehni	List	OR582	Costa Rica	KF570695	KF570900	KF571013	KF571139	KF571273
Chlorobalius leucoviridis	List	OR679	Australia	KX429760	KX429804	KX429856	KX429895	KX429947
Meiophisis micropennis	List	OR657	PNG	KF570812	KF570889	KF571074	KF571458	KX429948
Neobarrettia sp.	List	OR684	USA	–	–	–	KX429896	KX429949
Requena sp.	List	OR553	Western Australia	KF570696	KF570901	KF571008	KF571133	KF571267
Alloteratura sp.	Mecon	OR636	Malaysia	KF570703	KF570878	KF571055	KF571191	KF571325
Kuzicus megaterminatus	Mecon	OR635	India	KF570701	KF570877	KF571054	KF571190	KF571324
Meconema thalassinum	Mecon	OR685	USA	KX429761	KX429805	–	KX429897	KX429950

(*continued next page*)

Table 3. (*continued*)

Taxon	Sub	Voucher	Locality	18S	28S	COII	H3	WG
Phlugiola arborea	Mecon	OR666	Peru	KX429762	KX429806	–	KX429898	KX429951
Phlugis irregularis	Mecon	OR624	Bolivia	KF570755	KF570899	KF571045	KF571179	KF571314
Phlugis sp.	Mecon	OR583	Costa Rica	KF570754	KF570898	KF571014	KF571140	KF571274
Poecilomerus sp.	Mecon	OR608	Madagascar	KF570811	KF570907	KX429857	KF571164	KF571298
Xiphidiopsis sp.	Mecon	OR637	Malaysia	KF570702	KF570879	KF571056	KF571192	KF571326
Anoedopoda erosa	Mecop	OR600	Cameroon	KF570774	KF570952	KF571030	KF571157	KF571291
Eumecopoda cyrtoscelis	Mecop	OR385	PNG	KF570771	KF570912	–	KF571113	KF571248
Phrictaeformia insulana	Mecop	OR653	PNG	KF570773	KF570908	KF571070	KF571206	KF571342
Phrictaetypus viridis	Mecop	OR393	PNG	KF570772	KF570909	KF570997	KF571121	KF571256
Segestidea gracilis	Mecop	OR137	PNG	KX429763	KX429807	KX429858	KX429899	KX429952
Segestidea novaeguineae	Mecop	OR136	PNG	KX429764	KX429808	–	KX429900	KX429953
Zitsikama tessellata	Mecop	OR384	South Africa	KF570756	KF570881	KF570990	KF571112	KF571247
Acrometopa macropoda	Phan	OR043	Slovenia	KF570717	KF570853	KF570962	KF571082	KF571217
Acropsis tectiformis	Phan	OR626	Peru	KF570741	KF570834	KF571046	KF571181	KF571315
Aegimia sp.	Phan	OR619	Costa Rica	KF570749	KF570955	KF571040	KF571175	KF571309
Aganacris sp.	Phan	OR084	Bolivia	KF570720	KF570839	KF570971	KF571093	KF571228
Amblycorypha sp.	Phan	OR597	USA	KF570727	KF570842	KX429859	KF571154	KF571288
Anaulacomera sp.	Phan	OR627	Peru	KF570723	KF570841	KF571047	KF571182	KF571316
Anaulacomera sp.	Phan	OR146	Boliva	KX429765	KX429809	KX429860	KX429901	KX429954
Austrodontura capensis	Phan	OR671	South Africa	KX429766	KX429810	KX429861	KX429902	KX429955
Barbitistes constrictus	Phan	OR077	Germany	–	KX429811	KX429862	KX429903	KX429956
Barbitistes ocskayi	Phan	OR068	Slovenia	–	–	–	KX429904	KX429957
Barbitistes sp.	Phan	OR069	Germany	KF570742	KF570859	KF570964	KF571084	KF571219
Ceraia mytra	Phan	OR621	Panama	KF570728	KF570838	KF571042	KF571177	KF571311
Chloroscirtus forceps	Phan	OR617	Costa Rica	KF570729	KF570843	KF571038	KF571173	KF571307
Cnemidophyllum eximium	Phan	OR135	Peru	KX429767	KX429812	KX429863	KX429905	KX429958
Deflorita integra	Phan	OR647	Malaysia	KF570737	KF570847	KF571200	KF571449	KX429959
Dolichocercus sp.	Phan	OR625	Peru	KF570740	KF570855	KX429864	KF571180	KX429960
Ducetia japonica	Phan	OR644	India	KF570746	KF570862	KF571063	KX429906	KF571333
Dysmopha obesa	Phan	OR589	Malaysia	KF570739	KF570868	KF571020	KF571146	KF571280
Dysonia pirani	Phan	OR386	Peru	KF570722	KF570849	KF570991	KF571114	KF571249
Elimaea sp.	Phan	OR681	India	KX429768	KX429813	KX429865	KX429907	KX429961
Enochletica affinis	Phan	OR606	Cameroon	KF570716	KF570857	KF571034	KX429908	KF571296
Euceraia rufovariegata	Phan	OR663	Peru	–	KX429814	–	KX429909	–
Eulioptera reticulata	Phan	OR148	Zambia	KX429769	KX429815	KX429866	KX429910	KX429962
Eurycorypha sp.	Phan	OR672	South Africa	KX429770	KX429816	KX429867	KX429911	KX429963
Hemielimaea sp.	Phan	OR682	Malaysia	KX429771	KX429817	KX429868	KX429912	KX429964
Hemimirollia sp.	Phan	OR646	India	KF570735	KF570845	KF571065	KF571199	KF571335
Hemimirollia sp.	Phan	OR649	Malaysia	KF570736	KF570846	KF571066	KF571202	KF571338
Holochlora sp.	Phan	OR611	India	KF570724	KF570860	–	KF571167	KF571301
Horatosphaga sp.	Phan	OR604	Namibia	KF570733	KF570954	KF571033	KF571161	KF571294
Hueikaeana sp.	Phan	OR645	Malaysia	KF570734	KF570861	KF571064	KF571198	KF571334
Hyperphrona irregularis	Phan	OR669	Peru	KX429772	KX429818	–	KX429913	KX429965
Insara elegans	Phan	OR675	AZ, USA	KX429773	KX429819	–	–	KX429966
Insara sp.	Phan	OR616	Costa Rica	KF570744	KF570856	KF571037	KF571172	KF571306
Leptophyes punctatissima	Phan	OR044	Germany	KF570751	KF570851	KF570963	KF571083	KF571218
Letana megastridula	Phan	OR650	India	KF570748	KF570848	KF571067	KF571203	KF571339
Microcentrum rhombifolium	Phan	OR033	UT, USA	KX429774	KF570836	KF570960	KF571080	KF571215
Microcentrum sp.	Phan	OR620	Costa Rica	KF570721	KF570835	KF571041	KF571176	KF571310
Montezumina modesta	Phan	OR683	USA	KX429775	KX429820	KX429869	KX429914	–
Monticolaria sp.	Phan	OR552	Africa	KF570747	KF570854	–	KF571132	KF571266
Noia testacea	Phan	OR677	India	KX429776	KX429821	KX429870	KX429915	KX429967
Parapyrrhicia dentipes	Phan	OR670	Madagascar	KX429777	KX429822	KX429871	KX429916	KX429968
Parascudderia setrina	Phan	OR662	Peru	KX429778	KX429823	–	KX429917	KX429969
Phaneroptera falcata	Phan	OR076	Germany	KF570718	KF570864	–	KF571088	KF571223
Phylloptera sp.	Phan	OR388	Panama	KF570858	KX429824	KX429872	KF571116	KF571251
Poecilimon ornatus	Phan	OR074	Slovenia	KF570752	KF570852	KF570966	KF571086	KF571221
Polichne argentata	Phan	OR632	Australia	KF570719	KF570865	KF571051	KF571187	KF571321
Polysarcus denticauda	Phan	OR038	Slovenia	KX429779	KX429825	–	KX429918	KX429970

(*continued next page*)

Table 3. (*continued*)

Taxon	Sub	Voucher	Locality	18S	28S	COII	H3	WG
Quiva pulchella	Phan	OR661	Peru	KX429780	KX429826	–	KX429919	KX429971
Rectimarginalis ensis	Phan	OR648	Malaysia	KF570725	KF570850	KF571201	KF571450	KX429972
Scaphura elegans	Phan	OR668	Argentina	KX429781	KX429827	–	KX429920	KX429973
Scudderia sp.	Phan	OR551	LA, USA	KF570753	KF570837	KF571007	KF571131	KF571265
Steirodon sp.	Phan	OR618	Costa Rica	KF570732	KF570840	KF571039	KF571174	KF571308
Stenophyllia modesta	Phan	OR060	Chile	–	–	–	KX429921	KX429974
Stilpnochlora sp.	Phan	OR592	FL, USA	KF570731	KX429828	KF571023	KF571149	KF571283
Sympaestria sp.	Phan	OR678	Malaysia	KX429782	KX429829		KX429922	KX429975
Torbia viridissima	Phan	OR631	Australia	KF570750	KF570866	KF571050	KF571186	KF571320
Trigonocorypha sp.	Phan	OR378	Madagascar	KF570745	KF570844	KF570985	KF571107	KF571242
Trigonocorypha sp.	Phan	OR651	India	KF570726	KF570867	KF571068	KF571204	KF571340
Tylopsis sp.	Phan	OR607	South Africa	KF570738	KF570869	KX429873	KF571163	KF571297
Vossia obesa	Phan	OR387	Cameroon	KF570743	KF570863	KF570992	KF571115	KF571250
Weissenbornia sp.	Phan	OR605	Cameroon	KF570730	KX429830	KX429874	KF571162	KF571295
Phasmodes sp.	Phas	OR485	Australia	KF570817	KF570944	KF571001	KF571125	KF571259
Phyllophora sp.	Phyll	OR132	PNG	KF570816	KF570911	KF570974	KF571096	KF571231
Sasima sp.	Phyll	OR131	PNG	KF570770	KF570910	KF570973	KF571095	KF571230
Acanthodiphrus sp.	Pseud	OR584	Costa Rica	KF570709	KF570830	KF571015	KF571141	KF571275
Acanthoprion suspectum	Pseud	OR676	India	KX429783	KX429831	–	KX429923	KX429976
Acauloplacella sp.	Pseud	OR652	PNG	KF570808	KF570883	KF571069	KF571205	KF571341
Adenes obesus	Pseud	OR392	Ghana	KF570707	KF570823	–	KF571120	KF571255
Adenes obesus	Pseud	OR601	Cameroon	KF570704	KF570953	–	KF571158	KF571292
Balboana tibialis	Pseud	OR389	Costa Rica	KF570712	KF570827	KF570994	KF571117	KF571252
Calamoptera grandis	Pseud	OR585	Costa Rica	KF570710	KF570832	KF571016	KF571142	KF571276
Callimenellus apterus	Pseud	OR140	India	KX429784	KX429832	–	KX429924	KX429977
Championica sp.	Pseud	OR615	Peru	KF570705	KF570831	KF571036	KF571171	KF571305
Cymatomera denticollis	Pseud	OR139	Africa	KF570779	KF570885	KF570976	KF571098	KF571233
Diyllus sp.	Pseud	OR623	Costa Rica	KF570711	KF570828	KF571044	KX429926	KF571313
Goethalsiella tridens	Pseud	OR588	Costa Rica	KF570775	KF570913	KF571019	KF571145	KF571279
Ischnomela pulchripennis	Pseud	OR614	Costa Rica	KF570776	KF570914	KX429876	KF571170	KF571304
Leptotettix sp.	Pseud	OR665	Peru	KX429785	KX429833	–	–	–
Parasimodera saussurei	Pseud	OR673	Madagascar	KX429786	KX429834	KX429875	KX429925	KX429978
Panoploscelis sp.	Pseud	OR377	Peru	KF570713	KF570826	KF570984	KF571106	KF571241
Parapleminia sp.	Pseud	OR629	Brazil	KF570708	KF570824	KX429877	KF571184	KF571318
Phyllozelus infumatus	Pseud	OR643	India	KF570781	KF570888	KF571062	KF571197	KF571332
Pterophylla camellifolia	Pseud	OR658	KY, USA	KF570715	KF570833	KF571210	KF571459	KX429979
Sathrophyllia fuliginosa	Pseud	OR587	India	KF570780	KF570887	KF571018	KF571144	KF571278
Schedocentrus sp.	Pseud	OR630	Peru	KF570714	KF570906	KF571049	KF571185	KF571319
Stenampyx annulicornis	Pseud	OR602	Cameroon	KF570782	KF570886	KF571031	KF571159	KF571293
Teleutias sp.	Pseud	OR391	Peru	KF570815	KF570829	KF570996	KF571119	KF571254
Xiphophyllum sp.	Pseud	OR628	Bolivia	KF570706	KF570825	KF571048	KF571183	KF571317
Zabalius opthalmicus.	Pseud	OR138	Africa	KF570778	KF570884	KF570975	KF571097	KF571232
Mimetica tuberata	Pter	OR612	Costa Rica	KF570694	KF570945	–	KF571168	KF571302
Typophyllum sp.	Pter	OR196	Peru	KF570693	KF570946	KF570981	KF571103	KF571238
Clonia sp.	Sagi	OR201	South Africa	KF570699	KF570880	KF570983	KF571105	KF571240
Peringueyella sp.	Sagi	OR199	South Africa	KF570810	KF570905	KF570982	KF571104	KF571239
Afredestes sp.	Tett	OR603	South Africa	KF570809	KF570874	KF571032	KF571160	–
Anabrus sp.	Tett	OR034	NV, USA	KF570763	KF570890	KF570961	KF571081	KF571216
Ateloplus coconino	Tett	OR674	AZ, USA	KX429787	KX429835	KX429878	KX429927	KX429980
Atlanticus sp.	Tett	OR598	FL, USA	KF570761	KX429836	KF571028	KF571155	KF571289
Bicolorana kuntzeni	Tett	OR080	Slovenia	KX429788	KX429837	KX429879	KX429928	KX429981
Capnobotes sp.	Tett	OR591	Utah, USA	KF570759	KX429838	KF571022	KF571148	KF571282
Decticus verrucivorus	Tett	OR039	Slovenia	KX429789	KX429839	–	KX429929	–
Eobiana japonica	Tett	OR590	Japan	KF570760	KF570895	KF571021	KF571147	KF571281
Eupholidoptera chabrieri	Tett	OR042	Slovenia	KX429790	KX429840	KX429880	KX429930	KX429982
Metrioptera brachyptera	Tett	OR070	Germany	KX429796	–	KX429881	KX429931	KX429983
Pachytrachis gracilis	Tett	OR081	Slovenia	KF570769	KF570892	KF570968	KF571090	KF571225
Pachytrachis stridulatus	Tett	OR045	Slovenia	KX429791	KX429841	KX429882	KX429932	KX429984
Pediodectes sp.	Tett	OR659	Texas	KF570766	KF570939	KF571075	KF571211	KF571348

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Table 3. (*continued*)

Taxon	Sub	Voucher	Locality	18S	28S	COII	H3	WG
Pholidoptera griseoaptera	Tett	OR079	Germany	KF570767	KF570893	KF570967	KF571089	KF571224
Pholidoptera littoralis	Tett	OR041	Slovenia	KX429792	KX429842	KX429883	KX429933	KX429985
Plagiostira albonotata	Tett	OR594	UT, USA	KF570768	KF570894	KF571025	KF571151	KF571285
Platycleis affinis	Tett	OR071	Slovenia	KF570764	KF570891	KF570965	KF571085	KF571220
Platydecticus sp.	Tett	OR664	Chile	KX429793	KX429843	–	–	–
Roeseliana roeselii	Tett	OR046	Germany	–	–	KX429884	KX429934	KX429986
Sepiana sepium	Tett	OR072	Slovenia	KX429794	KX429844	KX429885	KX429935	KX429987
Steiroxys trilineata	Tett	OR593	WA, USA	KF570762	KX429845	KF571024	KF571150	KF571284
Tettigonia cantans	Tett	OR075	Germany	KF570765	–	–	KF571087	KF571222
Tettigonia veridissima	Tett	OR040	Germany	KX429795	KX429846	KX429886	KX429936	KX429988
Tympanophora sp.	Tymp	OR486	Australia	KF570777	KF570947	KF571002	KF571126	KF571260
Kawanaphila sp.	Zap	OR487	Australia	KF570700	KF570882	KF571003	KF571127	KF571261
OUTGROUPS								
Camptonotus carolinensis	Gryllacrididae	OR024	NC, USA	KF570818	KF570941	KF570958	KF571078	KF571213
Erythrogryllacris sp.	Gryllacrididae	OR390	Malaysia	KF570819	KF570942	KF570995	KF571118	KF571253
Cyphoderris monstrosa	Prophalangopsidae	OR021	Canada	KF570814	KF570943	KF570957	KF571077	KF571212
Troglophilus neglectus	Raphidophoridae	OR083	Slovenia	KF570820	KF570948	KF570970	KF571092	KF571227
Stenopelmatus fuscus	Stenopelmatidae	OR014	UT, USA	KF570813	KF570940	KF570956	KF571076	–

Table 4. Results of MANOVA analysis examining shape variation between leaf-like and non-leaf-like wings

A significant difference was found between the two wing types (P-value <0.001) and in wing size (P-value <0.001), but an interaction between wing shape and size was not found (P-value = 0.0156)

Effects	d.f.	F-value	P
Wing group	1	8.316	**<0.001**
Size	1	13.291	**<0.001**
Wing group: size	1	1.448	0.156

wings differed significantly in size (measured by the centroid; P-value < 0.001). No interaction between wing shape and size was found (P-value = 0.156) indicating that wing shape by group is not affected by size. Given these results, we conducted an LDA to determine with what accuracy we could delineate wing groups based on the input shape data (i.e. can these data reliably separate leaf-like and non-leaf-like into their respective groups). In the original model, the LDA correctly assigned 43 of 51 (84%) species to the leaf-like group and 63 of 68 (92%) species to the non-leaf-like group. The cross-validation procedure indicated that the model was robust to random data removal, correctly assigning 40 of 51 (78%) and 60 of 68 (88%) to the leaf-like and non-leaf-like group, respectively. Thus, modern GM techniques validate our thorax to wing ratio method, making this simple ratio an operational method for identifying the presence of leaf-like wings in katydids.

The topology produced was largely congruent though more detailed than the topology presented in Mugleston *et al*. (2013). The majority of katydid diversity is contained in two large subclades (Clades A and B; Fig. 6), and the relationships among the subfamilies within these clades are congruent with Mugleston *et al*. (2013). The present study provides a more detailed view of the position of Saginae and Pterochrozinae. This topology places Saginae as a well-supported sister group to the Australian clade consisting of Zaprochilinae, Tympanophorinae and Phasmodinae. The position of Pterochrozinae as a basal lineage was not well supported in earlier studies. This analysis places Pterochrozinae as sister to the remaining subfamilies in Clade A, though this position is still not well supported in this analysis. Optimising the leaf-like wings on the topology reveals 15 independent derivations of leaf-like wings in seven subfamilies of Tettigoniidae (Fig. 6*A*, *B*; with transitions to leaf-like wings labelled 1–15). The basal condition in Tettigoniidae is non-leaf-like wings. One transition (Fig. 6*A*; Tran. #1) is apparent in the basal katydid clade (genus *Tympanophora*). The bulk of katydid diversity is found within two major clades. Clade A comprises the subfamilies Pterochrozinae, Tettigoniinae, Meconematinae, Conocephalinae, Hexacentrinae, Hetrodinae, Listroscelidinae, Lipotactinae and Austrosaginae. Members of this clade are distributed worldwide with many species present in the temperate and subtropical regions (Fig. 6*A*). Our phylogenetic analysis indicates that many of these subfamilies are not monophyletic, but this is not surprising given prior analyses (Mugleston *et al*. 2013). While revisions are necessary in Tettigoniidae, details regarding the taxonomy of this group are beyond the scope of the present work. The majority of species within Clade A lack leaf-like wings, but our analysis revealed three transitions to leaf-like wings from a non-leaf-like ancestor within this clade. The first transition in Clade A (Fig. 6*A*, Tran. #2; Fig. 3*A*) is in the subfamily Pterochrozinae. These include katydids with very broad, leaf-like wings and are the quintessential leaf-like katydid. The second transition (Fig. 6*A*, Tran. #3; Fig. 3*B*) occurs within one lineage of Hexacentrinae. Hexacentrines are distributed throughout both the New and Old World tropics. The leaf-like tegmina are only found in males in some Indomalayan and Australasian taxa. The third transition (Fig. 6*A*, Tran. #4; Fig. 3*C–E*) occurs with the cosmopolitan subfamily Conocephalinae, the conehead katydids. Conocephalinae is the second largest subfamily of katydids, with over 1200 described species. We sampled this

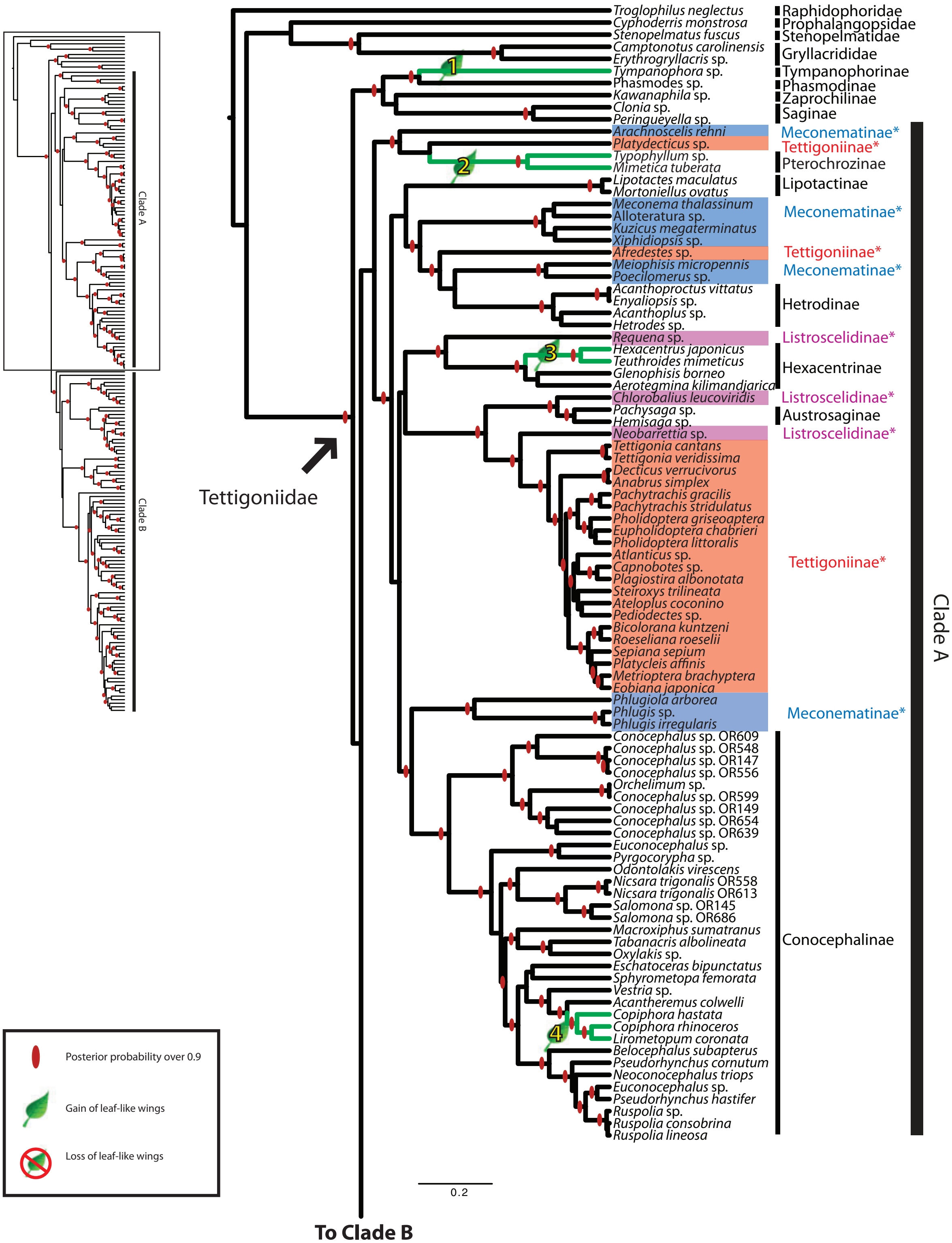

Fig. 6. BEAST tree (log-likelihood score of –99423.482) consisting of 185 ingroup taxa. Posterior probabilities over 90 are marked with a red bar at the node. Coloured boxes around terminals indicate paraphyletic subfamilies. Transitions to leaf-like wings are marked with a leaf icon, a number referenced in the text (Tran. 1–15) and subsequent branches are coloured in green. Lineages that have subsequently lost leaf-like wings are marked with a red no sign (⃠), numbered (R1–R4) and branches are coloured blue. Subfamilies are marked to the right of the tree. Monophyletic subfamilies are printed in black. Paraphyletic subfamily names are to the right of their respective taxa and coloured to match the boxes used to denote paraphyletic groups. Major clades A and B are marked for ease of discussion.

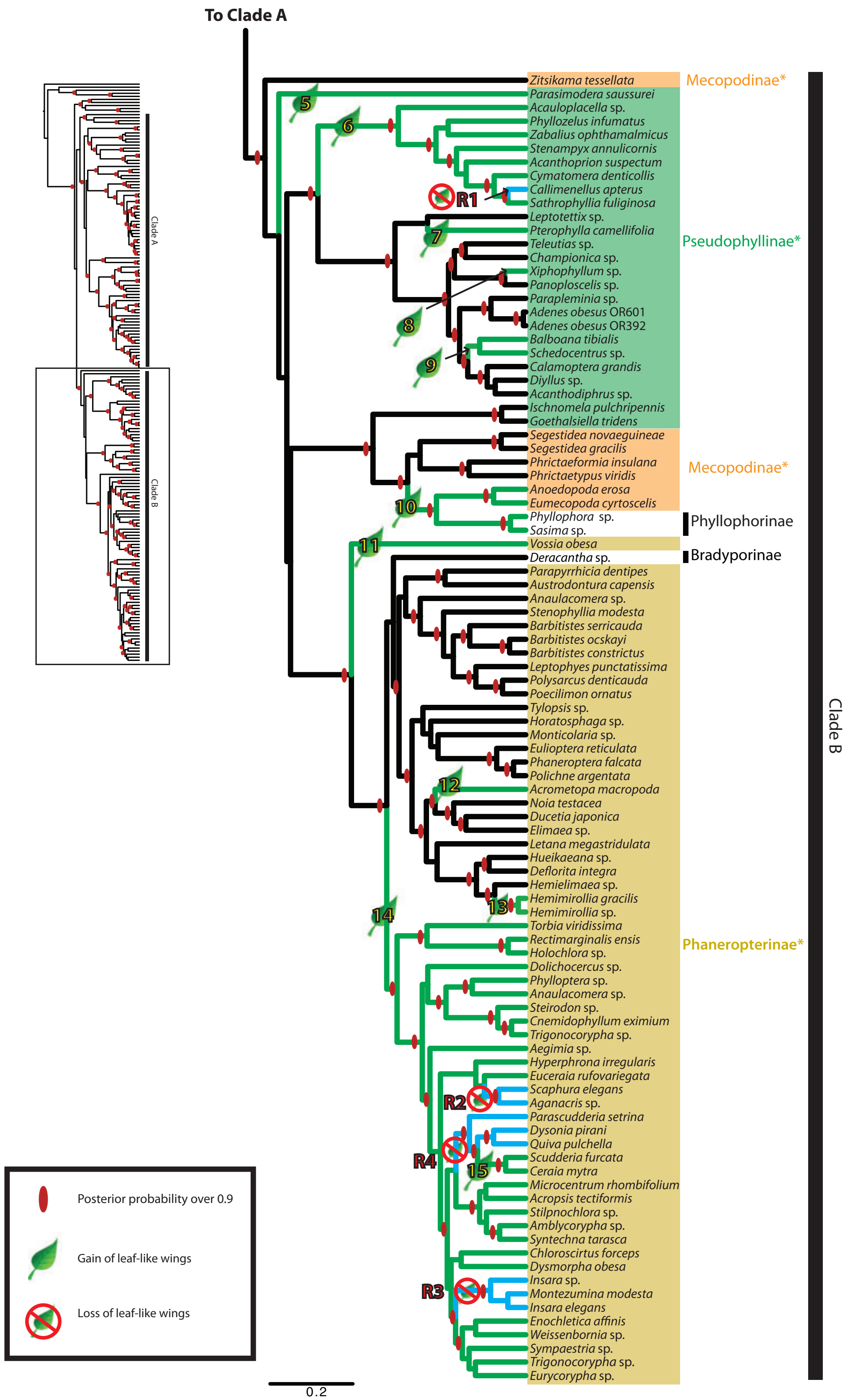

Fig. 6. (*continued*)

group extensively, including exemplars from all continents. Only a single derivation of leaf-like wings is known in this subfamily, in the lineage that gave rise to the genera *Copiphora* and *Lirometopum* in Central and South America.

Clade B comprises the subfamilies Mecopodinae, Pseudophyllinae, Phyllophorinae, Bradyporinae and Phaneropterinae (Fig. 6*B*). The basal condition for this clade is non-leaf-like, with 11 subsequent transitions to leaf-like wings. In Clade B, an early transition to leaf-like wings (Tran. #5) is seen in the lineage that gave rise to the Malagasy pseudophylline *Parasimodera saussurei*.

Most of the remaining Pseudophyllinae comprise two clades: one Old World and one predominantly New World. Leaf-like wings were developed early in the Old World pseudophyllines (Fig. 6*B*, Tran. #6; Fig. 3*F*). In this clade there is a later shift in memesis from leaf-like to bark-like wings in *Sathrophyllia fulginosa* (Fig. 3*AA*) and *Cymatomera denticollis* (Fig. 3*Z*). These taxa retain the shape of the leaf-like wings, but the venation and behaviour have adapted for hiding on bark. A loss of leaf-like wings in this clade is evident in brachypterous (short-winged) or apterous (wingless) species in the genus *Callimenellus* (Fig. 6*B*, #R1).

The second clade of Pseudophyllinae contains primarily New World taxa with the exception of *Adenes obesus* from western Africa. This clade has three independent derivations of leaf-like wings seen with the North American *Pterophylla camellifolia* (Fig. 6*B*, Tran. #7; Fig. 3*P*), the South American *Xiphophyllum* sp. (Fig. 6*B*, Tran. #8) and the clade comprised of *Balboana tibialis* + *Schedocentrus* sp. (Fig. 6*B*, Tran. #9). Transition #10 occurs in a clade of Mecopodinae + Phyllophorinae from the Old World tropics.

Within Phaneropterinae we found five transitions to leaf-like wings. The first is in the basal phaneropterine *Vossia obesa* (Fig. 6*B*, Tran. #11). The remaining Phaneropterinae are divided into two major clades. The first clade includes species that are primarily non-leaf-like. We found two transitions to leaf-like wings in *Acrometopa macropoda* (Fig. 6*B*, Tran. #12) and *Hemimirollia* spp. (Fig. 6*B*, Tran. #13). The second clade includes species that are primarily leaf-like, with the transition to the leaf-like form mapped at the base of the clade (Fig. 6*B*, Tran. #14) with three subsequent reversals to non-leaf-like wings. The first reversal (Fig. 6*B*, #R2) is in the spider wasp mimics *Scaphura elegans* and *Aganacris* sp. (Fig. 3*DD*). These katydids are remarkable in that their bodies are black, their wings are yellow, orange or black, and their behaviour and activity patterns mirror that of their model wasps and not other katydids (Belwood 1993). A second reversal occurs in the entire tribe of Insarini (Fig. 6*B*, #R3), represented in our analysis by *Insara* spp. (Fig. 3*HH*) and *Montezumina*. These gracile katydids are still cryptically coloured, but lack the broad wings typical of the leaf-like phaneropterines. A third reversal within Phaneropterinae occurs in the base of a clade that includes five taxa: *Parascudderia*, *Dysonia*, *Quiva*, *Scudderia* and *Ceraia* (Fig. 6*B*, #R4). *Dysonia* (Fig. 3*BB*), *Quiva* (Fig. 3*CC*) and *Parascudderia* are non-leaf-like and are cryptically coloured to resemble fungus or lichen and are often collected on bark (Belwood 1993). However, *Scudderia furcata* and *Ceraia mytra* have wings that are leaf-like, and these species are often collected on leaves. The presence of leaf-like wings in these genera represents the only instance of a gain, loss and subsequent regain of leaf-like wings that we have detected in Tettigoniidae.

Speciation rates within the non-leaf-like katydids are greater than that of the leaf-like katydids (Fig. 7; LRT $P = 0.01954$). When considering only Phaneropterinae, a subfamily that includes many tropical leaf-like forms, the speciation rates of the non-leaf-like katydids were nearly equal to the leaf-like katydids and the LRT comparing the two-rate model to the single-rate model was returned as non-significant (LRT $P = 0.5918$). In both Tettigoniidae as a whole, and in the Phaneropterinae subclade, leaf-like wings were not found to be a driver of speciation relative to non-leaf-like wings. Also, non-leaf-like wings were favoured by selection across Tettigoniidae as a whole (Fig. 8; LRT $P < 0.001$). Within Phaneropterinae the transition rates to the leaf-like form appear slightly greater relative to the non-leaf-like forms, but this difference was not significant (Fig. 8; LRT $P = 0.8472$) indicating neither wing form was favoured relative to the other in this subclade.

Discussion

While it is well known that many katydid species have leaf-like wings, previous workers generally have not explicitly stated their criteria for assigning leaf-like wings to individual species. For instance, in a work describing various types of katydid venation, Zeuner (1936) excluded the leaf-mimic species without indicating how they were distinguished from the non-leaf-like species. Belwood (1993) limited the leaf-like katydids to Phaneropterinae and Pterochrozinae with wings that resemble 'simple oval leaves'. Nickle and Castner (1995) also limited their definition of leaf-like Neotropical katydids to those with irregular leaf-shaped wings and lobed legs that resemble knotted twigs. Ragge (1955) considered broad-winged green or brown katydids as leaf-mimics. The difficulty in identifying leaf-like wings comes from the diversity of sizes and shapes of katydid wings, and because even in the definitive leaf-like katydids (i.e. Pterochrozinae), not all the wings converge on the same overall shape. Instead, the leaf-like wings of katydids vary considerably in overall shape, texture and size (Fig. 3). The ratio method presented in this work provides a simple metric for assessing whether a wing is leaf-like. We demonstrate that this method is operational, repeatable and validated via GM methods. Now that we have clarified a definition for leaf-like wings, we can proceed to a discussion of the evolution of this feature across katydids.

A general trend among katydids is that species with leaf-like wings are more common in the tropics. Considerable variation is found in the 15 lineages that independently derived the leaf-like wings. Katydids with leaf-like wings are not specialised on particular plant species and will roost among leaves that match their colour (Edmunds 1974; Castner and Nickle 1995*b*; Nickle and Castner 1995). This lack of specialisation among katydids may have contributed in part to the variety of leaf-like forms found in tettigoniid wings. Outside the tropics, cryptically coloured katydids are still common, but the leaf-like wings are much less prevalent. This general trend may be in part due to the lack of specialisation in roosting sites, differences in plant diversity and plant density outside the tropics, and the potential for additional niches outside the tropical regions. In our experience, sampling

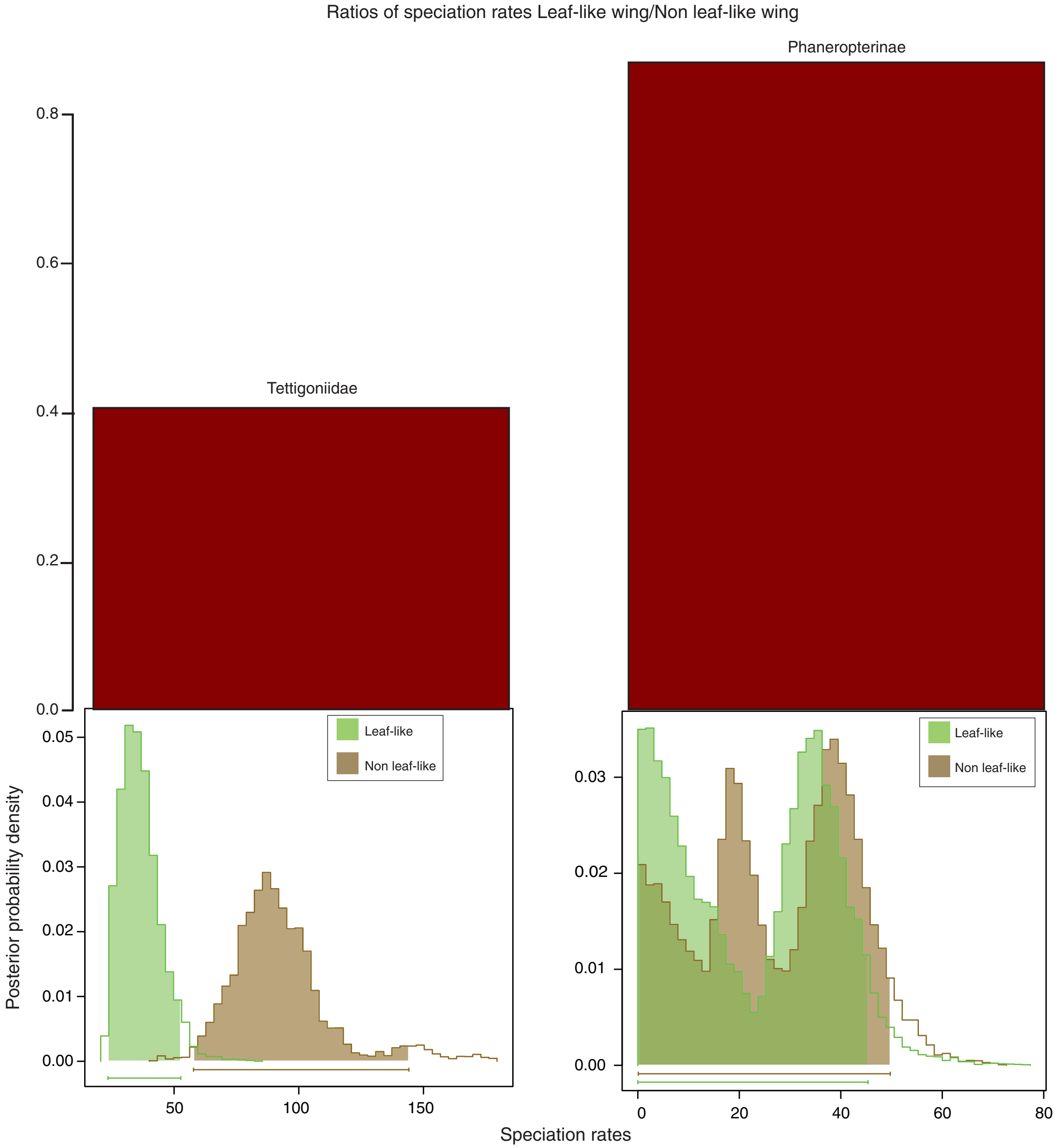

Fig. 7. Speciation rate ($\lambda_{leaf\text{-}like}/\lambda_{non\text{-}leaf\text{-}like}$) ratio from DivTree analyses. Values <1 suggest speciation rates are lower in the leaf-like forms in all Tettigoniidae (likelihood ratio tests, LRT $P = 0.01954$) and Phaneropterinae (LRT $P = 0.5918$). DivTree posterior probability distributions are shown below with green representing the leaf-like forms and brown the non-leaf-like forms.

katydids in temperate regions reveals several cryptically coloured, ground-dwelling katydids that take refuge in grasses, at the base of plants, or in burrows to escape diurnal predators. The large number of leaf-like transitions, particularly within the tropical taxa, makes it tempting to assume the leaf-like wings are driving speciation, but our results suggest this may not be the case throughout Tettigoniidae. Relative rates of speciation indicate the leaf-like lineages do not show an increased rate of speciation relative to the non-leaf-like forms. The transition rates to leaf-like wings in katydids vary depending on the clades focused on and the scope of the analysis. Throughout Tettigoniidae as a whole, selection favours transitions to the non-leaf-like forms. However, narrowing the scope to only the large subclade Phaneropterinae, there appears to be a greater rate of transition to the leaf-like form, though this rate was not found to be significant. This increased rate of transition to leaf-like wings

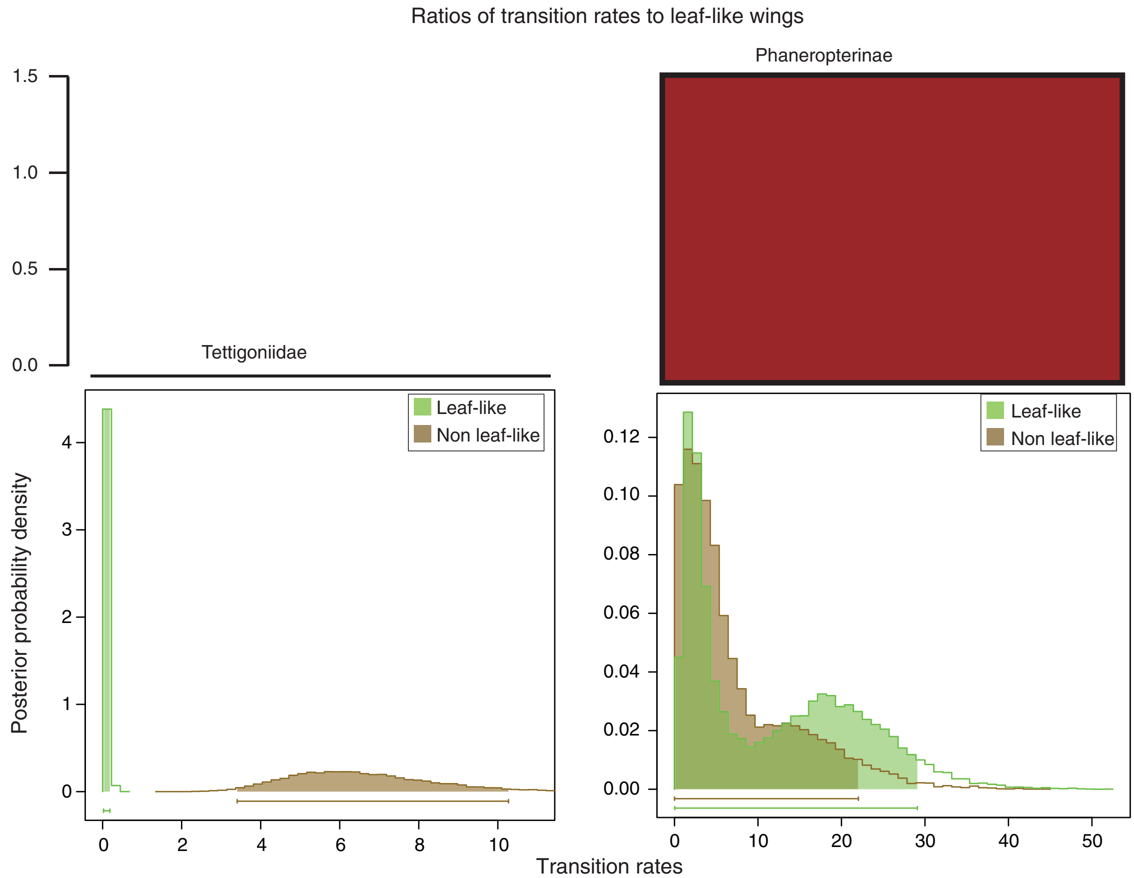

Fig. 8. Ratio of transition rates (q_transition from non-leaf-like to leaf-like/q_transition from leaf-like to non-leaf-like) from DivTree. A ratio greater than 1 is expected if selection favours the leaf-like form. In Tettigoniiae the ratio was near zero (0), indicating selection favours the non-leaf-like form (likelihood ratio tests, LRT $P < 0.0001$). The Phaneropterinae subclade has a greater transition rate to the leaf-like form (LRT $P = 0.8472$), indicating selection possibly favours the leaf-like form within this subfamily.

within Phaneropterinae suggests that, while not significantly greater overall, in at least some lineages selection may favour the leaf-like form.

Conclusions

Prior to this work the definition of leaf-like has been ambiguous, variable or specific to each study. Herein we present an operational method for quickly differentiating the leaf-like from the non-leaf-like katydid wings. A clearer view of katydid phylogenetic relationships was presented showing an obvious need for major revisionary work within Tettigoniidae.

Leaf-like wings have been independently derived in Tettigoniidae at least 15 times. Wings have been lost in four lineages and we found a single instance of leaf-like wings being gained, lost and subsequently regained. Overall, leaf-like wings are not a major driver of evolution within Tettigoniidae and, in general, selection favours the non-leaf-like wing. However, this pattern varies slightly in Phaneropterinae and selection possibly favours the leaf-like wing in this subfamily. Within this family the leaf-like wings are not a driver of speciation. Additional work is needed to determine whether this trend is shared in the other insect orders with leaf mimesis.

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References

Belwood, J. J. (1993). Anti-predator defences and ecology of neotropical forest katydids, especially the Pseudophyllinae. In 'The Tettigoniidae: Biology, Systematics, and Evolution'. (Eds W. J. Bailey and D. C. F. Rentz.) pp. 8–26. (Springer-Verlag: New York.)

Castner, J. L. (1995). Defensive behavior and display of the leaf-mimicking katydid *Pterochroza ocellata* (L.) (Orthoptera: Tettigoniidae: Pseudophyllinae: Pterochrozini). *Journal of Orthoptera Research* **4**, 89–92. doi:10.2307/3503462

Castner, J. L., and Nickle, D. A. (1995*a*). Notes on the biology and ecology of the leaf-mimicking katydid *Typophyllum bolivari* Vignon (Orthoptera: Tettigoniidae: Pseudophyllinae: Pterochrozini). *Journal of Orthoptera Research* **4**, 105–109. doi:10.2307/3503465

Castner, J. L., and Nickle, D. A. (1995*b*). Observations on the behavior and biology of leaf-mimicking katydids (Orthoptera: Tettigoniidae: Pseudophyllinae: Pterochrozini). *Journal of Orthoptera Research* **4**, 93–97. doi:10.2307/3503463

Colgan, D. J., McLauchlan, A., Wilson, G. D. F., Livingston, S. P., Edgecombe, G. D., Macaranas, J., Cassis, G., and Gray, M. R. (1998). Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Australian Journal of Zoology* **46**(5), 419–437. doi:10.1071/ZO98048[In English]

Conner, W. E. (2014). Adaptive sounds and silences: acoustic anti-predator strategies in insects. In 'Insect Hearing and Acoustic Communication'. pp. 65–79. (Springer: New York City.)

Crespi, B., and Sandoval, C. (2000). Phylogenetic evidence for the evolution of ecological specialization in Timema walking-sticks. *Journal of Evolutionary Biology* **13**(2), 249–262. doi:10.1046/j.1420-9101.2000.00164.x

Drummond, A. J., Suchard, M. A., Xie, D., and Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29**, 1969–1973.

Eades, D. C., Otte, D., Cigliano, M. M., and Braun, H. (2015). Orthoptera Species File Online. Version 5.0/5.0. Available at http://Orthoptera.SpeciesFile.org [Verified May 2016]

Edmunds, M. (1974). 'Defence in Animals: a Survey of Anti-predator Defences.' (Longman Publishing Group: Harlow, UK.)

Fabricant, S. A., and Herberstein, M. E. (2014). Hidden in plain orange: aposematic coloration is cryptic to a colorblind insect predator. *Behavioral Ecology* **26**, 38–44.

FitzJohn, R. G. (2012). Diversitree: comparative phylogenetic analyses of diversification in R. *Methods in Ecology and Evolution* **3**(6), 1084–1092.

Gwynne, D. T. (2001). 'Katydids and Bush-Crickets.' (Comstock Publishing Associates: Sacramento, CA.)

Hultgren, K., and Stachowicz, J. (2009). Evolution of decoration in majoid crabs: a comparative phylogenetic analysis of the role of body size and alternative defensive strategies. *American Naturalist* **173**(5), 566–578. doi:10.1086/597797

Johannesson, K., and Ekendahl, A. (2002). Selective predation favouring cryptic individuals of marine snails (Littorina). *Biological Journal of the Linnean Society. Linnean Society of London* **76**(1), 137–144. doi:10.1111/j.1095-8312.2002.tb01720.x

Katoh, K., Kuma, K.-i., Toh, H., and Miyata, T. (2005). MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Research* **33**(2), 511–518. doi:10.1093/nar/gki198

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., and Duran, C. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**(12), 1647–1649. doi:10.1093/bioinformatics/bts199

Kumar, S., Nei, M., Dudley, J., and Tamura, K. (2008). MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. *Briefings in Bioinformatics* **9**(4), 299–306. [In English] doi:10.1093/bib/bbn017

Lanfear, R., Calcott, B., Ho, S. Y. W., and Guindon, S. (2012). PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* **29**(6), 1695–1701. doi:10.1093/molbev/mss020

Maddison, W. P., and Maddison, D. R. (2015). Mesquite: a modular system for evolutionary analysis. Version 3.04. Available at http://mesquiteproject.org [Verified June 2016]

Maddison, W. P., Midford, P. E., and Otto, S. P. (2007). Estimating a binary character's effect on speciation and extinction. *Systematic Biology* **56**(5), 701–710. doi:10.1080/10635150701607033

Marshall, K. L., and Gluckman, T. L. (2015). The evolution of pattern camouflage strategies in waterfowl and game birds. *Ecology and Evolution* **5**(10), 1981–1991. doi:10.1002/ece3.1482

Mugleston, J. D., Song, H., and Whiting, M. F. (2013). A century of paraphyly: a molecular phylogeny of katydids (Orthoptera: Tettigoniidae) supports multiple origins of leaf-like wings. *Molecular Phylogenetics and Evolution* **69**(3), 1120–1134. doi:10.1016/j.ympev.2013.07.014

Nentwig, W. (1985). A tropical caterpillar that mimics faeces, leaves and a snake (Lepidoptera: Oxytenidae: *Oxytenis naemia*). *Journal of Research on the Lepidoptera* **24**, 136–141.

Nickle, D. A., and Castner, J. L. (1995). Strategies utilized by katydids (Orthoptera: Tettigoniidae) against diurnal predators in rainforests of northeastern Peru. *Journal of Orthoptera Research* **4**, 75–88. doi:10.2307/3503461

Pinheiro, C., and Freitas, A. (2014). Some possible cases of escape mimicry in neotropical butterflies. *Neotropical Entomology* **43**(5), 393–398. doi:10.1007/s13744-014-0240-y

R Core Team (2013). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at http://www.R-project.org/ [Verified July 2015]

Ragge, D. R. (1955). 'The Wing-Venation of the Orthoptera Saltatoria with Notes on Dictyopteran Wing-Venation.' (British Museum: London.)

Rajabizadeh, M., Adriaens, D., Kaboli, M., Sarafraz, J., and Ahmadi, M. (2015). Dorsal colour pattern variation in Eurasian mountain vipers (genus *Montivipera*): a trade-off between thermoregulation and crypsis. *Zoologischer Anzeiger* **257**(0), 1–9. doi:10.1016/j.jcz.2015.03.006

Rambaut, A., and Drummond, A. J. (2003). Tracer v1.3. Oxford, UK. Available at http://evolve.zoo.ox.ac.uk/ [Verified May 2016]

Robinson, M. H. (1969). The defensive behaviour of some orthopteroid insects from Panama. *Transactions of the Royal Entomological Society of London* **121**(7), 281–303. doi:10.1111/j.1365-2311.1969.tb00521.x

Rohlf, F. (2003). TpsRelw, relative warps analysis. 1.36 edn. Department of Ecology and Evolution, State University of New York at Stony Brook: Stony Brook, NY. Available at http://life.bio.sunysb.edu/morph/index.html [Verified June 2016].

Rohlf, F. (2005). TpsDig, digitize landmarks and outlines. 2.05 edn. Department of Ecology and Evolution, State University of New York at Stony Brook: Stony Brook, NY. Available at http://life.bio.sunysb.edu/morph/ [verified June 2016].

Rohlf, F. J., and Slice, D. (1990). Extensions of the procrustes method for the optimal superimposition of landmarks. *Systematic Zoology* **39**(1), 40–59. doi:10.2307/2992207

Ruxton, G. D., Sherratt, T. N., and Speed, M. P. (2004). 'Avoiding Attack: the Evolutionary Ecology of Crypsis, Warning Signals, and Mimicry.' (Oxford University Press: Oxford, NY.)

Schmidt, J. O. (1990). 'Insect Defenses: Adaptive Mechanisms and Strategies of Prey and Predators.' (SUNY Press, Albany, NY.)

Skelhorn, J., Rowland, H. M., and Ruxton, G. D. (2010*a*). The evolution and ecology of masquerade. *Biological Journal of the Linnean Society* **99**, 1–8.

Skelhorn, J., Rowland, H. M., Speed, M. P., and Ruxton, G. D. (2010*b*). Masquerade: camouflage without crypsis. *Science* **327**(5961), 51. doi:10.1126/science.1181931

Song, H., Amédégnato, C., Cigliano, M. M., Desutter-Grandcolas, L., Heads, S. W., Huang, Y., Otte, D., and Whiting, M. F. (2015). 300 million years of diversification: elucidating the patterns of orthopteran evolution based on comprehensive taxon and gene sampling. *Cladistics* **31**, 621–651. doi:10.1111/cla.12116

Stamatakis, A. (2006). RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**(21), 2688–2690. doi:10.1093/bioinformatics/btl446

Svanbäck, R., and Eklöv, P. (2011). Catch me if you can – predation affects divergence in a polyphenic species. *Evolution* **65**(12), 3515–3526. doi:10.1111/j.1558-5646.2011.01398.x

Svenson, G. J., and Whiting, M. F. (2004). Phylogeny of Mantodea based on molecular data: evolution of a charismatic predator. *Systematic Entomology* **29**(3), 359–370. doi:10.1111/j.0307-6970.2004.00240.x

Svenson, G. J., and Whiting, M. F. (2009). Reconstructing the origins of praying mantises (Dictyoptera, Mantodea): the roles of Gondwanan vicariance and morphological convergence. *Cladistics* **25**(5), 468–514. [In English] doi:10.1111/j.1096-0031.2009.00263.x

Vane-Wright, R., and Boppre, M. (1993). Visual and chemical signalling in butterflies: functional and phylogenetic perspectives. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **340**(1292), 197–205. doi:10.1098/rstb.1993.0058

Whiting, M. F. (2002). Mecoptera is paraphyletic: multiple genes and phylogeny of Mecoptera and Siphonaptera. *Zoologica Scripta* **31**(1), 93–104. doi:10.1046/j.0300-3256.2001.00095.x

Wild, A. L., and Maddison, D. R. (2008). Evaluating nuclear protein-coding genes for phylogenetic utility in beetles. *Molecular Phylogenetics and Evolution* **48**(3), 877–891. doi:10.1016/j.ympev.2008.05.023

Zeuner, F. E. (1936). The subfamilies of Tettigoniidae (Orthoptera). *Proceedings of the Royal Entomological Society of London. Series B, Taxonomy* **5**(5), 103–109.

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