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A note on the phylogenetic position of *Duartettix montanus* within the subfamily Melanoplinae

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Abstract

This research sheds light on the phylogenetic position of the recently described Caribbean species *Duartettix montanus*. Morphologically most similar to the Melanoplinae, especially the North American genus *Melanoplus*, it was assigned to that subfamily. The Orthoptera Species File, curiously, assigns *Duartettix* to the South American tribe Dichroplini. The present molecular phylogenetic analysis of portions of four mitochondrial genes, however, strongly rejects that allocation and instead supports an association with the northern melanoplines. Within the context of an "Out-of-South America" hypothesis proposed earlier for the subfamily's origin, it is speculated that the antecedents of *Duartettix* arose from ancestors on their northward movement, traversing a series of island arcs that intermittently connected the two Americas during the late Cretaceous. Of possible taxonomic interest, phylogenetic information is also provided for a number of South American melanoplines, notably members of the tribe Jivarini, which have not been previously analyzed using molecular methods.

Keywords

Orthoptera, Acrididae, Melanoplinae, *Duartettix*, phylogeny, biogeography, mitochondrial DNA

Introduction

Duartettix montanus, a recently described genus and species (Perez-Gelabert & Otte 2000), presents some interesting taxonomic difficulties. The species occurs in the high mountainous valleys of the Dominican Republic and, apparently, nowhere else. Morphologically, the species bears a superficial resemblance to North American melanoplines, specifically the genus *Melanoplus*; yet, it resembles no one species overall. Perez-Gelabert & Otte (2000) point to the species' geographic proximity to the North American continent as further evidence of its probable link with the northern melanoplines. For reasons not entirely clear, the Orthoptera Species File (OSF2) assigns *Duartettix* to the South American tribe Dichroplini (Otte *et al.* 2006). This note seeks to clarify the phylogenetic position of *Duartettix* in relation to a selection of melanopline grasshoppers distributed in North America, South America and Eurasia. The Neotropical taxa encompass material from two major tribes, Dichroplini and Jivarini. Including the latter may be significant because most members are also adapted to high altitudes (Rowell & Carbonell 1977). This study also includes the South American species, *Apacris rubritorax*, whose tribal affiliation is presently unknown (Amédégnato *et al.* 2003).

Materials and Methods

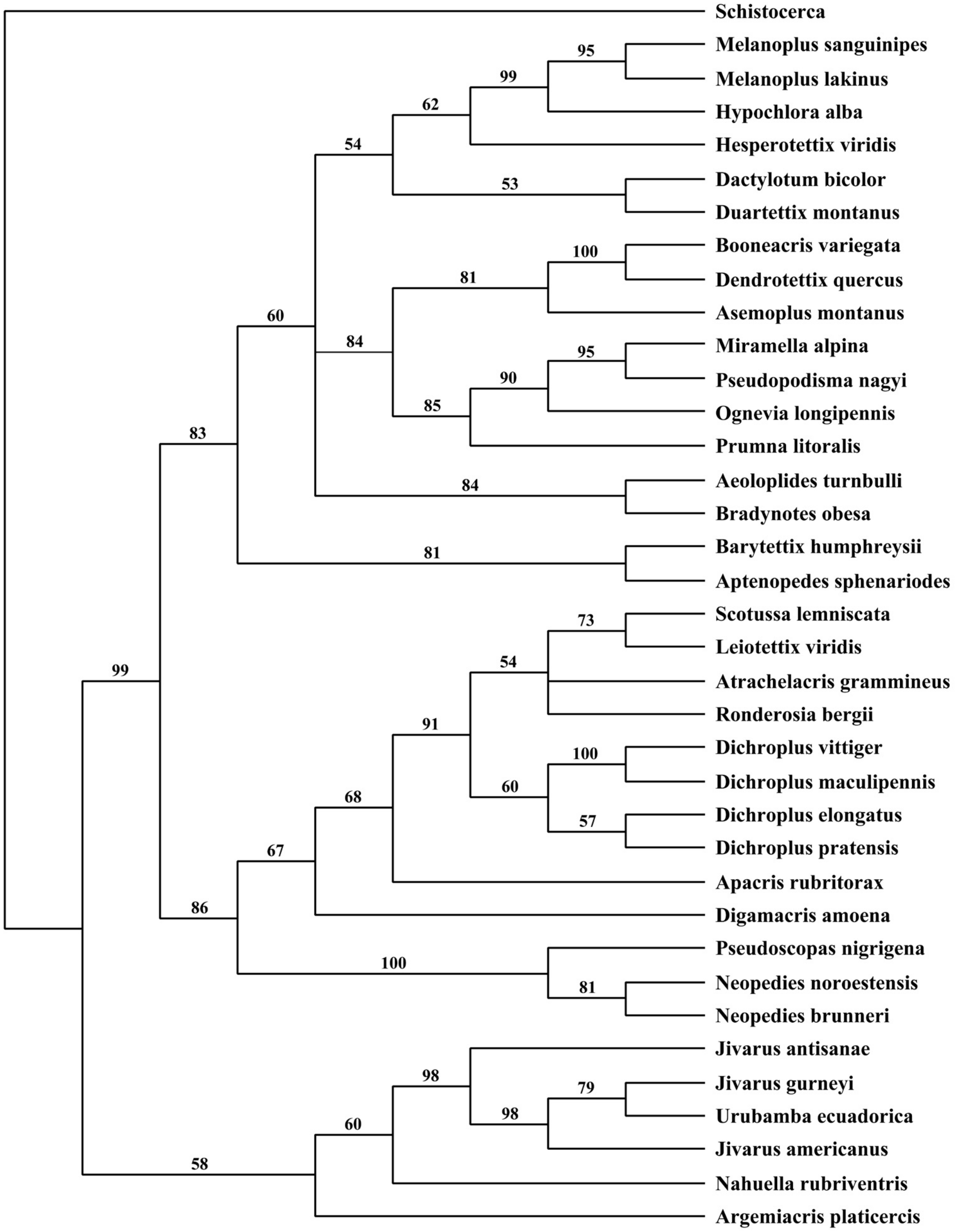
Species along with sources are listed in Table 1. Included are 19 South American species, 12 North American species, 4 Eurasian species and 1 specimen of *Duartettix montanus*. DNA was extracted using either the DTAB/CTAB method (Philips & Simon 1995) or the QIAGEN DNeasy tissue kit (Mississauga, Canada). Portions of the mitochondrial genes cytochrome b (cytb), cytochrome oxidase subunits I and II (COII and COII), and NADH dehydrogenase subunit II (ND2) were PCR amplified and sequenced. Parsimony, weighted parsimony analysis, maximum likelihood and Bayesian analyses were performed using the programs PAUP* (Swofford 2001) and MrBayes (Huelsenbeck & Ronquist 2001). Application of the program Modeltest (Posada & Crandall 1998) identified the model GTR+G+I as best fitting the data and accordingly this model was used in ML searches. *Schistocerca gregaria*, (Forskål) a member of the subfamily Cyrtacanthacridinae, served as the outgroup. Levels of branch support were estimated through 1000 bootstrap replicates using parsimony and by calculating Bayesian posterior probabilities (PP). Further procedures concerning molecular and data manipulation can be found elsewhere (Litzenberger & Chapco 2003).

Results and Discussion

Sequence data consisting of a maximum of 1716 bases have been deposited in GenBank (Table 1). Across the four genes, 926 sites were variable and of these, 538 were phylogenetically informative. ML yielded a single best tree with -LnL = 15658.84. Maximum resolution was achieved using Bayesian methods and weighted parsimony following Farris's (1969) iterative reweighting scheme and by counting transversions at third codon positions only. Figs 1A and 1B depict relationships uncovered by these two methods. The Bayesian approach considered a variety of models and the one that emerged with the highest likelihood was also GTR+G+I. The two techniques generally yielded the same broad associations with different levels of support.

Duartettix.— The base composition of mitochondrial DNA in *Duartettix* consists of 35.1% (A), 16.1% (C), 14.3% (G), and 34.4% (T), well within the range of all melanoplines thus far examined (Litzenberger 2002). Contrary to its tribal designation in OSF2, the species is not phylogenetically related to either South American tribe, Dichroplini or Jivarini. Instead, *Duartettix* is very strongly associated with the northern melanoplines, a result that supports Perez-Gelabert & Otte's (2000) viewpoint. Unfortunately, its exact

Fig. 1A. Relationships recovered using two different methods. Maximum parsimony tree obtained by scoring all substitutions at first two codon positions and transversions only at third codon positions. Homoplasy minimized by applying successive rounds of weighting using rescaled consistency indices. Numbers indicate bootstrap levels of support using 1000 replicates.



placement is indeterminate. Parsimony suggests an affiliation with *Dactylotum*, a member of the North American tribe Dactylotini (Vickery 1997, see also Litzenberger & Chapco 2003). Both species are, in turn, connected to the clade encompassing *Melanoplus*, *Hypochlora* and *Hesperotettix*. Bootstrap support for these associations, however, is not large. In analyses based on the Bayesian approach, *Duartettix* emerges as part of an unresolved polytomy within the northern group. In the context of the "Out-of-South America" scenario proposed for the subfamily's origin (Chapco *et al.* 2001, Amédégnato *et al.* 2003), if *Duartettix* had branched off from ancestors on their northward movement, traversing the series of "proto-Antilles" island arcs that intermittently connected the two Americas (Pitman *et al.* 1993), one might expect the species to be basal to the North American-Eurasian clade and internal to the South American clade. An alternative possibility, suggested—albeit weakly—by the parsimony result, is that *Duartettix* may have evolved more recently, from northern melanoplines. Application of an "orthopteroid clock" places, in rapid succession, the times separating *Duartettix* from South American taxa and from North American taxa at approximately 78 and 73 Mya, respectively (this clock was calibrated using transversional substitutions, for which there is evidence of linear accumulation over time, and fossil data that link Caelifera and Ensifera—see references and data in Chapco *et al.* 2001). These times would be in accord with the first scenario, but until a better resolution of relationships within the northern taxa—*Duartettix* cluster—is achieved, one can only speculate on the precise sequence of events.

Remaining taxa.—The inclusion of additional South American species in the analysis leads to results which further substantiate the "Out of South America" hypothesis for the origin of the Melanoplinae, given that the southern taxa occupy a basal and paraphyletic position

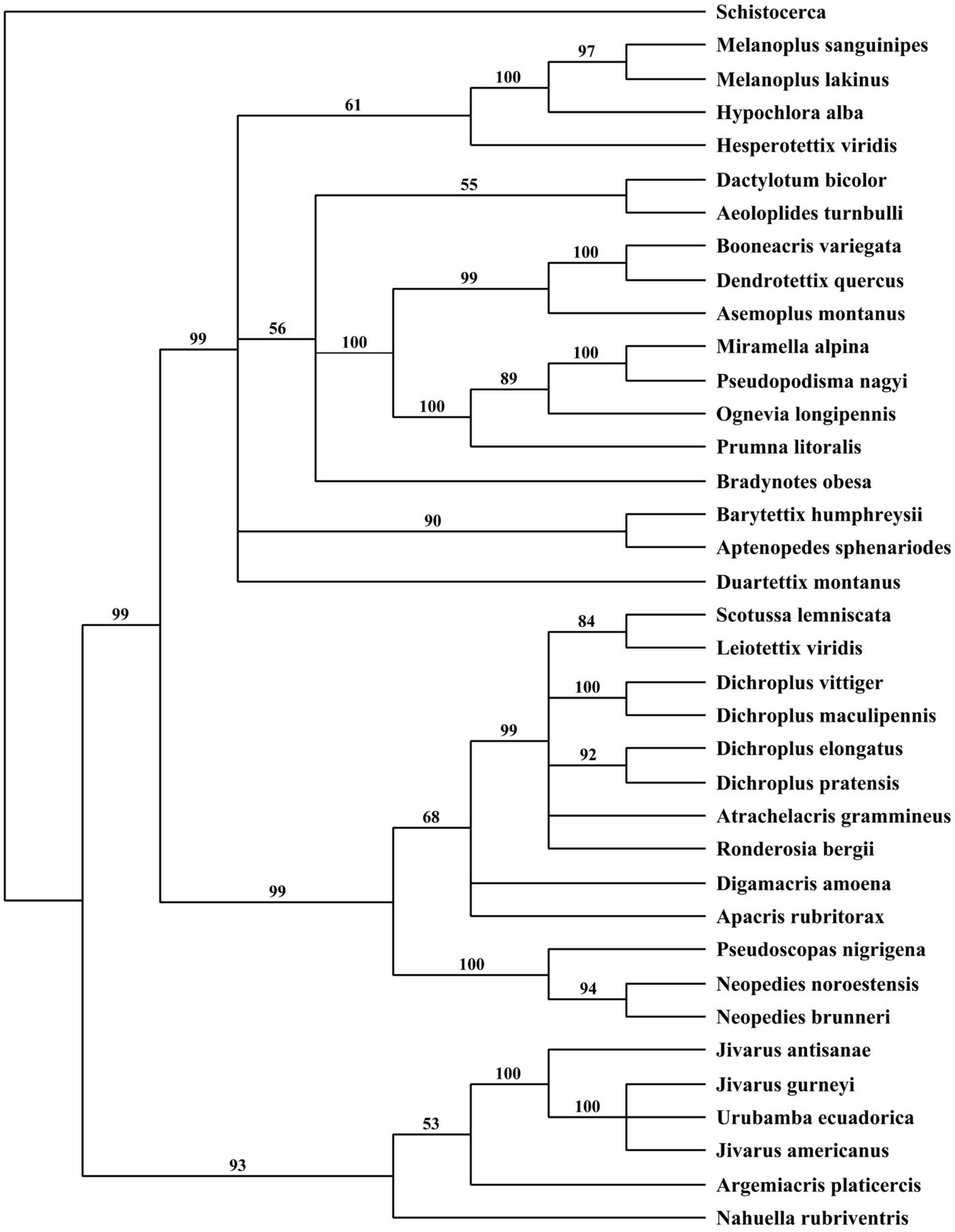


Fig. 1B. Bayesian tree based on GTR + G + I model. Eight Monte Carlo Markov chains, one cold and seven heated were run simultaneously for 3×106 generations. Trees, saved every 200 generations, yielded 15,000 saved trees; the last 2000 were used to estimate the topology, parameter values and posterior probabilities, indicated in the figure.

to the northern species. All analyses identify Jivarini as the more basal of the two South American tribes, and probably the more ancient (see also Amédégnato *et al.* 2003). Both tribes emerge as monophyletic assemblages.

Within Dichroplini, the grassland species *Scotussa*, *Leiotettix*, *Ronderosia* and *Atrachelacris*, comprise the "Paranense-Pampeano" group (Cigliano & Ronderos 1994). However, bootstrap support for this group's integrity is weak (54%), and support using Bayesian methods is nonexistent, although the four genera are strongly associated with four species of *Dichroplus* (99% PP). *Dichroplus* is clearly not monophyletic. Colombo *et al.* (2005) analyzed a greater number of species of *Dichroplus*, using two genes and morphology, and their investigation also found the genus to be polyphyletic. In the present work, *D. vittiger* and *D. maculipennis* consistently emerged together in all analyses. Both belong to the same species group, but to different subgroups (Otte *et al.* 2006). The two remaining *Dichroplus* species, *elongatus* and *pratensis,* were also linked, strongly in the Bayesian analysis, weakly using parsimony. Both belong to different species groups in the OSF2 (Otte *et al.* 2006). In the Colombo *et al.* (2005) investigation, these species were either part of a large unresolved polytomy when genes were analyzed, or were topologically separated when genes and morphology were analyzed in combination. Additional genes and, especially, species (see Zwickl & Hillis 2002) need to be studied to resolve relationships within that genus. *Apacris*, previously unassigned to tribe, could conceivably be regarded as part of Dichroplini.

While Jivarini is monophyletic, the genus *Jivarus* is not; instead, it is paraphyletic with *J. antisanae* occupying a position external to *Urubamba* and two other species of *Jivarus*. Using parsimony, *Argemiacris* is positioned externally to the remaining species, followed by *Nahuella*. Bayesian methods reverse those placements. According to Ronderos' (1978) morphological studies, *Argemiacris* is phylogenetically close to *Urubamba*, a finding not supported by the present analysis.

Table 1. Species analyzed, location and GenBank accession numbers of mtDNA sequences.

Tribe*	Species	Continental Affiliation	Accession Nos. (cytb, COII, ND2, COI)
Melanoplini	*Aeoloplides turnbulli*[1]	North America	AY014308, AY014309, AY014310, AY014311
	Aptenopedes sphenariodes	North America	DQ389235, DQ389212, DQ389221, DQ389225
	Hypochlora alba[2]	North America	AF260544, AF260545, AF260547, AF260548
	Melanoplus lakinus[3]	North America	AF317172 – AF317175
	Melanoplus sanguinipes[4,5,2]	North America	AF145499, AF145500, AF227279, AF260533
Dactylotini	*Hesperotettix viridis*[1]	North America	AY014306, AY014307, AY014312, AY014313
	Dactylotum bicolor[1]	North America	AY014314 - AY014317
Conalcaeini	*Barytettix humphreysii*[1]	North America	AF317194 - AF317197
Podismini	*Asemoplus montanus*[1]	North America	AY014334 - AY014337
	Bradynotes obesa[1]	North America	AY014305, AY014354 - AY014356
	Booneacris variegata[1]	North America	AY014326 - AY014329
	Dendrotettix quercus[1]	North America	AY014322 - AY014325
	Miramella alpina[5]	Eurasia	AF227291, AF227292, AF227293, AF260543
	Pseudopodisma nagyi[5]	Eurasia	AY004197 - AY004200
	Prumna litoralis[5]	Eurasia	AY004205 - AY004208
	Ognevia longipennis[5]	Eurasia	AY004176 - AY004179
Dichroplini	*Atrachelacris grammineus*[6]	South America	AY014357 - AY014360
	Dichroplus elongatus[2]	South America	AF260556, AF260549 - AF260551
	Dichroplus maculipennis	South America	DQ389237, DQ389214, DQ389222, DQ389227
	Dichroplus pratensis	South America	DQ389238, -, -, DQ389228
	Dichroplus vittiger	South America	DQ389236, DQ389213, -, DQ389226
	Digamacris amoenus[6]	South America	AY014338 - AY014341
	Leiotettix viridis[6]	South America	AY014350 - AY014353
	Neopedies brunneri	South America	DQ389240, DQ389216, DQ389224, DQ389230
	Neopedies noroestensis[6]	South America	AF539849 - AF539852
	Pseudoscopas nigrigena[6]	South America	AY014346 - AY014349
	Ronderosia bergii[6]	South America	AF539841 - AF539844
	Scotussa lemniscata	South America	DQ389239, DQ389215, DQ389223, DQ389229
	Duartettix montanus	Central America	AY147159 - AY147162
Jivarini	*Argemiacris platicercis*	South America	DQ389244, DQ389220, -, DQ389234
	Jivarus americanus	South America	DQ389243, DQ389219, -, DQ389233
	Jivarus antisanae[6]	South America	AF373002 - AF373005
	Jivarus gurneyi	South America	DQ389241, DQ389217, -, DQ389231
	Nahuella rubriventris[6]	South America	AF539837 - AF539840
	Urubamba ecuadorica	South America	DQ389242, DQ389218, -, DQ389232
Un-named	*Apacris rubritorax*[6]	South America	AF539845 - AF539848
Outgroup	*Schistocerca gregaria*[3]		AF145491, M83966, AF227278, AF260532

* - as allocated in the OSF2; "-" = no sequence

1= Litzenberger & Chapco 2003; 2 = Chapco *et al.* 2001; 3 = Chapco & Litzenberger 2002; 4 = Chapco *et al.* 1999; 5 = Litzenberger & Chapco 2001; 6 = Amédégnato *et al.* 2003

The strong connection between *Barytettix* and *Aptenopedes*, two species on opposite sides of the southern United States, is somewhat surprising. Perhaps one or both genera were, in the past, distributed more widely and over time became restricted in their distributions.

It should be noted that when sequences were scrutinized for possible internal stop codons or deletions, which could signify that nuclear sequences of mitochondrial origin or "Numts" (Bensasson *et al.* 2000) had been amplified, all but *Neopedies brunneri* were free of these features. The latter possessed two deletions within the ND2 gene, one three nucleotides long and another, one nucleotide long. Nevertheless, the two *Neopedies* species sequences, when aligned, were linked with high bootstrap and posterior probability values.

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