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A note on the molecular phylogeny of a small sample of Catantopine grasshoppers

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Abstract

Somewhat surprisingly, the rather speciose subfamily Catantopinae has largely evaded phylogenetic scrutiny. This note describes relationships among a small, but larger than that heretofore studied, subset of members based on an analysis of mitochondrial sequence characters.

Analysis of portions of 4 mitochondrial genes, some sequenced in this laboratory and some obtained from GenBank, was applied to 12 specimens of Catantopinae. Also included were representatives of the subfamilies Calliptaminae, Cyrtacanthacridinae, Eyprepocnemidinae, Melanoplinae and Oxyinae. *Pyrgomorpha conica* served as the outgroup. Sequences were analyzed by weighted and unweighted maximum parsimony and Bayesian methods.

Both weighted parsimony and Bayesian approaches, with some minor exceptions, yielded similar relationships. Although some clusters of catantopines do occur, molecular results appear to confirm earlier suspicions that the subfamily is not monophyletic and therefore should be subdivided. More work clearly needs to be devoted to the Catantopinae in order to determine the nature and extent of the subfamily's inevitable sundering.

Key words

Acrididae, Catantopinae, phylogeny, mitochondrial DNA

Introduction

As with many subfamilies that comprise the Acrididae, the Catantopinae have had a rather inconstant taxonomic past. Questions regarding membership, their nearest relations, and monophyly are far from settled. Some orthopterists (e.g., Bellman & Luquet 1995; Huo *et al.* 2007; Li *et al.* 2011) continue to adhere to Dirsh's (1975) system of clumping together and assigning subfamilies Calliptaminae, Catantopinae, Cyrtacanthacridinae, Eyprepocnemidinae and Oxyinae, among others, to the family "Catantopidae". This construct was adumbrated earlier by Mishchenko (1952) who divided the assemblage into more or less the same subsets, tribes in this case. Many of the latter are now regarded as *bona fide* subfamilies of Acrididae as documented in the current Orthoptera Species File (OSF – Eades *et al.* 2013). Various components of Catantopinae's contorted history are described by Vickery and Kevin (1985), Song and Wenzel (2008), and Li *et al.* (2011). The latter correctly points out that questions of relationship and monophyly cannot suitably be addressed without a proper phylogeny. The only extensive morphology-based phylogeny of Catantopinae appeared just recently (Li *et al.* 2011), but, as will be discussed later, findings are not entirely clear. Previously, small numbers of Catantopinae had been included in a few morphological studies in which the primary goals focused on the evolution of specific traits rather than on phylogeny (Eades 2000; Song & Wenzel 2008).

Analysis of DNA sequences has not been particularly illuminating either. Given the large number of catantopines worldwide (Otte 1995), there have been surprisingly few molecular phylogenetic studies of the subfamily. Within the past 6 years, about 10 reports have appeared which examine relationships among the aforementioned subfamilies, including Catantopinae. A major shortcoming of these studies is that only 3 or fewer catantopine genera were included. In fact, in some cases (e.g., Zhao *et al.* 2011), genera labelled as Catantopinae are instead members of the Melanoplinae. Sun *et al.* (2010) did publish the complete genome of a supposed catantopine, *Primnoa*, but it too turns out to be a melanopline, according to the OSF.

The aim of this note is to revisit questions of monophyly and relatedness among a somewhat greater sampling of the Catantopinae (*sensu* OSF) than that previously undertaken. From the outset it should be stated that by no means is this study exhaustive or to be viewed as definitive, given the relatively small number of taxa and tribes sampled. Nevertheless, it does represent one of the first studies that focuses primarily on the phylogenetics of the subfamily itself (*sensu* OSF).

Materials and methods

Species, along with sources, geographical ranges and subfamilial affiliations (*sensu* OSF) are listed in Table 1. Of the subfamily's 18 tribes, 3 are represented here; one genus, *Apalacris*, is unassigned. Three genera are restricted to Australia; the rest are more widely distributed in the Old World. Representatives of Calliptaminae, Cyrtacanthacridinae, Eyprepocnemidinae, Melanoplinae and Oxyinae are also included. *Pyrgomorpha* served as the outgroup.

This investigation was based on sequences obtained from specimens sent to this laboratory by colleagues; other sequences (those of the first 6 taxa and *Oxya*), were obtained from GenBank. DNA samples analyzed in this laboratory were extracted from specimens using a QIAGEN DNeasy tissue kit (Mississauga, Canada). Portions of the mitochondrial genes encoding cytochrome oxidase subunits I (CO1) and II (CO2), cytochrome b (cytb) and NADH dehydrogenase subunit V (ND5) were amplified and sequenced. Primer sequences, PCR gene amplification conditions, as well as DNA sequencing methods, are described elsewhere (Litzenberger & Chapco 2001a, 2001b; Contreras & Chapco 2006).

Sequences were easily aligned by visual inspection, transferred into MacClade (Maddison & Maddison 2004) and analyzed using the software packages PAUP* (version 4.0b8 – Swofford 2003) and MrBayes (Version 3.2 – Ronquist *et al.* 2011). Both standard maximum parsimony (MP) and weighted parsimony (wMP), as described by Farris (1969), were conducted. Searches were repeated using all

Table 1. Species analyzed, locations and GenBank Accession numbers of mtDNA sequences.

Subfamily/Tribe	Species	Source	Accession Numbers CO1, CO2, CYTB, ND5
Catantopinae			
/Unassigned	<i>Apalacris varicornis</i>	Loc?, China ^{CE}	EU3660777, FJ554811, DQ366762, -
/Catantopini	<i>Ecphantacris mirabilis</i>	Loc?, China ^C	FJ571157, FJ554819, -, -
	<i>Xenocatantops humilis</i>	Loc?, China ^{CDEF}	EU366111, FJ554817, EU366112, -
	<i>Catantops sp.</i>	Loc?, China ^{DEF}	EU366109, -, EU366082, -
	<i>Diabolocantantops pinguis</i>	Loc?, China ^{CDE}	FJ571154, FJ554815, -, -
	<i>Stenocatantops spendens</i>	Loc?, China ^{CEF}	FJ571155, FJ554816, -, -
	<i>Coryphistes ruricola</i>	Savannah, QLD, AU ^F	-, JX033918, JX033925, JX033937
	<i>Goniaea vocans</i>	Silver Valley Rd, QLD, AU ^F	JX033911, JX033919, JX033926, JX033938
	<i>Theomolpus pulcher</i>	Moomin, QLD, AU ^F	JX033914, *, JX033929, JX033941
/Pezotettigini	<i>Pezotettix giornae</i>	Sierra Nevada, Spain ^{CD}	JX033912, JX033920, JX033927, JX033939
	<i>Sphenophyma rugulosa</i>	Malatya, Turkey ^C	JX033913, JX033921, JX033928, JX033940
/Trauliini	<i>Traulia minuta</i>	Loc?, China ^{CE}	FJ571149, DQ099597, DQ366763, -
Calliptaminae			
/Calliptamini	<i>Calliptamus barbarus</i>	Sierra Nevada, Spain ^{CD}	JX033916, JX033923, JX033932, JX033944
Cyrtacanthacridinae			
/Cyrtacanthacridini	<i>Anacridium aegyptium</i>	Sierra Nevada, Spain ^{CDE}	JX033915, JX033922, JX033930, JX033942
	<i>Schistocerca gregaria</i>	SFU ^{ABCD}	AF260532, M83966, JX033931, JX033943
Eyprepocnemidinae			
/Eyprepocnemidini	<i>Eyprepocnemis plorans</i>	Nerja, Spain ^{CDE}	JX033917, JX033924, JX033936, JX033947
Melanoplinae			
/Melanoplini	<i>Melanopus sanguinipes</i>	Regina, SK, Can ^A	AF260533, AF145500, JX033933, JX033945
/Podismini	<i>Miramella alpina</i>	Bavaria, Germany ^C	AF260543, AF227292, JX033935, JX033946
/Dichroplini	<i>Scotussa lemniscata</i>	Benito Juarez, Argentina ^B	DQ389229, DQ389215, JX033934, -
Oxyinae			
/Oxyini	<i>Oxya chinensis</i>	Loc?, China ^{CDEF}	EF437157
Outgroup	<i>Pyrgomorpha conica</i>	Malatya, Turkey ^{ABCDE}	EU031776-9

Loc? =location unknown; dashes signify no sequence obtained or available; * - see Appendix; Biogeographical Regions: A = Nearctic, B = Neotropics, C = Palearctic, D = Africa, E = Oriental, F = Australia; QLD, AU = Queensland, Australia; SK, Can = Saskatchewan, Canada; SFU = Simon Fraser University. The first 6 sequences and that of *Oxya* were obtained from GenBank

substitutions at the first 2 codon positions but only transversional substitutions at the third position (methods referred to as MP123TV and wMP123TV, respectively). All (heuristic) parsimony searches employed the TBR branch-swapping option with 10 random addition replicates. Bayesian analysis applied default settings with respect to prior distributions, number of chains, start trees, etc. Eight Monte Carlo Markov chains, 1 cold and 7 heated, were run simultaneously for 50 million generations when convergence had been attained. Trees were sampled every 500 generations; consensus of the last 10000 trees was calculated. Data were partitioned according to codon position and gene; each partition was allowed to follow its own GTR + G + I evolutionary model. Levels of support for parsimony-derived relationships were estimated through 1000 bootstrap replicates. Maximum likelihood procedures were not performed owing to the excessive run-times that would be required for bootstrapping. For Bayesian analyses, measures of nodal support appeared in the form of posterior probabilities (PP). For all analyses, the 4 sequences were treated as a combined unit, a procedure that, as in our previous studies, always yielded trees with greater resolution and support when compared to those based on single genes.

Results

Sequences analyzed here are devoid of inserts and/or deletions and are indeed believed to be mitochondrial and not nuclear pseu-

dogene inserts. Moreover, the sequences adhere to other criteria set out previously by Zhang and Hewitt (1996) for ensuring that they are mitochondrial.

Of the different parsimony searches, wMP123TV yielded the tree with the greatest resolution, presented as a bootstrap consensus tree in Fig. 1. In general, very similar relationships were uncovered by the Bayesian method (Fig. 2). Members of Catantopinae, singly or in groups, are located in 5 areas of both trees. At the tree's apex, 6 catantopines form a clade, 5 belonging to the tribe Catantopini. In a second location, *Pezotettix* (tribe Pezotettigini) is tightly linked to a member of the subfamily Eyprepocnemidinae; together they are basal to the above sextet. Another group of catantopines, external to the aforementioned, consists of the Australian trio: *Coryphistes*, *Goniaea*, *Theomolpus*. Although the cluster has excellent support, their inter-associations differ between the 2 trees. The phylogenetic position of *Traulia* (tribe Trauliini) is unclear. In the parsimony tree, the genus has a moderately strong association with *Calliptamus*, whereas in the Bayesian tree it is as part of a polytomy at the crown end. *Sphenophyma* is the first catantopine to diverge, occupying a position at the tree's base. It belongs to the tribe Pezotettigini but is distant from the latter's only other member, *Pezotettix*.

Among the other subfamilies, members of Oxinae and Melanoplinae are next to diverge after *Sphenophyma*. The *Calliptamus* branch follows, but as stated above, it is linked with *Traulia* in the parsimony tree; in the Bayesian topology it appears alone. With respect to the 2 members of the Cyrtacanthacridinae, parsimony analysis places the

pair at the base of the crown sextet plus *Platyphyma-Eyprepocnemus* and inside the Australian group, albeit with weak support. In the Bayesian tree, the pair is part of a large polytomy as seen in Fig 2.

Discussion

Clearly, the subfamily Catantopinae (and the 2 tribes Catantopini and Pezotettigini for that matter) cannot be regarded as monophyletic. Historically, Catantopinae have been variously labelled as "le groupe artificiel" (Amédégno 1974), a "heterogeneous group" (Song & Wenzel 2008; see also Dirsh 1975) and – owing to its variability – a subfamily that "should be subdivided" (Vickery 1997). Lack of monophyly had already been inferred by Ma *et al.* (2006), Wang *et al.* (2008) and Liu *et al.* (2008); however, fewer taxa and shorter DNA sequences were analyzed and branch support values on the whole were low. Curiously, Li *et al.* (2011) in their morphological study claimed that 4 of the 7 subfamilies of Catantopidae are monophyletic, including Catantopinae. Yet this is clearly not the case, which even a cursory examination of their trees should reveal. *Catantops*, *Stenocatantops* and *Xenocatantops* do indeed cluster together as they do here, but other catantopines in their study, such as *Pachyacris*, *Ecphantacris*, *Fer* and *Bannacris*, are sprinkled throughout their topologies.

Catantopinae can be added to a seemingly growing list of other subfamilies recently deemed to be non-monophyletic: Acridinae, Gomphocerinae and Oedipodinae (Chapco & Contreras 2011), and perhaps Melanoplineae (Chintauan-Marquier *et al.* 2011) but not, apparently, Cyrtacanthacridinae (Song & Wenzel 2008) or Proctolabinae (Rowell & Flook 2004). As expressed by Key and Colless (1993) [see also Amédégno 1993; Rowell 2005], many traits commonly used for taxonomic purposes are particularly subject to evolutionary convergence (*e.g.*, by "tracking" environmental changes) and may, for some reason, be more common in orthopteran insects than previously acknowledged.

It is difficult to compare the present findings with those in the literature owing to the spotty occurrence of catantopines in those works. A few points of interest warrant mention however.

The crown clade.—The linking of *Catantops* and *Xenocatantops* mirrors the morphological results of Li *et al.* (2011) and molecular findings of Wang *et al.* (2008). With respect to the tribally unassigned *Apalacris*, both studies position the genus far from the aforementioned pair (see also Liu *et al.* 2008), albeit with low bootstrap support. Mishchenko (1952) placed *Apalacris* within the tribe Coptacrinini, which has since been elevated to a subfamily of Acrididae (Coptacridinae). There may indeed be some support for an association with the latter if the morphology-based phylogeny derived by Li *et al.* (2011) is upheld by comprehensive molecular studies in the future. [It should be noted that the analysis of a short segment of the CO2 gene by Ma and Huang (2006) also suggested an association with the Coptacridinae]. It will, therefore, be interesting to see if including Coptacridinae sequences will alter the current placement of *Apalacris*.

Traulia.—The obvious lack of affinity between *Traulia* and other members of the Catantopinae, appears to agree with the findings of Eades (2005) and Liu *et al.* (2008) who, respectively, examined orthopteran phallic anatomy and mitochondrial ribosomal sequences. The genitalic anatomy of *Traulia* is shown to be quite different from that of other catantopines; instead, structures appear to resemble those of the Oedipodinae to which Eades contemplates making a

possible taxonomic shift. Liu *et al.* (2008) also distanced *Traulia* from other catantopines, but unlike Eades, discovered that it was far removed from the oedipodines (see also supporting molecular evidence by Zhao *et al.* 2011). In any case, it is presently uncertain where *Traulia* belongs.

Pezotettix and Sphenophyma.—Both belong to the tribe Pezotettigini, which evidently is not monophyletic. There is very little mention of these genera in the molecular literature apart from the use of the *Pezotettix* as an outgroup in 2 studies (Hochkirch & Görzig 2009; Chintauan-Marquier *et al.* 2011).

Sphenophyma is currently geographically restricted to Asia Minor (Fishelson 1985) and is the first genus to diverge from the common ancestor of the entire body of taxa studied. This region may have been the place of origin for these subfamilies whose antecedents dispersed from there. Another plausible scenario is that the common ancestor was widespread and some elements evolved into the present day, range-limited, *Sphenophyma*. Other ancestors gave rise to, in turn, Oxyinae, Melanoplineae, and so on (see Figs for branching sequences).

The Australian genera.—It is interesting that the 3 Australian genera analyzed here do form a clade, but given this miniscule sample it is impossible, although tempting, to make any inferences about their biogeographical origins.

Relationship among the "spine-throats".—After the divergence of *Sphenophyma*, the temporal sequence of branching of the other subfamilies (some scattered among the catantopines) appears to be: Oxyinae, Melanoplineae, Calliptaminae, Cyrtacanthacridinae and Eyprepocnemidinae. This sequence is somewhat similar to the branching order discovered by Liu *et al.* (2008) and Zhao *et al.* (2011) who, respectively, analyzed sequences of 12S+16S rDNA and those of the mitochondrial control region (see also Rowell & Flook 1998 who also included 18S sequences). In all investigations, Catantopinae is shown to be more closely related to the Cyrtacanthacridinae than either is to Melanoplineae or Oxyinae. With respect to the position of Calliptaminae, present results agree with those of Rowell and Flook (1998) but not with the findings of Zhao *et al.* (2011). Of the 3 papers, only Rowell and Flook include Eyprepocnemidinae and here it occupies an unresolved position relative to the other subfamilies.

Based on this admittedly limited study, it can be stated that Vickery's (1989) suggestion that Catantopinae should be subdivided rings true. Clearly much more work needs to be done to discover what those dividing lines should be.

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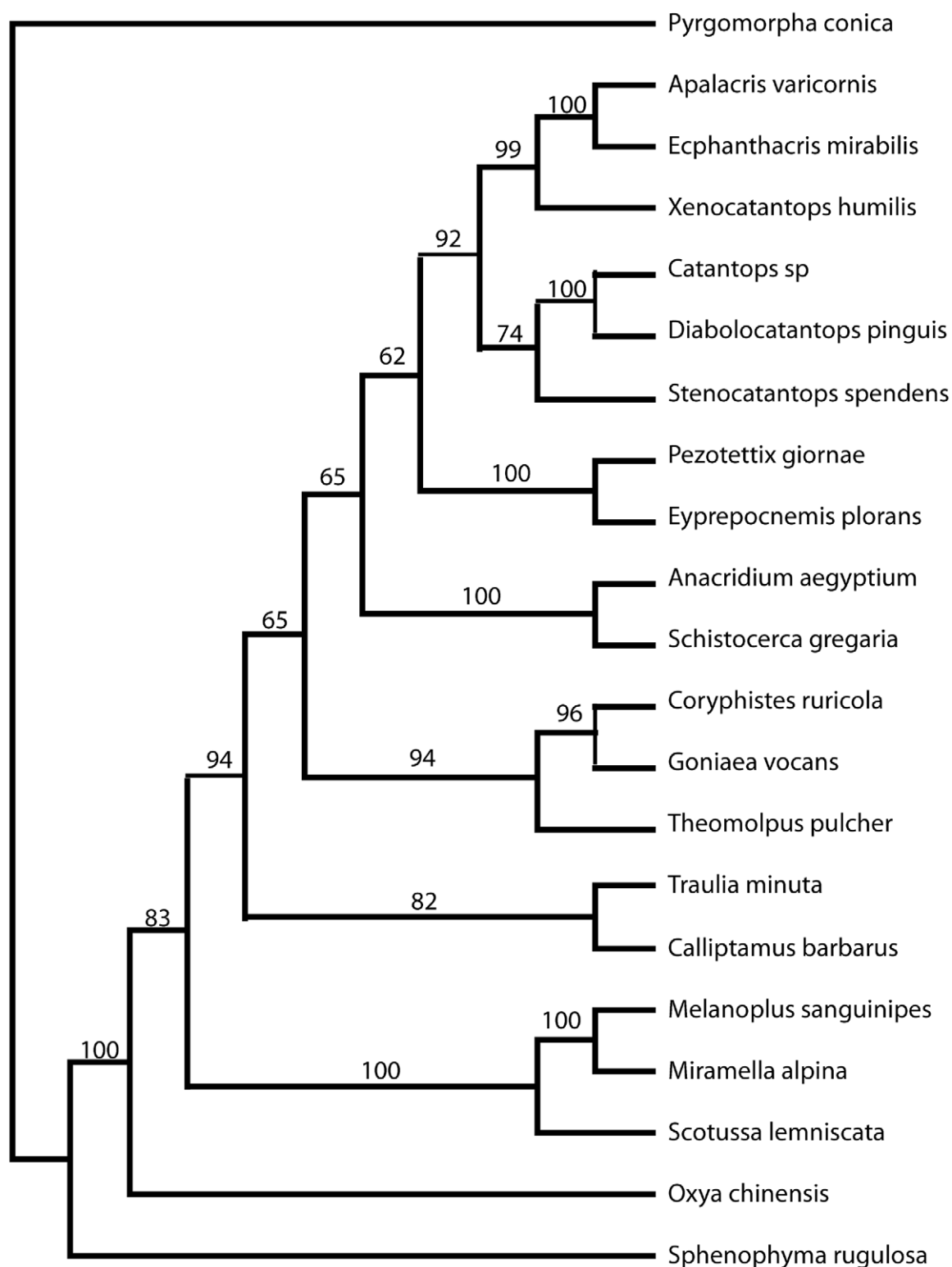


Fig. 1. Bootstrap consensus tree recovered using weighted maximum parsimony. Substitutions at the first 2 codon positions and transversions at third codon positions were scored. Numbers (> 50 %) indicate bootstrap levels of support using 1000 replicates.

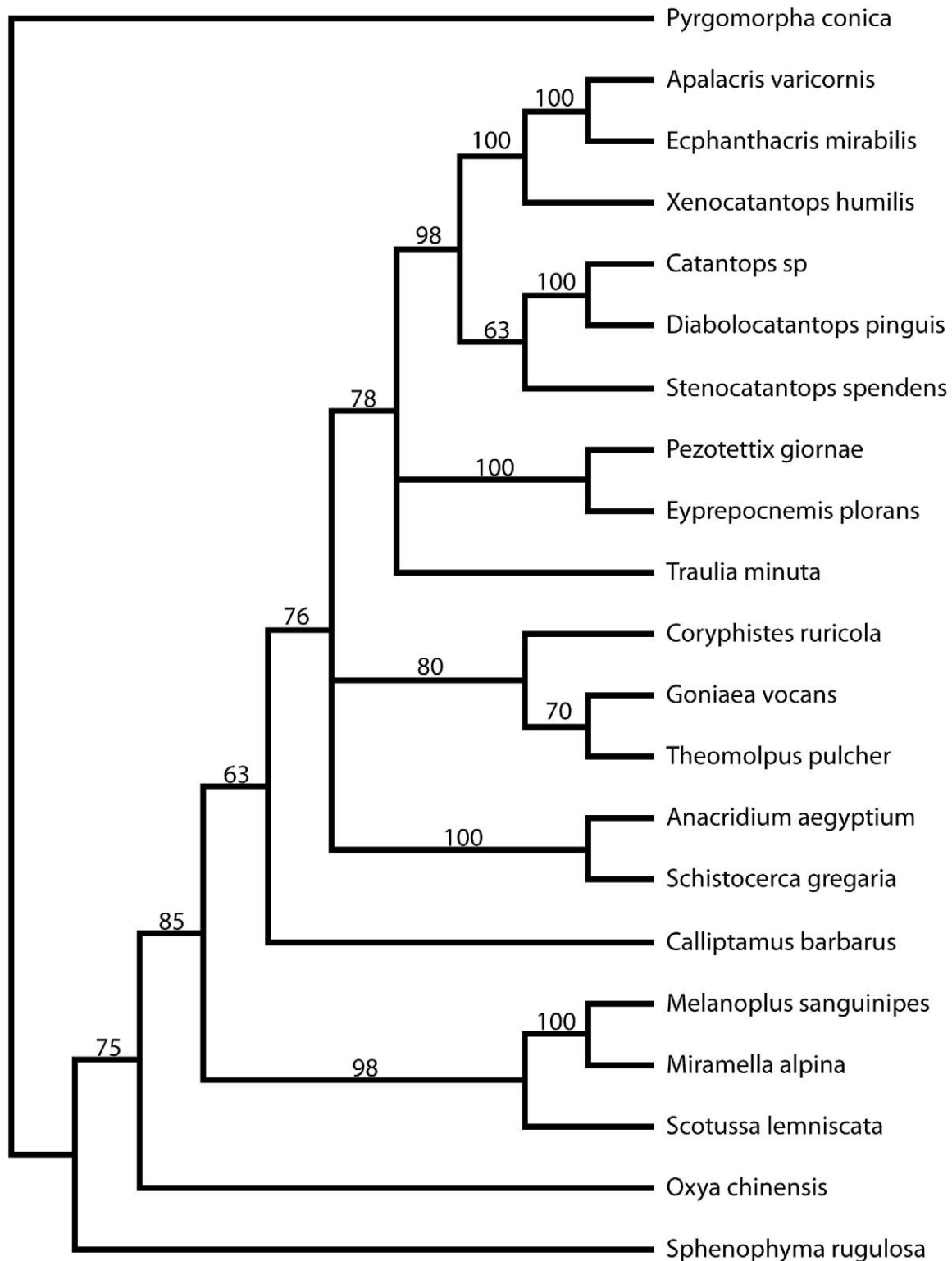


Fig. 2. Bayesian tree. Data were partitioned according to codon position and gene; each partition was allowed to follow its own GTR + G + I evolutionary model. Eight Monte Carlo Markov chains, 1 cold and 7 heated, were run simultaneously for 50 million generations when convergence had been reached. Trees were sampled every 500 generations; consensus of the last 10000 trees was calculated. Posterior probabilities (> 50%) are indicated in the figure.

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Appendix 1. The following sequence, owing to Genbank's limitations regarding sequence length, is reproduced here.

Theomolpus pulcher COII gene (translation starts at base 1):

GATACTTACATAACAACAGAAAAAGAAATTAATAATAATGAATTCCGATTACTTGATGTTGATAATCGAACAATCTTACCCATAAATACAGAAG-TACGAGTATTAAGTAGAGCATCTGATGTACTTCATTCATGAGCAGTTCGCCGACTGGGAGTAAAAATTGATGCTACACCAGGACGACTCAAT