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One hundred years of instability in ensiferan relationships

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Abstract. Although Ensifera is a major insect model group, its phylogenetic relationships have been understudied so far. Few phylogenetic hypotheses have been proposed, either with morphological or molecular data. The largest dataset ever used for phylogeny reconstruction on this group is molecular (16S rRNA, 18S rRNA and 28S rRNA sequences for 51 ensiferan species), which has been used twice with different resultant topologies. However, only one of these hypotheses has been adopted commonly as a reference classification. Here we re-analyse this molecular dataset with different methods and parameters to test the robustness and the stability of the adopted phylogeny. Our study reveals the instability of phylogenetic relationships derived from this dataset, especially for the deepest nodes of the group, and suggests some guidelines for future studies. The comparison between the different classifications proposed in the past 70 years for Ensifera and our results allows the identification of potential monophyletic clades (katydids, mole crickets, scaly crickets + *Malgasia*, true crickets, leaf roller crickets, cave crickets) and the remaining unresolved clades (wetas, Jerusalem crickets and most of the highest rank clades) in Ensifera phylogeny.

Introduction

Natural classifications need stability (Carpenter, 2003), but they must also reflect progress made with new phylogenetic hypotheses (Dominguez & Wheeler, 1997). Traditionally, the robustness of any phylogenetic hypothesis is assessed through bootstrap, jackknife (JS) or Bremer support (BS) values (Felsenstein, 1985; Bremer, 1994; Farris *et al*., 1996), and more recently through posterior probabilities. Lately, robustness has been evaluated through clade stability, a condition showing the stability of clades under different analytical criteria. Support and stability are usually, but not always, correlated (Giribet, 2003). They are complementary and both are necessary to evaluate thoroughly the robustness of a hypothesis.

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An explicit and robust classification scheme for the Orthoptera has not been forthcoming. Orthoptera comprise two suborders, Caelifera (grasshoppers, ~11 000 species) and Ensifera (crickets and katydids, ~15 000 species). The Ensifera, which include important model organisms in evolutionary biology, such as *Gryllus* spp., *Ephippiger* spp. or *Hemideina* spp. (Gwynne & Morris, 1983; Greenfield, 2002), are affected particularly by the lack of a robust phylogenetic hypothesis. Ensiferan relationships have been inferred previously in several prephylogenetic and hennigian studies using morphological characters (Chopard, 1920; Ander, 1939; Zeuner, 1939; Judd, 1948; Ragge, 1955; Gorochov, 1986, 1995), but only recently through phylogenetic analyses using a formal data matrix and explicit algorithms (Mishler, 2005). Nevertheless, two formal analyses were carried out based on morphological characters (Gwynne, 1995; Desutter-Grandcolas, 2003), but both studies were deficient because they included high-level terminals that were not clearly monophyletic. Likewise, ensiferan relationships have seldom been investigated with molecular data. Either some ensiferan terminals were included in caeliferan phylogenetic analyses as outgroups (Flook & Rowell, 1997; Flook *et al*., 1999), or

molecular analyses focused only on subfamilial (Robillard & Desutter-Grandcolas, 2006) or lower taxonomic group relationships (Shaw, 1996; Huang *et al*., 2000; Brettschneider, 2006; Pratt *et al*., 2008).

The first comprehensive molecular phylogenetic analyses of Ensifera are those of Jost (2002) and Jost & Shaw (2006). These authors analysed almost identical datasets (Table 1), but found different phylogenetic relationships, suggesting instability in ensiferan relationships. Furthermore, even if 51 ingroup taxa were sampled for one to three molecular markers – the most extensive taxonomical sampling of Ensifera so far – this represents less than 0.5% of ensiferan specific diversity (Jost & Shaw, 2006: table 2) and half of the terminals contain missing data. In addition, Cooloolidae and several cricket and katydid subfamilies are not represented in the dataset, whereas other subfamilies, the monophyly of which is well established, are over-represented. Theoretical and empirical studies have shown how inadequate sampling can affect stability and accuracy in phylogenetic analyses (e.g. Grandcolas & D'Haese, 2001; Zwickl & Hillis, 2002). Nonetheless, Jost & Shaw's (2006) hypothesis has been mostly adopted as a reference for the classification of this group by the Orthoptera Species File Online, a taxonomic catalogue of Orthoptera (Eades & Otte, 2008). As systematists and evolutionary biologists, we question whether this hypothesis is sufficiently stable to form a basis for classification and evolutionary studies.

Status of the classification of Ensifera

Here we do not use a priori taxonomic entities because there is no universally accepted classification of Ensifera today (see below) and we discuss phylogenetic results only according to the terminals of the data matrix.

In his textbook, Scudder (1897) separated Tettigoniidae, Gryllidae and Acridiidae as families of Orthoptera together with Forficulidae (now Dermaptera), Phasmidae (now Phasmatodea), Blattidae (now Blattaria) and Mantidae (now Mantodea), assigning them the same taxonomic rank. This system has been used for many years, even though Ensifera was recognized as a separate entity including either Gryllidae and Phasgonuridae (=Tettigoniidae, Chopard, 1920), or Gryllidae, Gryllacridae (sic), Tettigoniidae and Prophalangopsidae (Chopard, 1938). Ensifera are now considered a monophyletic clade within Orthoptera s.s. (Kevan, 1977, 1982; Kristensen, 1981, 1995; Rentz, 1991; Flook *et al*., 1999; contra Hennig, 1981; Jost & Shaw, 2006).

Within Ensifera, crickets and their immediate allies – mole crickets, scaly crickets, ant-loving crickets – have been readily separated either as a family (Chopard, 1920, 1938), a superfamily (Ander, 1939; Chopard, 1949; Ragge, 1955; Beier, 1972; Hennig, 1981; Rentz, 1991) or an infraorder (Vickery, 1977; Desutter, 1987; Desutter-Grandcolas, 2003). Ensifera other than crickets s.l. have been gathered most often into 'katydids and their allies', with katydids recognized as a family or a superfamily. The phylogenetic position of the 'katydid allies' remains the most intractable problem in ensiferan taxonomy (Hubbell, 1978; Hennig, 1981). Katydid allies have been considered a 'primitive' lineage because they are unable to stridulate with their forewings as crickets and katydids do. They have been united previously in a heterogeneous assemblage, the Gryllacrididae or Gryllacridoidea, but this assemblage has been dissolved by recent phylogenetic studies (Gwynne, 1995; Desutter-Grandcolas, 2003). Some of these allies form putative monophyletic entities – cave crickets, leaf rollers crickets, sand crickets – but others remain ill-defined, such as the Jerusalem crickets (Stenopelmatidae s.s.) and wetas (Anostostomatidae *sensu* Johns, 1997), the monophyly of which has yet to be corroborated [but see Pratt *et al*. (2008) for Anostostomatidae]. Sand crickets have been associated with crickets s.l. (Gwynne, 1995; Jost & Shaw, 2006). To increase confusion, a few genera are of uncertain affinity, including *Cyphoderris* Uhler, 1864, *Prophalangopsis* Walker, 1871, *Lezina* Walker, 1869 and *Cooloola* Rentz, 1980. *Cyphoderris* and *Prophalangopsis* are traditionally considered extant members of mostly fossil groups (Haglidae or Prophalangopsidae, according to authors), and seen as 'primitive' taxa. By contrast, morphological phylogenies cluster *Cyphoderris* and *Lezina* with a derived clade comprising Tettigoniidae and Anostostomatidae (Gwynne, 1995; Desutter-Grandcolas, 2003), consistent with Ander's (1939) Tettigoniaemorpha.

This situation results in two main problems. First, ensiferan taxonomists use dissimilar classification systems, adding to the confusion in ensiferan systematics. Second, biologists cannot analyse evolutionary questions in Ensifera unambiguously because they lack an adequate reference system at hand. Today, the classification most often used is an online catalogue (Eades & Otte, 2008) with all the usefulness and potential biases observed in such tools (e.g. Dubois, 2007). Classificatory levels are arbitrary, but today's criteria for systematics imply that these levels are based on monophyletic entities and that a

Table 1. Comparison of the three most recent molecular datasets used to infer ensiferan relationships: Jost (2002), Jost & Shaw (2006) and our dataset.

	No. taxa (ingroup + outgroup)	Alignment length (bp)	No. and position of deleted bases	No. informative characters	% informative characters
Jost (2002)	51 + 3	2775	55 (282–336)	939	33.8
Jost & Shaw (2006)	51 + 4	2596	255 (270–331; 1405–1480; 2628–2682; 2711–2772)	712	27.4
Present study	51 + 4	2499	282 (245–312; 1309–1385; 2399–2468; 2484–2550)	691	27.7

taxonomic correspondence exists for the equivalent nodes of the topology (e.g. Eldredge & Cracraft, 1980). To construct a phylogeny-based classification of Ensifera, one will also have to deal with different 'taxonomic schools', which attribute to the same group either family or superfamily ranks, following Scudder (1897) and/or Chopard (1967) (but see Chopard's foreword in his 1967 catalogue), or Chopard (1949, 1969) and Vickery (1977), respectively.

Aims of the study

Our aim was to estimate the robustness of the phylogenetic relationships of Ensifera and to investigate its usefulness as a reference system for classificatory and evolutionary purposes. We value Jost & Shaw's (2006) work as the most complete molecular study of Ensifera to date, despite some limitations with taxon sampling, methodology and evolutionary inference. We will not emphasize these points because, independent of the methods used, phylogenetic results tend to converge towards the same topology when data are adequate and no reconstruction artefact is suspected (Morrison & Ellis, 1997).

Instead, we re-analysed Jost & Shaw's (2006) data critically, using both traditional analyses with a static alignment and the direct optimization algorithm for tree reconstruction with dynamic alignment procedures (Wheeler, 1996). For the latter, we designed a sensitivity analysis to evaluate the robustness of the resultant clades (Wheeler, 1995; Giribet & Wheeler, 1999) and calculated BS and JS values to assess clade robustness. We discuss the current state of ensiferan phylogeny and provide some guidelines for future studies. Finally, we compare our results with the systems proposed for Ensifera by different authors (Ander, 1939; Chopard, 1969; Gorochov, 1995; Gwynne, 1995; Desutter-Grandcolas, 2003; Eades & Otte, 2008) and estimate the level of support for hypothesized clades.

Materials and methods

Taxon and character sampling

Jost & Shaw's (2006) dataset included 51 ensiferan terminals and four caeliferan outgroups sequenced for three partial molecular markers (16S rRNA, ~475 bp; 18S rRNA regions GAD and CEF, ~1700 bp; 28S rRNA region C, ~350 bp), each taxon comprising between 378 and 2624 bp. To fill gaps in the data matrix, especially for outgroup taxa, we added six sequences available in GenBank with the following accession numbers: X95741 (18S of *Acheta domesticus*), AF514628 and AF514657 (18S of *Myrmecophilus* sp.), Z97589 and AJ011974 (18S and 16S of *Trigonopteryx hopei*, respectively), Z97560 (18S of *Acrida turrita*) and AY239108 (16S of *Brachytrupes portentosus*). For the latter, the data matrix included sequences from two different species of *Brachytrupes*.

Phylogenetic analyses

First, we conducted 'traditional' analyses, with data aligned a priori of the phylogenetic reconstruction, in parsimony and Bayesian frameworks. Then we carried out dynamic analyses and assessed the stability of the phylogenetic results under different parameter sets.

Alignment and static analyses

We were unable to obtain Jost & Shaw's (2006) nucleotide alignment upon request, so we generated a multiple alignment using MUSCLE v3.6 (Edgar, 2004) under default settings. To facilitate repeatability and given that the usefulness of secondary structure in refining alignment is controversial (e.g. Giribet & Wheeler, 2001; Legendre, 2004; Wheeler *et al*., 2006), we did not modify the MUSCLE output to refine homology hypotheses, but we deleted a few nucleotides, as Jost & Shaw (2006) did, in order to compile comparable datasets. Thus, 282 bp were excluded from the static alignment due to 'poor sequence conservation' and 'great difficulty aligning the nucleotides' (Table 1). Similarly, the options used in parsimony and Bayesian analyses follow those used by Jost & Shaw (2006) to facilitate comparison.

A parsimony analysis was conducted using PAUP*4.0b10 (Swofford, 1998). The search strategy included 1000 replicates of random addition sequences with a tree-bisection-reconnection branch swapping algorithm. No MaxTrees value was specified and gaps were coded as missing data. An analysis in Bayesian inference was carried out using MRBAYES v3.1.2 (Huelsenbeck & Ronquist, 2001). Following Jost & Shaw (2006), a general time reversible model with a proportion of invariant sites and a gamma distributed rate variation among sites (GTR + I + G) was selected. The parameters of this model were estimated independently during the tree search procedure for the three different partitions – one for each gene – using the command 'unlink'. Four chains were run for 1 000 000 generations and sampled every 100 generations. The burn-in was estimated by plotting the number of generations against the log likelihood scores of the sampled trees. Two replicates with these parameters allowed a check for convergence in the results.

Direct optimization and sensitivity analyses

Direct optimization parsimony analyses were performed (Wheeler, 1996) as implemented in POY 4 build 2602. Powerful algorithms, such as treefusing (Goloboff, 1999) and ratcheting (Nixon, 1999b), were used in each analysis, increasing the possibility that a global optimum was reached. The 16S and 18S sequences were partitioned according to highly conserved regions in order to speed up the analyses and to avoid misleading alignments when portions of genes were lacking, but no nucleotide was excluded from the dynamic analyses. A sensitivity analysis (Wheeler, 1995) was conducted to

assess the stability of the phylogenetic results to different weighting schemes. Nine parameter combinations (gaps : transversions : transitions) were tested – 1 : 1 : 1, 2 : 1 : 1, 4 : 1 : 1, 2 : 2 : 1, 4 : 2 : 1, 8 : 2 : 1, 4 : 4 : 1, 8 : 4 : 1 and 16 : 4 : 1. The parameters' landscape was limited to close variations around the 1 : 1 : 1 combination to explore the dataset within a definite and consistent range of variation.

The equal weighting analysis (1 : 1 : 1) consisted of 100 replicates coupled with swapping and fusing algorithms. The swapping strategy alternated subtree-pruning-regrafting and tree-bisection-reconnection algorithms and tried all possible join positions [command '*swap(all)*']. The fusing strategy included 200 rounds, each round being followed by a swapping session {command '*fuse*[*iterations:200, swap(all)*]'}. The most-parsimonious tree was selected and ratchet algorithms were performed. The ratcheting session included ten iterations, during which 20% of the static characters [command *'transform(static_approx)'*] were upweighted by a factor of four [*command* '*ratchet:(0.20,4)*']. The most-parsimonious tree, called the 'preferred' tree, was selected and its length was better approximated using the '*report(static_approx)*' command. A few statistics linked to this tree were reported: consistency and retention indices were calculated with the '*report(ci, ri)*' command, whereas the '*report (seq_stats:all)*' command estimated the level of conservation of each molecular marker. An implied alignment was generated in the Hennig86 format (command *'phastwincladfile'*). This implied alignment was used to estimate branch lengths and also to compute partitioned Bremer supports (PBS; Baker & DeSalle, 1997). For branch lengths, the Hennig86 file was read in WINCLADA v1.00.08 (Nixon, 1999a) and characters were optimized on the preferred tree (command '*optimize/unambiguous only*'). For PBS, three partitions corresponding to the three markers were used and the default parameters in TREEROT v2b (Sorenson, 1999) and PAUP 4.0b10 (Swofford, 1998) were followed.

Two additional analyses were performed in POY 4 to compute BS and JS values. BS values were calculated following 20 replicates with swapping algorithms. All visited trees were stored [command *'swap(all, visited)'*] and their lengths were compared with the preferred tree. In this study, BS values are not the sum of PBS values, because the latter have been computed from an implied alignment whereas the former were calculated in a direct optimization framework. Nevertheless, BS and the sum of PBS are still tightly correlated and follow the same pattern. JS was calculated with 100 pseudoreplicates in which 30% of the dynamic homology characters were removed {command'*calculate_support*[*jackknife:*(*remove:30, resample:100*)]'}.

For the sensitivity analysis, ten searches were performed for each parameter combination. Each search included 30 replicates with treefusing and swapping algorithms {commands *'build(30) swap*(*all*) *fuse*[*iterations:120, swap*(*all*)]'}. The most-parsimonious trees of each ten searches were assembled and 100 extra rounds of treefusing were performed. The optimal tree was then selected for each parameter combination. The results of this sensitivity analysis were plotted on our preferred tree with diagrams of stability at each node. The darker the diagram, the more stable the results.

Results

Molecular marker statistics

Four markers were used in this analysis: two portions of 18S (hereafter referred to as 18Sa and 18Sb), a portion of 28S and a portion of 16S. The statistics computed on raw data (i.e. no data excluded) illustrated the variability in homologous sequence length and allowed an estimation of the level of marker conservation (Table 2). Fragment 18Sa4 had the largest range (from 294 to 379 bp), being far longer in true crickets than in other Ensifera, whereas fragments 16S4, 18Sb, 18Sa4 and 28S were the most divergent in this sampling, having higher average uncorrected pairwise distances. When divided by the average lengths, the 'normalized' average distances obtained allowed an estimation of the level of conservation of each fragment. The fragments 16S1–4, 18Sa4 and 28S appeared as less conserved, whereas the remaining

Table 2. Statistics for the ten dynamic characters (i.e. portions of genes) used in the present study.

Sequences	Maximum length (bp)	Minimum length (bp)	Average length (bp)	Maximum distance	Minimum distance	Average distance	Normalized average distance
18Sa1	465	437	447.1	70	0	29.3	0.066
18Sa2	150	146	147.9	15	0	4.2	0.028
18Sa3	104	103	103.9	16	0	5.3	0.051
18Sa4	379	294	324	125	0	48.8	0.151
18Sb	663	650	655.3	112	0	49.6	0.076
28S	407	328	339.8	132	3	47.5	0.140
16S1	75	60	64.3	30	4	15.3	0.238
16S2	77	76	76	16	1	8.7	0.114
16S3	81	77	79.4	26	4	16.5	0.208
16S4	265	224	253.2	97	22	65.1	0.257

The maximum and minimum distances are the absolute number of sequence differences. Normalized average distance = average distance/average length.

18S fragments (18Sa1, 18Sa2, 18Sa3 and 18Sb) were the most conserved. Not surprisingly, more conserved markers provided proportionately less parsimony-informative characters than less conserved markers ($r = 0.564$, $P < 0.05$).

PBS revealed that 28S was the least informative of the three genes used (Table 3). It provided less than 10% of the total support, whereas 16S and 18S each brought around 45% of the signal. 28S still appeared the least informative gene when support was normalized according to the number of informative characters, but the difference with the other markers became smaller, suggesting that 28S is mostly uninformative because of its short sequenced portion. 18S provided most of the support for clade B (58% – clades A–F refer to terminal groups as defined in Fig. 3), whereas 16S provided most of the support for clades D and E (78 and 61%, respectively). 18S also supported the monophyly of clade A, but 16S was responsible for resolving its internal relationships (78%). The difference in signal between the different markers cannot be explained by only the amount of missing data, as 11, nine and four ingroup taxa were not documented for 28S, 18S and 16S, respectively (Jost & Shaw, 2006: table 2).

Static analyses: alignment and optimal topologies

The four markers were aligned individually before a 2781 bp long concatenated alignment was constructed. Following Jost & Shaw (2006), four regions, for a total of 282 bp, were excluded due to dubious homology hypotheses: positions 245–312 (16S), 1309–1385 (18S), 2399–2468 and 2484–2550 (28S) of the concatenated alignment. The final alignment was 2499 bp long and included 691 parsimony-informative characters (~28%, see Table 1). The parsimony analysis resulted in 29 most-parsimonious trees (length = 4221, consistency index = 0.40, retention index = 0.48), the strict consensus of which was poorly resolved, especially for the deepest nodes (Fig. 1). Ensifera were paraphyletic due to the nested position of a caeliferan Batrachideidae sp. as sister group to clade F, whereas true crickets, mole crickets, leaf roller crickets and cave crickets were all monophyletic.

The majority-rule consensus obtained in the Bayesian framework is depicted in Fig. 2 (burn-in = 17 000 generations; −log likelihood = 22185.06). Ensifera were retrieved as monophyletic with a high posterior probability, but, again, the deepest relationships within Ensifera were unresolved, displaying a polytomy of four branches. Several groups were monophyletic: mole crickets, true crickets, cave crickets, leaf roller crickets, katydids and wetas. Three more inclusive clades were retrieved as monophyletic: clades B, D and F as defined below.

Both parsimony and Bayesian topologies placed the crickets s.l. clade (clade F) on the longest branch in the tree.

Dynamic analyses: structure, stability and support of ensiferan phylogenetic relationships

The equally weighted analysis resulted in one most-parsimonious tree (length = 5558, consistency index = 0.54, retention index = 0.63, Fig. 3) where the Ensifera were monophyletic with moderate support (BS = 15, JS = 54). Within Ensifera, cave crickets (clade A) constituted the first diverging lineage. This clade was strongly supported (BS = 15, JS = 72), but the remaining Ensifera were weakly supported. In the latter clade, we identified five main clades (B–F). Clade B included two sister taxa: crickets s.l. (clade F) and sand crickets, represented here by *Comicus* sp. only. This clade had low support values (BS = 9, JS = 36). Within this clade, mole crickets and true crickets were both retrieved as monophyletic with high support values (BS = 24, JS = 90 and BS = 14, JS = 80, respectively). Clade C, which was weakly supported (BS = 8, JS = 32), comprised two monophyletic groups, clades D and E. In both clades, most of the deepest nodes had very low support values. Clade E included katydids and was moderately supported (BS = 12, JS = 49), whereas clade D was a heterogeneous, weakly supported (BS = 9, JS = 29) group comprising leaf roller crickets, Jerusalem crickets, wetas, *Lezina* sp. and *Cyphoderris monstrosus*. Leaf roller crickets constituted a well-supported

Table 3. Level of information of the different markers for the whole 'preferred' topology and for its five main clades.

Markers	No. characters	No. informative characters	% informative characters	Σ Bremer	% Bremer	Σ Bremer/no. informative characters
18S	1940	358	18.5	213.2	45.2	0.60
28S	557	101	18.1	44.5	9.4	0.44
16S	870	305	35.1	214.3	45.4	0.70
Total	3367	764	22.7	472	100	

Markers	Clade A	Clade B	Clade D	Clade E
18S	6 (19%)	159 (58%)	13.2 (31%)	21 (20%)
28S	1 (3%)	23 (8%)	−4 (−10%)	19.5 (19%)
16S	25 (78%)	91 (33%)	32.8 (78%)	62.5 (61%)
Total	32 (100%)	273 (100%)	42 (100%)	103 (100%)

For each marker i, $\%_i\text{Bremer} = \sum_i \text{Bremer}/\sum_{\text{TOTAL}} \text{Bremer}$. The higher $\%_i$ support, the more information i brings. Clades are labelled as in Fig. 3.

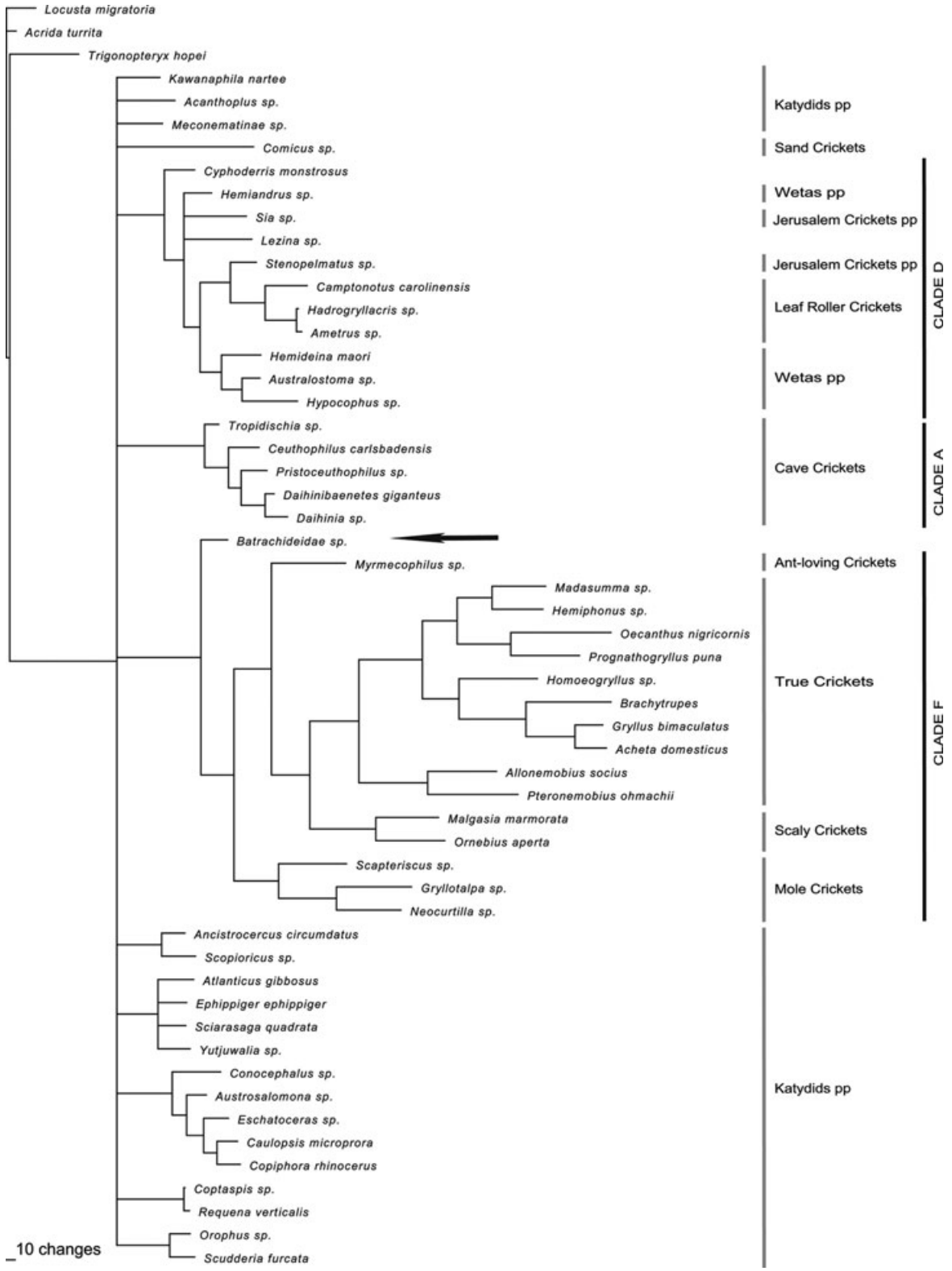

Fig. 1. Strict consensus of 29 most-parsimonious trees obtained from a combined static alignment with gaps treated as missing data (length = 4221, consistency index = 0.40, retention index = 0.48). Clades labelled as in Fig. 3. The arrow points to the caeliferan Batrachideidae sp., which is nested inside the ingroup.

Fig. 2. Majority-rule consensus tree obtained using Bayesian inference after alignment of the combined data. Clades labelled as in Fig. 3. Bayesian posterior probabilities are represented for each node.

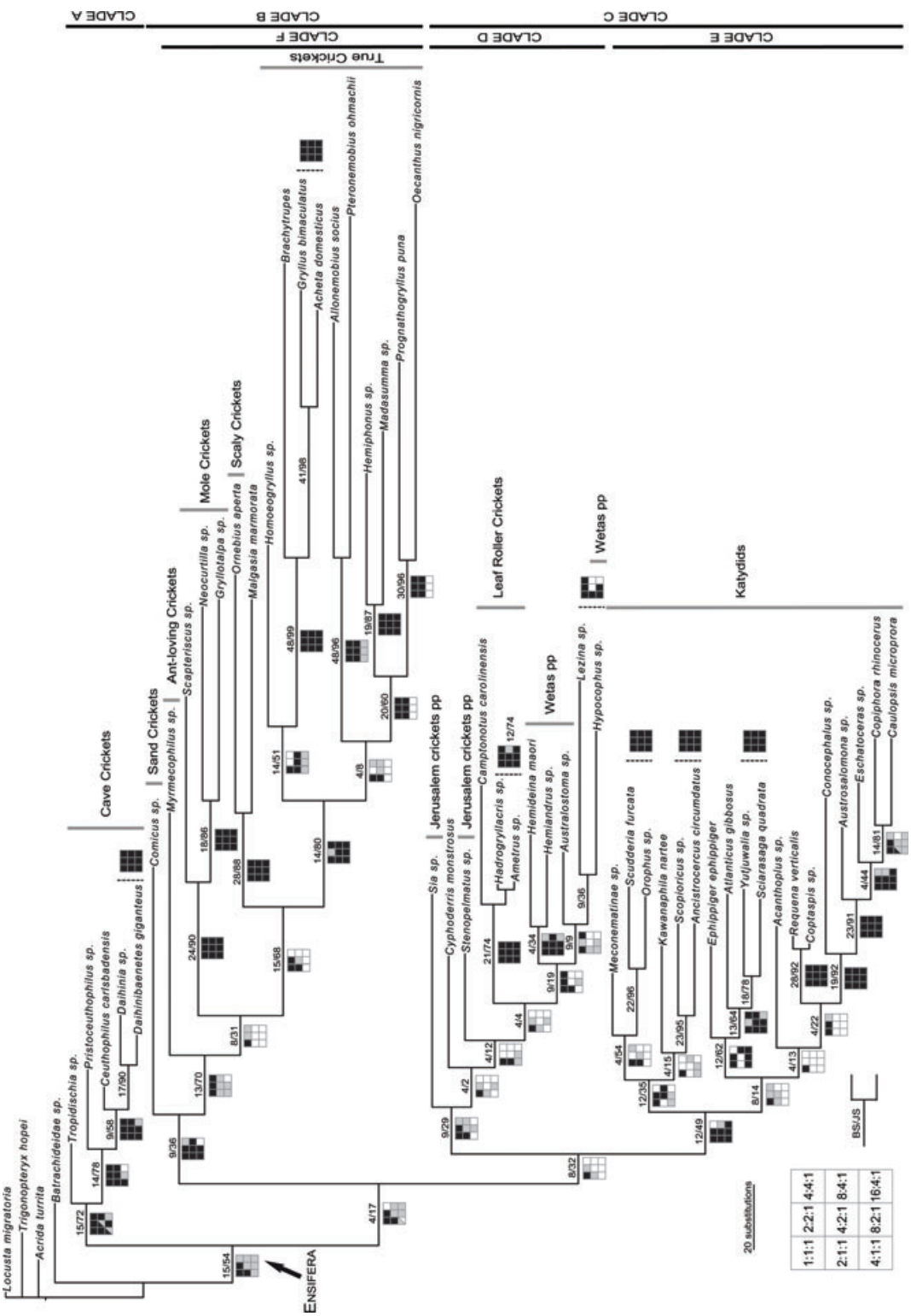

Fig. 3. Most optimal topology found in the direct optimization analysis with the parameter set 1 : 1 : 1 (length = 5558, consistency index = 0.54, retention index = 0.63). The results of the sensitivity analysis are depicted at each node with the following notation: monophyly in black, paraphyly in grey, polyphyly in white. The numbers above the branches are Bremer support/jackknife support. Clade A = cave crickets; clade B = crickets s.l. + sand crickets; clade C = clade D + clade E with clade D = leaf roller crickets + wetas + Jerusalem crickets + *Lezina* sp. + *Cyphoderris* sp. and clade E = katydids; clade F = crickets s.l. This topology is the 'preferred' topology in the present study.

monophylum (BS = 21, JS = 74), but Jerusalem crickets and wetas formed a polyphyletic group. *Lezina* sp. was nested within wetas, whereas *Cyphoderris* was the second diverging taxon in clade D. Finally, we found that clade B had longer branches than the other clades and that its internal nodes had higher BS than those of clades A, D and E (Fig. 3).

Our analysis shows the stability of the relationships inferred by the equally weighted analysis (Fig. 3). Ensifera were paraphyletic under six of nine parameter sets (4 : 1 : 1, 4 : 2 : 1, 4 : 4 : 1, 8 : 2 : 1, 8 : 4 : 1 and 16 : 4 : 1), but were never retrieved as polyphyletic. Clades A, B and E appear very stable: they were monophyletic under most of the parameter sets tested. In contrast, clade C is extremely unstable, being paraphyletic or polyphyletic in all the analyses except with the 1 : 1 : 1 parameters. This instability results from the fluctuating position of the members of clade D – katydid allies. Two different positions were found according to the parameters used: either as the sister group to a clade comprising the members of clades B and E or dispersed as several independent lineages diverging early within a nonmonophyletic Ensifera (see Figure S1). In the latter topology, cave crickets (with or without *Cyphoderris* included) were no longer the first diverging lineage within Ensifera. At a lower scale, mole crickets, true crickets and leaf roller crickets form three very stable monophyletic groups. The position of *Lezina* sp. appears relatively stable, associated with Jerusalem crickets and wetas in eight of nine analyses. As for *Cyphoderris*, it was placed in three very different positions according to the parameters used: as a member of the katydid allies more or less close to Jerusalem crickets (1 : 1 : 1, 2 : 1 : 1, 2 : 2 : 1, 8 : 4 : 1), as the sister group to cave crickets (4 : 1 : 1) or as sister taxon to the remainder of nonmonophyletic Ensifera (4 : 2 : 1, 4 : 4 : 1, 8 : 2 : 1, 16 : 4 : 1). *Malgasia marmorata* is always retrieved as sister taxon to scaly crickets.

Are the clades proposed by Jost & Shaw (2006: fig. 14) and their pattern of relationships supported by our sensitivity analysis?

Jost & Shaw (2006) proposed a phylogenetic hypothesis of Ensifera comprising nine families, the placement of *Lezina* being unsolved. Some of these families were well supported by our sensitivity analyses and were monophyletic in all or most analyses (Fig. 4). This was the case of their Gryllacrididae, Gryllotalpidae, Rhaphidophoridae and Tettigoniidae. On the other hand, their Anostostomatidae (i.e. *Australostoma* sp. + *Hemiandrus* sp. + *Hemideina maori* + *Hypocophus* sp.) was most often paraphyletic or polyphyletic, and their 'Stenopelmatidae' (i.e. *Sia* sp. + *Stenopelmatus* sp.) was always polyphyletic. Their Gryllidae were moderately supported, being found to be monophyletic with only three parameter sets (2 : 1 : 1, 2 : 2 : 1 and 4 : 2 : 1), but found to be polyphyletic with a single parameter set (16 : 4 : 1). Their Haglidae and Schizodactylidae were each represented by only

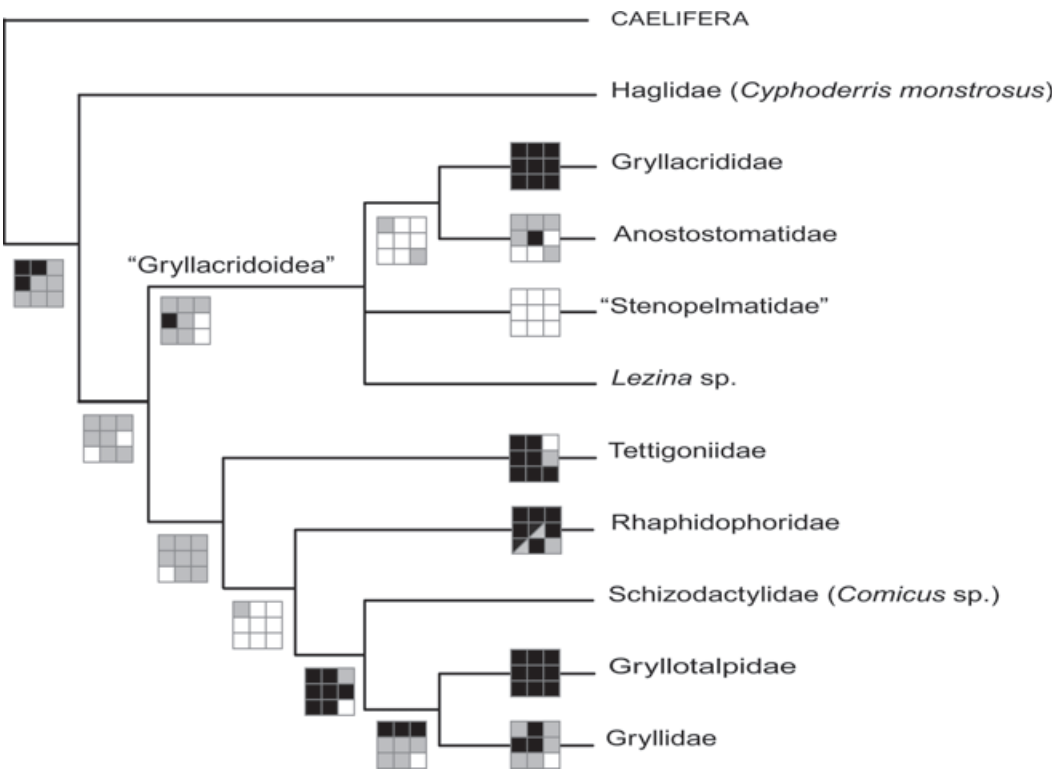

Fig. 4. Classificatory system derived by Jost & Shaw (2006: fig. 14) from their phylogenetic analysis with the support of our phylogenetic analysis to their different clades. The results of the sensitivity analysis are depicted as in Fig. 3.

one taxon (*Cyphoderris* and *Comicus*, respectively), so their monophyly could not be tested.

The interfamilial relationships were very weakly supported by our sensitivity analyses (Fig. 4). All the internal nodes of the Jost & Shaw (2006) classification corresponded to paraphyletic or polyphyletic assemblages, except their clade [Schizodactylidae (Gryllidae + Gryllotalpidae)], which was found to be monophyletic in seven analyses. Ensifera were retrieved as monophyletic in only one-third of the analyses, and the position of *Cyphoderris* as the first diverging lineage within Ensifera was never supported. Their 'Gryllacridoidea' appeared to be monophyletic in only one weighting scheme (2 : 1 : 1, Fig. 4), whereas their clade (Gryllacrididae + Anostostomatidae) was most often polyphyletic. Their clade comprising Tettigoniidae, Rhaphidophoridae, Schizodactylidae, Gryllidae and Gryllotalpidae was never monophyletic. In fact, both the sister relationship of Rhaphidophoridae with [Schizodactylidae (Gryllidae + Gryllotalpidae)] on one hand, and that of Tettigoniidae with {Rhaphidophoridae [Schizodactylidae (Gryllidae + Gryllotalpidae)]} on the other hand were weakly corroborated.

Discussion

Instability in ensiferan relationships

Despite the use of almost similar phylogenetic matrices and regardless of the reconstruction method, we found different topologies than those of Jost & Shaw (2006). In addition, each topology appeared to be extremely sensitive to parameter settings (Figs 3, 4) and this instability was coupled with low support values. For instance, in Jost & Shaw's (2006) strict consensus tree (their fig. 12), 32% of the resolved nodes had bootstrap values lower than 50%. Stability plots (Figs 3, 4) show that the deepest nodes were most affected by instability. Neither the bifurcation documented by Jost & Shaw (2006), with *Cyphoderris* as the sister group to all other Ensifera, nor the relationships between our three main clades (named A, B and C) is robust.

Similarly, the most inclusive groups proposed by previous authors for Ensifera classification are the less stable (Fig. 5). Traditionally, katydids and their allies (i.e. cave crickets, Jerusalem crickets, leaf roller crickets, wetas, *Lezina* sp. and *Cyphoderris* sp.) have been grouped together, as has a large cricket clade (i.e. true crickets, mole crickets, scaly crickets, ant-loving crickets and, according to authors, sand crickets). This cricket clade s.l. is almost always retrieved, whereas the katydid clade s.l. is not, for different reasons. For instance, in our equally weighted analysis, the early divergence of the cave cricket clade explains this nonmonophyly (Fig. 3).

Within katydid allies, none of the classifications proposed up to now, from Ander's to that of Jost & Shaw (2006), are well supported (Fig. 5): the Gryllacridoidea *sensu* Chopard, 1949 is always polyphyletic, whereas a clade comprising leaf roller crickets, *Sia*, *Stenopelmatus*, *Lezina* and wetas (= Stenopelmatoidea *sensu* Eades & Otte, 2008), is usually paraphyletic, being monophyletic only for the parameter setting 2 : 1 : 1. The Tettigoniaemorpha (*sensu* Ander, 1939), which are supported by morphological studies (Gwynne, 1995; Desutter-Grandcolas, 2003), were never monophyletic here. The Mimnermidae of Gorochov (1995), which unites wetas and *Lezina* sp., were monophyletic with four parameter sets (1 : 1 : 1, 2 : 1 : 1, 2 : 2 : 1, 4 : 4 : 1), but a group comprising *Lezina* and leaf roller crickets (Eades & Otte, 2008) was not supported by the sensitivity analysis. Jerusalem crickets, represented here by *Sia* sp. and *Stenopelmatus* sp., were never monophyletic. Wetas (Anostostomatidae *sensu* Johns, 1997) were monophyletic only under the parameter setting 4 : 2 : 1. Finally, true katydids (clade E – Tettigoniidae), which have been separated previously by most authors, were retrieved as monophyletic, except for parameter settings 4 : 4 : 1 and 8 : 4 : 1 (Figs 3, 5).

Thus, mainly subordinate groups seem to be supported unambiguously within katydids s.l. Leaf roller crickets (Gryllacrididae/Gryllacridinae) were monophyletic in all analyses. Similarly, cave crickets, which have been separated as the Raphidophoridae, constitute a very stable and strongly supported entity, although their position within Ensifera is less obvious.

Finally, what is the phylogenetic position of *Lezina* and *Cyphoderris*? That of *Lezina* appears rather stable, as it clustered with wetas in 11 optimal topologies. On the contrary, *Cyphoderris*' position varied with parameter setting. It is either sister taxon to cave crickets, or close to some wetas, or sister taxon to a paraphyletic assemblage of all other Ensifera. Thus, the phylogenetic position of *Cyphoderris* is highly unstable and its previously proposed position as sister taxon to all the other Ensifera should be considered with caution.

In the cricket clade s.l., the phylogeny seems more stable, although some deep nodes are unstable. First, our study supports a monophyletic assemblage comprising sand crickets (here *Comicus* only), mole crickets, ant-loving crickets, scaly crickets (+*Malgasia*) and true crickets (Fig. 3: clade B). Second, in both Jost & Shaw's (2006) topology and our 1 : 1 : 1 hypothesis, *Comicus* was the sister taxon to crickets s.l. (our clade F), and in nine of 12 optimal topologies in our sensitivity analysis, *Comicus* was the sister taxon to mole crickets. This position of *Comicus* prevents the recovering of a 'katydid clade s.l.', even though a true katydid clade and a partial katydid allies clade (i.e. leaf roller crickets, Jerusalem crickets, wetas and *Lezina*) are usually retrieved. Third, some subordinate groups, such as mole crickets (Gryllotalpidae), 'true crickets' or (scaly crickets + *Malgasia*) were confirmed as monophyletic in at least eight of nine parameter combinations with high support values (Figs 3, 5).

To sum up, most less inclusive groups had high support values and were robust to variation in parameter settings and/or methods, except those concerning wetas and Jerusalem crickets. Actually, most were already well supported by morphological and behavioural characters. Only cave crickets, which have never been clearly supported by any morphological synapomorphy (Desutter-Grandcolas, 2003), formed a stable monophyletic group. It should be noted, however, that four

Fig. 5. Support of our phylogenetic analysis to the ensiferan classifications proposed by Ander (1939), Chopard (1949), Gorochov (1995), Gwynne (1995), Desutter-Grandcolas (2003) and Eades & Otte (2008). Abbreviations: T, taxonomy; pP, prephylogenetic system; P, phylogenetic hypothesis. The taxa in the left column represent our ingroup. The taxa in parentheses could not be tested for monophyly. The results of the sensitivity analysis are depicted as in Fig. 3.

of the five cave crickets sampled seemed to be very close taxonomically, which may weaken the test of cave cricket monophyly. By contrast, relationships above family level are ill-defined and the present dataset is not informative at this level.

Therefore, it seems that the phylogeny of Ensifera is still largely unresolved, and that no stable classification can be proposed for this group yet. Our analyses clearly show how unstable the phylogenetic relationships within Ensifera (Fig. 3) are with the dataset at hand.

What information do we get from clade support and sensitivity analyses?

To increase the sampling of any phylogenetic study sounds like a trivial recommendation, but it is especially important for Ensifera, provided that it is done in a comprehensive way. Some taxa and characters should be targeted for future studies in this field and a detailed analysis of clade stability and support should help in this respect.

The situation seems different for the five main clades presented in our 1 : 1 : 1 topology (Fig. 3). The monophyly of clade A, and to a lesser extent of clade E, was rather stable to parameter variations, and well supported. By contrast, the monophyly of clade B was stable, but weakly supported, which could mean that this clade is defined by few but uncontradicted characters (Giribet, 2003). Given that JS were calculated by removing dynamic characters (see section 'Phylogenetic analyses'), this report suggests that only one or two of the ten portions of markers used hold these characters. A detailed analysis of the PBS showed that this fragment is 16S4 (data not shown). Finally, the monophyly of clades C and D was weakly supported and highly variable when parameter settings were altered.

Within clade B, the relationships were generally supported better than those within clades A, C, D and E. This clade also had longer branches than the others and PBS showed that 18S was mainly responsible for the internal resolution of this clade. The statistics reported in Table 2 show that fragment 18Sa4 has high length variability. A closer look at the sequences revealed that this fragment is longer for the species belonging to true crickets (members of clade B) than for species of other groups, which means that more characters are available to resolve this clade. Branch lengths and BS were consequently higher in this clade than in the others. Nevertheless, such differences in branch lengths across the whole tree could affect the pattern of relationships. For instance, long-branch attraction artefact could explain the instability of the deepest nodes in clade B (Giribet, 2003). Long branches could be broken up by the inclusion of new data, and taxa from nonsampled cricket families or subfamilies could be very useful in this context. Conversely, branches in clades C, D and E were short. This implies a lack of informative data, a problem that can only be solved with the addition of data.

As shown in Fig. 3, most of the deepest nodes were weakly supported and very unstable, a common pattern in many higher-level insect phylogenies (see Whitfield & Kjer, 2008). Two main reasons, not mutually exclusive, could explain such short branch lengths. Given the character sampling, the most probable hypothesis is that the markers used did not provide enough information for this level of relationship, even though an evolutionary radiation hypothesis cannot be eluded either. So, new markers are needed and not only molecular characters, but also morphological, behavioural and ecological data. We expect that such an integrative approach (Dayrat, 2005; Will *et al*., 2005) will result in a more stable and robust hypothesis, as in a study of Blaberidae (Blattodea) relationships, where molecular analyses (Maekawa *et al*., 2003; Inward *et al*., 2007; Pellens *et al*., 2007) brought less stable hypotheses than an analysis combining morphological, molecular and behavioural data (Legendre, 2007).

Conclusion

Most low-scale clades, such as mole crickets, leaf roller crickets, scaly crickets + *Malgasia*, true crickets, katydids and cave crickets, are quite resilient to parameter variation. Whatever their taxonomic levels, these groups seemed to form stable monophyletic clades, even though the present taxonomic sampling was neither optimal to test cave cricket monophyly nor the relationships within crickets s.l. Wetas and Jerusalem crickets [Jost & Shaw's, (2006) Stenopelmatidae and Anostostomatidae, respectively] are rarely recovered as separate monophyletic clades, a result contradicting recent advances in stenopelmatid classification (Johns, 1997). Instead, they are most often gathered with *Lezina* and leaf roller crickets in a clade not only corresponding partly to the classical 'katydid allies' (Hubbell, 1978), but also invalidating the Stenopelmatoidea *sensu* Gorochov (2001).

At higher taxonomic levels, the situation is no better with Jost & Shaw's (2006) Gryllacridoidea, i.e. Stenopelmatoidea *sensu* Eades & Otte (2008), retrieved as monophyletic only once in our sensitivity analysis. Other superfamilies that can be inferred from Jost & Shaw (2006: fig. 14) and Eades & Otte's (2008) online catalogue do not provide any supplementary information about Ensifera classification as, with the exception of Grylloidea, they are all monofamilial: Hagloidea for the haglid *Cyphoderris*, Raphidophoridoidea for cave crickets, Schizodactyloidea for *Comicus*, Tettigonioidea for katydids. This illustrates the lack of phylogenetic evidence for deeper relationships in Ensifera.

Overall, Ensifera phylogenetics is still largely unresolved and at present no stable high-level classification can be proposed for this group.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: DOI: 10.1111/j.1365-3113.2009.00519.x

Figure S1. Most-parsimonious trees obtained under sensitivity analysis. Parameter sets and tree costs appear at the top of each tree.

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References

Ander, K. (1939) *Vergleichend anatomische und phylogenetische Studien über die Ensifera (Saltatoria)*. Berlingska Boktryckeriet, Lund.

Baker, R.H. & DeSalle, R. (1997) Multiple sources of character information and the phylogeny of Hawaiian *Drosophila*. *Systematic Biology*, **46**, 654–673.

Beier, M. (1972) *IV. Band: Arthropoda – 2. Hälfte: Insecta (Zweite Auflage). 2. Teil: Spezielles. 9. Saltatoria (Grillen und Heuschrecken)*. Walter de Gruyter, Berlin.

Bremer, K. (1994) Branch support and tree stability. *Cladistics*, **10**, 295–304.

Brettschneider, H. (2006) *Systematics of Southern African Anostostomatidae (Orthoptera) Based on Morphological and Molecular Data*. Faculty of Natural and Agricultural Sciences. University of Pretoria, Pretoria.

Carpenter, J.M. (2003) Critique of pure folly. *Botanical Review*, **69**, 79–92.

Chopard, L. (1920) *Recherches sur la conformation et le développement des derniers segments abdominaux chez les Orthoptères*. Thèse de la Faculté des Sciences de Paris. Imprimerie Oberthur, Rennes.

Chopard, L. (1938) *La Biologie des Orthoptères*. Paul Lechevalier, Paris.

Chopard, L. (1949) Ordre des orthoptères. *Traité de Zoologie, IX* (ed. by P. P. Grassé), pp. 617–722. Masson, Paris.

Chopard, L. (1967) *Pars 10. Fam. Gryllidae: Subfam. Gryllinae*. Dr. W. Junk, Gravenhage.

Chopard, L. (1969) *The Fauna of India and Adjacent Countries. Orthoptera. Volume 2. Grylloidea*. Baptist Mission Press, Calcutta.

Dayrat, B. (2005) Towards integrative taxonomy. *Biological Journal of the Linnean Society*, **85**, 407–415.

Desutter, L. (1987) Structure et évolution du complexe phallique des Gryllidea (Orthoptera) et classification des genres néotropicaux de Grylloidea.1ère partie. *Annales de la Société Entomologique de France (N.S.)*, **23**, 213–239.

Desutter-Grandcolas, L. (2003) Phylogeny and the evolution of acoustic communication in extant Ensifera (Insecta, Orthoptera). *Zoologica Scripta*, **32**, 525–561.

Dominguez, E. & Wheeler, Q.D. (1997) Taxonomic stability is ignorance. *Cladistics*, **13**, 367–372.

Dubois, A. (2007) Naming taxa from cladograms: some confusions, misleading statements, and necessary clarifications. *Cladistics*, **23**, 390–402.

Eades, D.C. & Otte, D. (2008) *Orthoptera Species File Online*. Version 2.0/3.4. [WWW document]. URL http://orthoptera.speciesfile.org/.

Edgar, R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, **32**, 1792–1797.

Eldredge, N. & Cracraft, J. (1980) *Phylogenetic Patterns and the Evolutionary Process, Method and Theory in Comparative Biology*. Columbia University Press, New York, New York.

Farris, J.S., Albert, V.A., Källersjö, M., Lipscomb, D. & Kluge, A.G. (1996) Parsimony jackknifing outperforms neighbor-joining. *Cladistics*, **12**, 99–124.

Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, **39**, 783–791.

Flook, P.K. & Rowell, C.H.F. (1997) The phylogeny of the Caelifera (Insecta, Orthoptera) as deduced from mtDNA gene sequences. *Molecular Phylogenetics and Evolution*, **8**, 89–103.

Flook, P.K., Klee, S. & Rowell, C.H.F. (1999) Combined molecular phylogenetic analysis of the Orthoptera (Arthropoda, Insecta) and implications for their higher systematics. *Systematic Biology*, **48**, 233–253.

Giribet, G. (2003) Stability in phylogenetics formulations and its relationship to nodal support. *Systematic Biology*, **52**, 554–564.

Giribet, G. & Wheeler, W.C. (1999) On gaps. *Molecular Phylogenetics and Evolution*, **13**, 132–143.

Giribet, G. & Wheeler, W.C. (2001) Some unusual small-subunit ribosomal RNA sequences of metazoans. *American Museum Novitates*, **3337**, 1–14.

Goloboff, P.A. (1999) Analysing large data sets in reasonable times: solutions for composite optima. *Cladistics*, **15**, 415–428.

Gorochov, A.V. (1986) System and morphological evolution of crickets from the family Gryllidae (Orthoptera) with description of new taxa. Communications 1, 2. *Zoologicheskij Zhurnal*, **65**, 516–527, 851–858.

Gorochov, A.V. (1995) Contribution to the system and evolution of the order Orthoptera. *Zoologicheskij Zhurnal*, **74**, 39–45 [in Russian].

Gorochov, A.V. (2001) The higher classification, phylogeny and evolution of the superfamily Stenopelmatoidea. *The Biology of Wetas, King Crickets and their Allies* (ed. by L. H. Field), pp. 3–33. CAB International, New York, New York.

Grandcolas, P. & D'Haese, C. (2001) The phylogeny of cockroach families: is the current molecular hypothesis robust? *Cladistics*, **17**, 48–55.

Greenfield, M.D. (2002) *Signalers and Receivers. Mechanisms and Evolution of Arthropod Communication*. Oxford University Press, New York, New York.

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*, **4**, 203–218.

Gwynne, D.T. & Morris, G.K. (1983) *Orthopteran Mating Systems*. Westview Press, Boulder, Colorado.

Hennig, W. (1981) *Insect Phylogeny*. John Wiley & Sons, New York, New York.

Huang, Y., Orti, G., Sutherlin, M., Duhachek, A. & Zera, A. (2000) Phylogenetic relationships of North American field crickets inferred from mitochondrial DNA data. *Molecular Phylogenetics and Evolution*, **17**, 48–57.

Hubbell, T.H. (1978) The classification of "Gryllacrididae". *Metaleptea*, **1**, 1–7.

Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogeny. *Bioinformatics*, **17**, 754–755.

Inward, D., Beccaloni, G. & Eggleton, P. (2007) Death of an order: a comprehensive molecular phylogenetic study confirms that termites are eusocial cockroaches. *Biology Letters*, **3**, 331–335.

Johns, P.M. (1997) The Gondwanaland weta: family Anostostomatidae (formerly in Stenopelmatidae, Henicidae or Mimnermidae): nomenclatural problems, world checklist, new genera and species. *Journal of Orthoptera Research*, **6**, 125–138.

Jost, M.C. (2002) *Phylogeny of Crickets (Orthoptera: Ensifera: Gryllidae) with Comparative Studies on the Evolution of Acoustic Communication*. Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts.

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.

Judd, W.W. (1948) A comparative study of the proventriculus of orthopteroid insects with reference to its use in taxonomy. *Canadian Journal of Research*, **26**, 93–161.

Kevan, D.K.McE. (1977) The higher classification of the orthopteroid insects. *Memoir – Lyman Entomological Museum and Research Laboratory*, **4**, 1–27.

Kevan, D.K.McE. (1982) Orthoptera. *Synopsis and Classification of Living Organisms* (ed. by S. P. Parker), pp. 352–379. McGraw Hill, New York, New York.

Kristensen, N.P. (1981) Phylogeny of insect orders. *Annual Review of Entomology*, **26**, 135–157.

Kristensen, N.P. (1995) Forty years' insect phylogenetics. *Zoologische Beiträge N.F.*, **36**, 83–124.

Legendre, F. (2004) *Etude phylogénétique et biogéographique du genre Cryptocercus Scudder, 1862 (Dictyoptera : Blattaria)*. Masters thesis, Université Pierre et Marie Curie - Paris VI, Paris.

Legendre, F. (2007) *Phylogénie et comportement du comportement social chez les blattes et les termites*. PhD thesis, Université Pierre et Marie Curie - Paris VI, Paris.

Maekawa, K., Lo, N., Rose, H. & Matsumoto, T. (2003) The evolution of soil-burrowing cockroaches (Blattaria: Blaberidae) from wood-burrowing ancestors following an invasion of the latter from Asia into Australia. *Proceedings of the Royal Society of London, Series B*, **270**, 1301–1307.

Mishler, B.D. (2005) The logic of the data matrix in phylogenetic analysis. *Parsimony, Phylogeny and Genomics* (ed. by V. A. Albert), pp. 57–70. Oxford University Press, Oxford.

Morrison, D.A. & Ellis, J.T. (1997) Effects of nucleotide sequence alignment on phylogeny estimation: a case study of 18S rDNAs of Apicomplexa. *Molecular Biology and Evolution*, **14**, 428–441.

Nixon, K.C. (1999a) *WinClada ver. 1.0000*. Published by the author, Ithaca, New York.

Nixon, K.C. (1999b) The parsimony ratchet, a new method for rapid parsimony analysis. *Cladistics*, **15**, 407–414.

Pellens, R., D'Haese, C., Bellés, X., Piulachs, M.D., Legendre, F., Wheeler, W.C. & Grandcolas, P. (2007) The evolutionary transition from subsocial to eusocial behaviour in Dictyoptera: phylogenetic evidence for modification of the "shift-in-dependent-care" hypothesis with a new subsocial cockroach. *Molecular Phylogenetics and Evolution*, **43**, 616–626.

Pratt, R.C., Morgan-Richards, M. & Trewick, S.A. (2008) Diversification of New Zealand weta (Orthoptera: Ensifera: Anostostomatidae) and their relationships in Australasia. *Philosophical Transactions of the Royal Society of London, Series B*, **363**, 3427–3437.

Ragge, D.R. (1955) *The Wing Venation of the Orthoptera Saltatoria, with Notes on Dictyopteran Wing-venation*. British Museum (Natural History), London.

Rentz, D.C.F. (1980) A new family of ensiferous Orthoptera from the coastal sands of southeast Queensland. *Memoirs of the Queensland Museum*, **20**, 49–63.

Rentz, D.C.F. (1991) Orthoptera. *Insects of Australia. A Textbook for Students and Research Workers*, 2nd edn (ed. by I. D. Nauman, P. B. Carne, J. F. Lawrence, E. S. Nielsen, P. Spradbery, R. W. Taylor, M. J. Whitten and M. J. Littlejohn), pp. 369–393. Melbourne University Press, Carlton.

Robillard, T. & Desutter-Grandcolas, L. (2006) Phylogeny of the cricket subfamily Eneopterinae (Orthoptera, Grylloidea, Eneopteridae) based on four molecular loci and morphology. *Molecular Phylogenetics and Evolution*, **40**, 643–661.

Scudder, S.H. (1897) *Guide to the Genera and Classification of the North American Orthoptera*. Edward W. Wheeler, Cambridge.

Shaw, K.L. (1996) Sequential radiations and patterns of speciation in the Hawaiian cricket genus *Laupala* inferred from DNA sequences. *Evolution*, **50**, 237–255.

Sorenson, M.D. (1999) *TreeRot, Version 2*. Boston University, Boston, Massachusetts.

Swofford, D.L. (1998) *PAUP* – Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Sinauer Associates, Sunderland, Massachusetts.

Uhler, P.R. (1864) Orthopterological contributions. *Proceedings of the Entomological Society of Washington*, **2**, 543–555.

Vickery, V.R. (1977) Taxon ranking in Grylloidea and Gryllotalpoidea. *Memoir – Lyman Entomological Museum and Research Laboratory*, **4**, 32–43.

Walker, F. (1869) *Catalogue of the Specimens of Dermaptera Saltatoria, and Supplement to the Blattariae, in the Collections of the British Museum*. British Museum (Natural History), London.

Walker, F. (1871) *Catalogue of the Specimens of Dermaptera Saltatoria in the Collections of the British Museum. Part V, Supplement to the Catalogue of Dermaptera Saltatoria*. British Museum (Natural History), London.

Wheeler, W.C. (1995) Sequence alignment, parameter sensitivity, and the phylogenetic analysis of molecular data. *Systematic Biology*, **44**, 321–331.

Wheeler, W. (1996) Optimization alignment: the end of multiple sequence alignments in phylogenetics? *Cladistics*, **12**, 1–9.

Wheeler, W.C., Aagesen, L., Arango, C.P. *et al*. (2006) *Dynamic Homology and Phylogenetic Systematics: a Unified Approach using POY*. American Museum of Natural History, New York, New York.

Whitfield, J.B. & Kjer, K.M. (2008) Ancient rapid radiations of insects: challenges for phylogenetic analysis. *Annual Review of Entomology*, **53**, 449–472.

Will, K.W., Mishler, B.D. & Wheeler, Q.D. (2005) The perils of DNA barcoding and the need for integrative taxonomy. *Systematic Biology*, **54**, 844–851.

Zeuner, F.E. (1939) *Fossil Orthoptera Ensifera*. British Museum (Natural History), London.

Zwickl, D.J. & Hillis, D.M. (2002) Increased taxon sampling greatly reduces phylogenetic error. *Systematic Biology*, **51**, 588–598.

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