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PHYLOGENY OF THE WESTERN HEMISPHERE OZAENINI (COLEOPTERA: CARABIDAE: PAUSSINAE) BASED ON DNA SEQUENCE DATA

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ABSTRACT

Western Hemisphere species of bombardier beetles in the carabid tribe Ozaenini are distributed from southern Arizona and southern Texas to southern Chile, with more than 80% of described species being endemic to the tropics. Recent taxonomic works variously classify the 145 described species into six or 17 genera, indicating general disagreement on what constitutes natural lineages in the group. This study provides the first phylogenetic analysis of the Western Hemisphere Ozaenini using DNA sequence data from the mitochondrial and nuclear genomes. In the resulting trees, the following three suprageneric clades are supported by high bootstrap and posterior probability values: the *Ozaena* group (*Ozaena* Olivier + *Platycerozaena* Bänninger + *Goniotropis* Gray), the *Tropopsis* group (*Tropopsis* Solier + *Entomoantyx* Ball and McCleve), and the *Pachyteles* group (*Pachyteles* Perty + *Physea* Brullé + *Tachypeles* Deuve + *Inflatozaena* Deuve + *Filicerozaena* Deuve + *Proozaena* Deuve + *Serratozaena* Deuve). Close relationship among genera within the *Ozaena* group, and among genera within the *Pachyteles* group were not predicted and have not previously been hypothesized. Among the most surprising results is that *Pachyteles* (*sensu lato*) and *Goniotropis* are not sister groups, but rather represent well-defined and distantly related clades. The phylogeny inferred from molecular sequence data led to discovery of morphological characters unique to the *Pachyteles* group that clearly distinguish members of *Pachyteles (sensu lato)* from *Goniotropis*. These morphological characters are described and illustrated. Results provide evidence for polyphyly of the genera *Pachyteles*, *Tachypeles* and *Goniotropis* as they are currently defined. Adults of *Tachypeles moretianus* Deuve and *Serratozaena* Deuve are reported for the first time to be myrmecophilous.

KEY WORDS: flanged bombardier beetles, molecular phylogeny, Ozaenina, Ozaenini, paussine beetles, structural features.

INTRODUCTION

The pantropical tribe Ozaenini (Carabidae: Paussinae) is comprised of approximately 152 described species classified in 25 genera (Lorenz 2005; Deuve 2004, 2005a, 2007b). The Western Hemisphere contains the richest fauna and recent phylogenetic analysis of the subfamily Paussinae revealed that the New World members of the Ozaenini represent a monophyletic group, the subtribe Ozaenina (Moore 2006).

Like most members of the family Carabidae, ozaenine adults are night active predators on arthropods. Adults of most species are found under stones, under bark, in rotting wood, or on trees at night as they probe holes in the bark in search of prey (Moore and Di Giulio 2006). Members of the genus *Physea* Brullé are myrmecophiles (obligate associates of ants). Adults are commonly collected at night in and around refuse piles of Neotropical leaf-cutting ants in the genus *Atta* Fabricius (Formicidae: Myrmicinae: Attini) (Emden 1936; Eidmann 1937). This study revealed three new associations between ants and members of the tribe Ozaenini. Adults of *Tachypeles moretianus* Deuve and *Serratozaena paraphysea* Deuve were collected during excavations of *Atta cephalotes* (Linnaeus) nests in Costa Rica (W. Porras, personal communication), and adults of a new species of *Serratozaena* were observed entering *Atta colombica* (Guérin-Méneville) nests in Panama (A.E. Arnold, personal communication).

As is true for most large groups of organisms, Western Hemisphere ozaenine genera can be divided into two broad categories – those that are morphologically distinctive based on external morphological features and readily identified to genus by eye without the need for dissections or examination under high magnification, and those that are far less distinctive and their identification is more challenging. *Ozaena* Olivier, *Platycerozaena* Bänninger, *Physea* Brullé, and *Entomoantyx* Ball and McCleve are well-defined genera with distinctive diagnostic characters, whereas *Goniotropis* Gray, *Pachyteles* Perty, and *Tropopsis* Solier are not distinctive and lack clear morphological boundaries separating them from one another. The most commonly cited character used to distinguish members of *Goniotropis* from *Pachyteles* involves the intercoxal extensions of the mesosternum and metasternum. They are either well-developed, separating the middle coxae (as in *Goniotropis*), or they are not well-developed, with the middle coxae contiguous (as in most *Pachyteles*). Whereas members of the genus *Tropopsis* look most like *Pachyteles* species in terms of habitus and size, they have well-separated middle coxae as do members of *Goniotropis*, and they can only be distinguished from both genera by the lack of ventral projections on the forefemora. All members of *Pachyteles* and *Goniotropis* exhibit such ventral forefemoral projections.

Of all New World Ozaenine genera, *Pachyteles* (*sensu lato*) is most diverse in terms of species numbers and general structural features. Specimens of many undescribed species are in collections. At this time, keys to species are

TABLE 1. Lorenz (2005) classification of New World ozaenines, authors and type species.

Synonyms appear in gray font. The number of described species is provided for each genus and taxon sampling is indicated. Small letter "a" in the taxon sampling column denotes taxa included in the taxon sampling from pinned, museum specimens.

Genus	Subgenus	Author	Type Species	Described Species	Taxon Sampling
Crepidozaena		DEUVE, 2001	*Pachyteles gracilis* CHAUDOIR, 1868	1	
Entomoantyx		BALL AND MCCLEVE, 1990	*Ozaena cyanipennis* CHAUDOIR, 1852	1	1a
Filicerozaena		DEUVE, 2001	*Filicerozaena moreti* DEUVE, 2001	7	2a
Gibbozaena		DEUVE, 2001	*Gibbozaena mirabilis* DEUVE, 2001	2	
Inflatozaena		DEUVE, 2001	*Pachyteles inflatus* BATES, 1886	1	1a
Mimozaena		DEUVE, 2001	*Pachyteles virescens* CHAUDOIR, 1868	1	
Ozaena		OLIVIER, 1812	*Ozaena dentipes* OLIVIER, 1812	12	4
Ictinus		LAPORTE DE CASTELNAU, 1834	*Ictinus tenebrionoides* LAPORTE DE CASTELNAU, 1834		
Pachyteles	*Pachyteles*	PERTY, 1830	*Pachyteles striola* PERTY, 1830	51	5
	Goniotropis	GRAY, 1832	*Goniotropis brasiliensis* GRAY, 1832	26	8
	Scythropasus	CHAUDOIR, 1854	*Scythropasus elongatus* CHAUDOIR, 1854		
	Tropopsis	SOLIER, 1849	*Tropopsis marginicollis* SOLIER, 1849	2	2
Physea		BRULLÉ, 1834	*Trachelizus rufus* BRULLÉ, 1834	6	1
			[junior synonym of *Physea testudinea* (KLUG, 1834)]		
Coeloxenus		WASMANN, 1925	*Coeloxenus guentheri* WASMANN, 1925		
Physeomorpha		OGUETA, 1963	*Physeomorpha vianai* OGUETA, 1965	1	
Platycerozaena		BÄNNINGER, 1927	*Ozaena brevicornis* BATES, 1874	4	1, 1a
Proozaena		DEUVE, 2001	*Pachyteles parallelus* CHAUDOIR, 1848	8	4
Tachypeles		DEUVE, 2001	*Pachyteles arechavaletae* CHAUDOIR, 1868	20	5
Serratozaena		DEUVE, 2001	*Serratozaena paraphysea* DEUVE, 2001	1	2
			Totals	144	37

not available and most species cannot be identified from published descriptions.

The most comprehensive taxonomic work on the New World Ozaenini, based on adult morphological characters, addressed the identity of 14 species distributed in the southwestern United States and Mexico, and provided a solid foundation on which to base a synthetic revision of this group (Ball and McCleve 1990). Although that paper did not include a phylogenetic analysis, it included discussion of more than 50 morphological characters that may be phylogenetically informative. Based on this study, Ball and McCleve (1990) synonymized *Goniotropis* and *Tropopsis* with *Pachyteles*, since the differences between these taxa are relatively slight compared to the differences among other genera in the tribe, and they retained all three names as subgenera.

Most recently, Deuve (2001) described eight new genera to house former members of the large genus *Pachyteles* (*sensu lato*) including *Tachypeles* Deuve, *Inflatozaena* Deuve, *Filicerozaena* Deuve, *Proozaena* Deuve, *Serratozaena* Deuve, *Crepidozaena* Deuve, *Mimozaena* Deuve, and *Gibbozaena* Deuve. Many of these new genera are defined by the form of the female gonocoxae, which are strikingly diverse among the species in this group (see Ball and McCleve 1990; Deuve 1993, 2001, 2004).

The two most recent taxonomic treatments classify species into either six (Ball and McCleve 1990) or 17 separate genera (Deuve 2001, 2004, 2005), indicating general disagreement on what constitutes natural lineages in the group. At the present time, the only modern classification of ozaenine species appears in a world list of Carabidae (Lorenz 2005). This classification is a hybrid of the views of ozaenine taxonomy presented in Ball and McCleve (1990) and Deuve (2001), which does not include the taxa described simultaneously by Deuve (2005). The genera recognized by Lorenz (2005) are presented in Table 1 with their authors, type species, and number of species assigned to each genus. Despite the interesting attributes of ozaenines, and their relative ubiquity in tropical habitats, our knowledge of the classification of the Ozaenini

TABLE 2. Taxonomic and geographic information for the specimens used in this study and GenBank accession numbers for each sequence *(continued on next page).*

Voucher Species	Voucher Number	Locality and Collection Data	28S GenBank Accession Number	COI GenBank Accession Number
Metrius contractus ESCHSCHOLTZ	0138	USA: California, Marin Co., Lagunitas Creek, 0.1 mile below spillway of Nicasio Dam, summer 1993, collected by D.H. Kavanaugh.	AF398687	EF694886
Metrius explodens BOUSQUET AND GOULET	0787	USA: Montana: Ravali Co., Bitterroot Mountains, collected by M.A. Ivie.	EF694841	EF694887
Mystropomus regularis BÄNNINGER	0796, 1230	AUSTRALIA: Queensland, Mt. Abbott, 800-1000m, 20º06'S 47º45'E, 9-12.iv.1997, collected by Monteith, Cook and Janetzki.	EF694842	EF694888
Mystropomus subcostatus CHAUDOIR	0786	AUSTRALIA: New South Wales, Lansdowne, iii.1997, collected by G. Williams.	EF694843	EF694889
Itamus sp.	a0011	SRI LANKA: Colombo Dist., Ratmalana, 26-27. iv.1968, collected by T.L. Halstead.	EF424238	
Pseudozaena tricostata opaca (CHAUDOIR)	0788	MALAYSIA: Sabah, Sabah Parks, Poring Hot Springs, 2000m, ii.2000, collected by M. Zhjra.	EF694844	EF694890
Sphaerostylus (Sphaerostylus) goryi (LAPORTE DE CASTELNAU)	0785	MADAGASCAR: Fianarantsoa Prov., Ranomafana National Park, Talatakely area, 900m, mixed tropical forest, 14.iv.1998, 21°14.9'S 47°25.6'E, collected by D.H. Kavanaugh.	EF694845	EF694891
Sphaerostylus (Afrozaena) luteus (HOPE)	a0012	CAMEROON: Yaounde Prov., Congo-Guinean rainforest, 700m, 14.ii- 5.iii.1980, collected by R.L. Aalbu.	EF424232	
Entomoantyx cyanipennis (CHAUDOIR)	a0006	COSTA RICA: Guanacaste Prov., Est. Maritza, lado Vol. Orosi, 600m, 10°57.45'N 85°29.42'W, i-iv.1992.	EF424230	
Goniotropis kuntzeni BÄNNINGER	0799	USA: Arizona, Santa Cruz Co., Walker Canyon, 31°22.819'N 111°03.994'W, 1214m, 24.vii.1999, collected by W. Moore.	EF694846	EF694892
Goniotropis parca (LECONTE)	0828	USA: Arizona, Santa Cruz Co., Walker Canyon 31°22.819'N 111°03.994'W, 1214m, 2.vii.2000, collected by W. Moore.	EF694847	EF694893
Goniotropis parca (LECONTE)	1194	MEXICO: Baja California Sur, 10.viii.2000, collected by C. Smith.	EF694848	EF694894
Goniotropis morio (KLUG)	0889	PANAMA: Barro Colorado Island, 9°09'N 79°51'W, 14-16.vii.1999, collected by A.E. Arnold.	EF694849	EF694895
Goniotropis nr. *seriatoporus* (CHAUDOIR)	0808	ECUADOR: Sucumibos Prov., 175 km ESE of Coca, La Selva Station, 250m, 15.vi.1997, collected by H. Greeney.	EF694850	EF694896
Goniotropis nr. *seriatoporus* (CHAUDOIR)	1200	ECUADOR: Orellana Prov., Tiputini Biodiversity Station, 0°38'S 76°9'W, 5.x.2000, collected by W. Moore.		EF694897
Goniotropis nr. *seriatoporus* (CHAUDOIR)	0964	ECUADOR: Orellana Prov., Tiputini Biodiversity Station, 0°38'S 76°9'W, 27.ix.2000, collected by W. Moore.	EF694851	EF694898
Goniotropis (Scythropasus) tiputinica DEUVE	1197	ECUADOR: Orellana Prov., Tiputini Biodiversity Station, 0°38'S 76°9'W, 4.x.2000, collected by W. Moore and T.L. Erwin.	EF694852	EF694899
Goniotropis simplicicollis DEUVE	1199	ECUADOR: Orellana Prov., Tiputini Biodiversity Station trails, 0°38'S 76°9'W, 30.ix.2000, collected by W. Moore.	EF694853	EF694900

TABLE 2 *(continued)*. Taxonomic and geographic information for the specimens used in this study and GenBank accession numbers for each sequence *(continued on next page)*.

Voucher Species	Voucher Number	Locality and Collection Data	28S GenBank Accession Number	COI GenBank Accession Number
Goniotropis (Scythropasus) olivieri (CHAUDOIR)	1196	ECUADOR: Orellana Prov., Tiputini Biodiversity Station, 0°38'S 76°9'W, 4.x.2000, collected by W. Moore and T.L. Erwin.		EF694901
Goniotropis (Scythropasus) olivieri (CHAUDOIR)	1198	ECUADOR: Orellana Prov., Tiputini Biodiversity Station, 0°38'S 76°9'W, 29.ix.2000, collected by W. Moore.	EF694854	EF694902
Goniotropis (Scythropasus) nicaraguensis (BATES)	0888	PANAMA: Barro Colorado Island, 9°09'N 79°51'W, 7-9.vii.1999, collected by A.E. Arnold.	EF694855	EF694903
Goniotropis (Scythropasus) nicaraguensis (BATES)	0804	PANAMA: Barro Colorado Island, 9°09'N 79°51'W, 25.vi.1999, collected by A.E. Arnold.	EF694856	EF694904
Goniotropis (Scythropasus) nicaraguensis (BATES)	1379	COSTA RICA: Guanacaste Prov., Estacion Monte Alto, 10°01'N 85°23'W, 9.vii.2002, collected by W. Porras.	EF694857	EF694905
Goniotropis (Scythropasus) nicaraguensis (BATES)	1382	COSTA RICA: Guanacaste Prov., San Pablo Nandayure, Finca Agua Fria, 20m, 10°01.14'N 85°16.09'W, 17.i.2002, collected by W. Porras.	EF694858	EF694906
Goniotropis (Scythropasus) nicaraguensis (BATES)	1383	COSTA RICA: Guanacaste Prov., San Pablo Nandayure, Finca Agua Fria, 20m, 10°01.14'N 85°16.09'W, 17.i.2002, collected by W. Porras.	EF694859	EF694907
Pachyteles nr. *striola* PERTY	0346	ECUADOR: Sucumbios Prov., Cuyabeno Faunal Reserve, Laguna Grande, 22-27.iv.1994, collected by W.P. Maddison.	AF012517	EF694908
Pachyteles nr. *striola* PERTY	1232	COSTA RICA: Osa Peninsula, Corcovado Station, La Leona, 8°27'N 83°29'W, 25.vi.2001, collected by W. Moore and W. Porras.	EF694860	EF694909
Pachyteles nr. *granulata* (DEJEAN)	0827	PANAMA: Barro Colorado Island, 9°09'N 79°51'W, 7.vii.1999, collected by A.E. Arnold.	EF694861	EF694910
Pachyteles nr. *trinidadensis* DEUVE	1189	ECUADOR: Orellana Prov., Tiputini Biodiversity Station, 0°38'S 76°9'W, 4.x.2000, collected by W. Moore.	EF694862	EF694911
Inflatozaena inflata (BATES)	a2017	COSTA RICA: Guanacaste Prov., Estacion Pitilla 9 km S. de Santa Ceceila, 10°59.25'N 85°25.37'W, 700m x.1996, collected by C. Moraga.	EF694863	EF694912
Filicerozaena pseudovignai DEUVE	1190	ECUADOR: Orellana Prov., Tiputini Biodiversity Station, 0°38'S 76°9'W, 3.x.2000, collected by W. Moore.	EF694865	EF694914
Filicerozaena moreti DEUVE	a2015	ECUADOR: N. of Valladalid, 2200-2600m, 24.v.1998, collected by A. Jasinski.		EF694915
Filicerozaena tapiai DEUVE	a2016	ECUADOR: Pichincha Prov., Alluriquin, 16.v.1997, collected by A. Jasinski.		EF694916
Tachypeles gonioderoides DEUVE	1187	ECUADOR: Orellana Prov., Tiputini Biodiversity Station, Heliport, 0°38'S 76°9'W, 6.x.2000, collected by W. Moore.	EF694864	EF694913
Tachypeles gonioderoides DEUVE	1188	ECUADOR: Orellana Prov., Tiputini Biodiversity Station, Heliport, 0°38'S 76°9'W, 6.x.2000, collected by W. Moore.	EF694866	EF694917
Tachypeles limonensis DEUVE	0890	COSTA RICA: Heredia Prov., La Selva, 55m, 10°25.78'N 84°1.0'W, 22.xii.1999, collected by W. Moore.	EF694867	EF694918
Tachypeles moretianus DEUVE	0807	PANAMA: Barro Colorado Island, 9°09'N 79°51'W, 14.vii.1999, collected by A.E. Arnold.	EF694868	EF694919

TABLE 2 *(continued)*. Taxonomic and geographic information for the specimens used in this study and GenBank accession numbers for each sequence *(continued on next page)*.

Voucher Species	Voucher Number	Locality and Collection Data	28S GenBank Accession Number	COI GenBank Accession Number
Tachypeles moretianus DEUVE	1308	COSTA RICA: Guanacaste Prov., Zapotal de Nandayure, Cerro Santa Rita, 600-800m, 10°01.14'N 85°16.09'W, collected by W. Porras from *Atta cephalotes* (Linnaeus) nest.	EF694869	EF694920
Tachypeles nr. *boulardi* DEUVE	1378	COSTA RICA: Guanacaste Prov., Estacion Monte Alto, 10°01'N 85°23'W 9.vii.2002, collected by W. Porras.	EF694870	EF694921
Proozaena parallela (CHAUDOIR)	0805	COSTA RICA: Heredia Prov., La Selva, 55m, 10°25.78'N 84°1.0'W 22.x.1999, collected by W. Moore.	EF694871	EF694922
Proozaena mooreae DEUVE	0963	ECUADOR: Orellana Prov., Tiputini Biodiversity Station, Heliport, 0°38'S 76°9'W, 6.x.2000, collected by W. Moore.	EF694872	EF694923
Proozaena flavonigra DEUVE	1185	ECUADOR: Orellana Prov., Tiputini Biodiversity Station, 0°38'S 76°9'W, 27.ix.2000, collected by W. Moore.	EF694873	EF694924
Proozaena funcki (CHAUDOIR)	0851	PANAMA: Barro Colorado Island, 9°09'N 79°51'W, 30.vi.1999, collected by A.E. Arnold.		EF694925
Serratozaena nr. *paraphysea* DEUVE	1113	PANAMA: Barro Colorado Island, 9°09'N 79°51'W, 18-26.x.2001, collected by A.E. Arnold from *Atta colombica* (Guerin-Meneville) nest.	EF694874	EF694926
Serratozaena paraphysea DEUVE	1310	COSTA RICA: Guanacaste Prov., Zapotal de Nandayure, Cerro Santa Rita, 600-800m, 10°01.14'N 85°16.09'W, 10.vii.2002, collected by W. Porras from *Atta cephalotes* (Linnaeus) nest.	EF694875	EF694927
Physea new species	1152	PANAMA: Barro Colorado Island, 9°09'N 79°51'W, 18-26.x.2001, collected by A.E. Arnold from *Atta colombica* (Guerin-Meneville) nest.	EF694876	EF694928
Physea new species	1584, 1596	COSTA RICA: Guanacaste Prov., BN Diria Vista de Mar Torre Cocesna Santa Cruz, 900-1000m, 10°08.03'N 85°38.01'W, 10.viii.2002, collected by W. Porras from *Atta cephalotes* (Linnaeus) nest.	EF694877	EF694929
Physea new species	2004	TRINIDAD: St. Andrews Co., Cumuto Mnt. Rd. 10°29.4'N 61°13.2'W 67m 4.vii.2005, collected by S.C. Crews.	EF694878	EF694930
Platycerozaena panamensis (BÄNNINGER)	0720	PANAMA: Barro Colorado Island, 9°09'N 79°51'W, 2.vii.1999, collected by A.E. Arnold.	EF694879	EF694931
Platycerozaena magna (BATES)	a0007	ECUADOR: Rio Palenque, 6.vi.1992, collected by H. Greeney.	EF424231	
Ozaena lemoulti BÄNNINGER	0719	USA: Arizona, Santa Cruz Co., Walker Canyon, 31°22.819'N 111°03.994'W, 1214m, 24.vii.1999, collected by W. Moore.	EF694880	EF694932
Ozaena ecuadorica BÄNNINGER	0682	ECUADOR: Sucumibos Prov., 175 km ESE of Coca, La Selva Station, 250m, 16.vii.1997, collected by H. Greeney.	EF694881	

TABLE 2 *(continued)*. Taxonomic and geographic information for the specimens used in this study and GenBank accession numbers for each sequence.

Voucher Species	Voucher Number	Locality and Collection Data	28S GenBank Accession Number	COI GenBank Accession Number
Ozaena ecuadorica BÄNNINGER	1151	ECUADOR: Orellana Prov., Tiputini Biodiversity Station, 0°38'S 76°9'W, 6.x.2000, collected by W. Moore.		EF694933
Ozaena martinezi OGUETA	1485	BOLIVIA: Parque Nacional Amboro, 1-9. xi.2002, collected by R. Leschen.	EF694882	
Ozaena dentipes OLIVIER	1306	ECUADOR: Orellana Prov., Yasuni Biological Reserve Station, 0º40'S, 76º24'W, 4.xi.2002, collected by E.M. Fisher.	EF694883	EF694934
Tropopsis biguttatus SOLIER	1372	CHILE: IX Region, Villarrica National Park, 1240m, 39º23'00"S, 71º56'54"W, 11.i.2003, collected by K.W. Will.	EF694884	EF694935
Tropopsis marginicollis SOLIER	1371	CHILE: X Region, Puyeheu National Park, 40º39'59"S, 72º10'19"W, 22.i.2003, collected by K.W. Will.	EF694885	EF694936

is sparse. In order to develop a meaningful classification, we need hypotheses of the phylogenetic history of these organisms.

Goals of the Present Study.—This study uses molecular sequence data to infer the evolutionary history of the Western Hemisphere Ozaenini based on the mitochondrial protein-coding gene, COI (cytochrome c oxidase subunit I), and a nuclear ribosomal gene, 28S. Morphological characters diagnostic of some well-supported molecular clades are described and illustrated. Results are preliminary but provide a framework for continuing research on this group (Deuve and Moore, in prep). Ultimately we will produce a classification that reflects evolutionary history and an identification key to Western Hemisphere ozaenine genera (Deuve and Moore, in prep).

MATERIALS AND METHODS

Molecular Phylogenetics

Taxon Sampling.—The goal for taxon sampling was inclusion of at least two species of each Neotropical ozaenine genus. This goal was nearly met (Table 1) with the exception of several genera recently described by Deuve (2001), for which there are only a few known specimens. The speciose genera *Goniotropis* and *Pachyteles* (sensu lato) were sampled more thoroughly in order to test competing classifications and evolutionary relationships suggested in the literature. Two species of Metriini, two species of Mystropomini, and four species of Old World Ozaenini were included as out-group taxa. Most specimens used in this study were collected recently and were preserved specifically for molecular work in 95-100% EtOH. Six pinned museum specimens were chosen for DNA analysis to fill in holes in the taxon sampling (Tables 1 and 2). These specimens were borrowed from the collections noted below (curators are indicated in parentheses). A list of taxa sampled and details of each voucher specimen are provided in Table 2. Thierry Deuve (Museum National d'Histoire Natrelle, Paris) identified the voucher specimens of *Pachyteles* (sensu lato) and *Goniotropis*, and he described 7 new species from the voucher material (Deuve 2007b).

CASC – Department of Entomology, California Academy of Sciences, Golden Gate Park, San Francisco, California, 94118, USA (D.H. Kavanaugh).
DRMC – David R. Maddison Insect Collection. University of Arizona, Department of Entomology, Tucson, Arizona, 85721-0036, USA (D.R. Maddison).
MNHP – Entomologie, Museum National d'Histoire Naturelle, Paris 75005, France (T. Deuve).
USNM – Department of Entomology, National Museum of Natural History, Smithsonian Institution, Washington D.C., 20560, USA (T.L. Erwin).
WMIC – Wendy Moore Insect Collection. University of Arizona, Department of Entomology, Tucson, Arizona, 85721-0036, USA (W. Moore).

DNA Extraction.—Different DNA extraction protocols were used on pterothoracic muscle tissue taken from specimens preserved specifically for molecular work in 90-100% EtOH, and tissue taken from dry museum specimens. Total genomic DNA was extracted from specimens preserved for molecular work by using the Qiagen DNeasy tissue kit with ATL buffer, proteinase K, and the protocol "DNA Extraction for Animal Tissues" provided with the kit. Extractions from dry museum specimens were conducted in a laboratory dedicated to research on samples that contain low amounts of DNA. Extraction protocols for these specimens followed those outlined in Gilbert *et al.* (2007).

Gene Choice, PCR Amplification and Sequencing.— Two independent genes were selected for this study. Approximately 1000 base pairs of 28S ribosomal DNA and 800 base pairs of the mitochondrial protein-coding gene cytochrome oxidase I (COI) were amplified from total genomic DNA. Gene fragments were PCR amplified in 50 µl volume reactions with Eppendorf Hot Master Mix, 10 mmol dNTP, 5 pmol primers each, and 0.2 µl of Taq polymerase (Hot Master Eppendorf). Double stranded amplification reactions were performed either with an Eppendorf Thermal Cycler or a MJ Research Thermal Cycler.

The D2-D3 region of 28S rDNA was amplified using either 5' primer LS30F (ACCCCCTRAATTTAAGCATAT) or LS58F (GGGAGGAAAAGAAACTAAC) in conjunction with the 3' primer LS998R (GAAAGATGGTGAACTATGC) or LS1066R (CTGACGTGCAAATCGGTCG) and the following PCR conditions: 5 min at 94°C, and then 30s at 94°C, 30s at 50°C-54°C, and 1 min at 65°C for 30 cycles with a 10 minute final extension at 65°C. A shorter piece of 28S rDNA, 280-300 base pairs, was amplified from the dry museum specimens. For these samples the forward primers used were LS757F (5'AGGACCCGTCTTGAAACACGG, annealing temperature 54°C), LS773F (5'CACGGACCAGGGAGTCTAGCAT, annealing temperature 50°C), or LS776F (5' GGACCAGGGAGTCTAGCAT, annealing temperature 48°C), and they were paired with reverse primer LS1126R (5'GCATAGTTCACCATCTTTC).

The COI gene was amplified using the 5' primer JER (CAACATTTATTTTGATTTTTTGG) in conjunction with the 3' primer PAT (TCCAATGCACTAATCTGCCATATTA) and the following PCR conditions: 5 min at 94°C, and then 30s at 94°C, 30s at 55°C or 56°C, and 1 min at 65°C for 30 cycles, with a 10 minute final extension at 65°C.

PCR products were sequenced either at the University of Arizona's Genomic and Technology Core Facility using either a 3730 or 3730 XL Applied Biosystems automatic sequencer or at the California Academy of Science's Osher Molecular Systematics Laboratory using an ABI 3100 Automated Sequence Analyzer. Chromatograms were assembled into contigs, and base calls were checked using Sequencher 4.5. New sequences were deposited in GenBank (see Table 2 for accession numbers).

Sequence Alignment.—Ribosomal sequence data were aligned with ClustalX 1.83 (Thompson *et al*. 1997) using a gap opening cost of 10, an extension cost of 2, and a transition/transversion ratio of 0.1. The resulting alignment was improved by scanning data by eye and manually adjusting the alignment in MacClade 4.08 (Maddison and Maddison 2005). The lack of insertion and deletion events in the history of the evolution of COI within Ozaenina made the alignment of sequences straightforward. COI nucleotides were aligned with the pairwise sequence alignment tool in MacClade 4.08.

Phylogenetic Analysis.—Data sets for the two genes were analyzed separately and in combination. Data sets of single genes and the concatenated matrix were submitted to parsimony and Bayesian searches. All characters were included.

Parsimony searches were conducted in PAUP*4.0d81 (Swofford 2001) using the heuristic algorithm, TBR branch swapping, and equal character weighting, in either one analysis or two consecutive analyses. In the first analysis 1000 random addition sequence replicates were performed, starting trees were made by stepwise addition, and no more than 100 trees of a length greater than or equal to 1 were saved in each replicate. If, at the end of this search, more than 99 trees were found on one island, a second search was conducted using all most parsimonious trees found in the first search as starting trees. Bootstrap analyses were performed to assess nodal support. Heuristic searches were conducted on 1000 bootstrap replicates, each with 10 random addition sequence replicates, TBR branch swapping, and the limitation that no more than 100 trees of a length greater than or equal to one were saved in each replicate.

Bayesian estimation of phylogeny was performed in

TABLE 3. Diagnostic morphological characters and states for three suprageneric clades.

Character	*Ozaena* Group	*Tropopsis* Group	*Pachyteles* Group
1	0	0	1
2	0	0	0, 1
3	0	0	1
4	0	1	1
5	1	0	1

Character List

1. Mesosternum and mesepisternum
   0. distinct, clearly separated by a mesopleurosternal suture (Fig. 5B)
   1. completely fused (Fig. 5A)
2. Mesosternum and metasternum, intercoxal processes
   0. firmly or loosely articulated but completely separate the middle coxae (Fig. 5B)
   1. reduced such that the middle coxae touch medially (Fig. 5A)
3. Metasternum, metakatepisternal sulcus shape
   0. straight near the discriminal line (Fig. 5D)
   1. sinuate near the discriminal line (Fig. 5C)
4. Metasternum, metakatepisternal sulcus length
   0. reaches metepimeron (Fig. 5F)
   1. obliterated near metepimeron (Fig. 5D)
5. Forefemur, ventral projection
   0. absent (see Ball and McCleve 1990, figs. 57, 58, 62)
   1. present (see Ball and McCleve 1990, figs. 59-61)

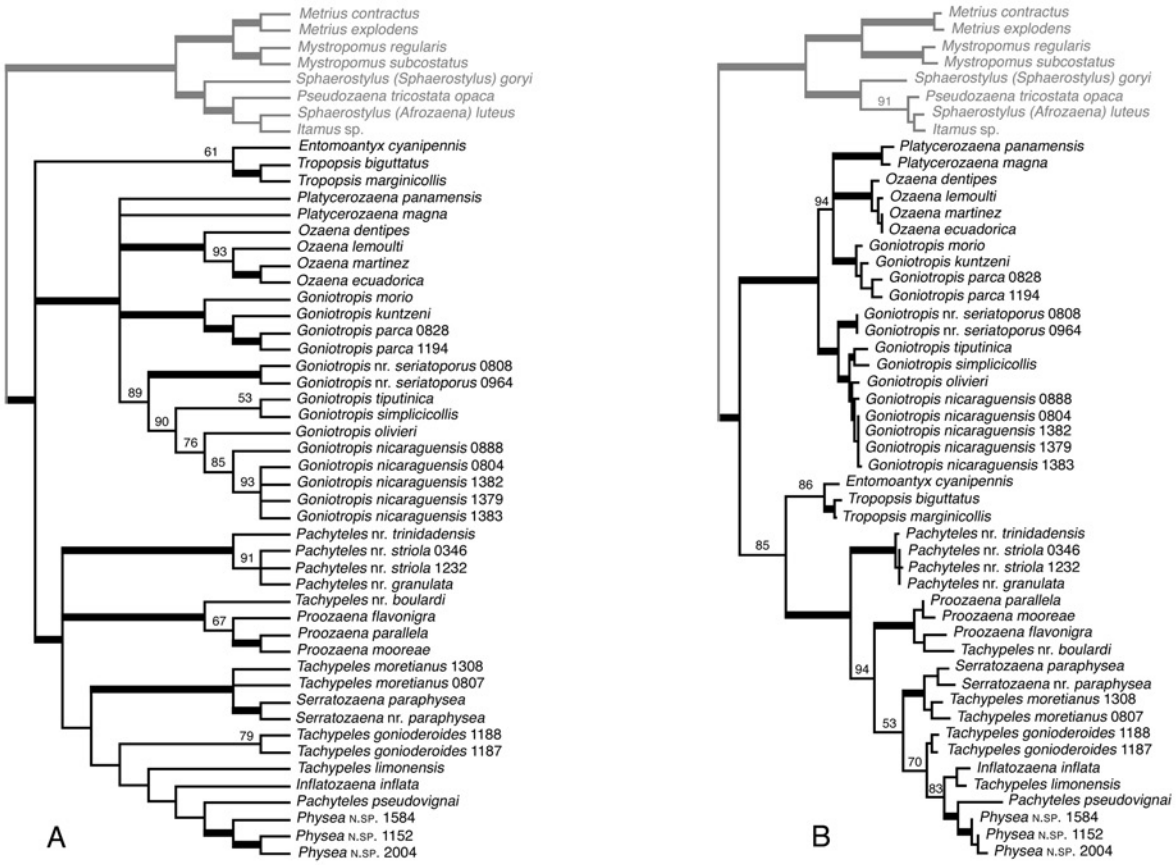

Fig. 1.—Optimal trees found for the 28S rDNA dataset. Bootstrap values and posterior probability values are provided above each branch for values greater than 50 and less than 95; values greater than or equal to 95 are indicated by thick branches. **A**, strict consensus of the 104 most parsimonious trees (TL = 1716; CI = 0.56; RI = 0.85); **B**, Bayesian consensus tree.

MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). The COI data set was separated into three partitions, one for each codon position. The concatenated dataset was separated into four partitions, one for 28S and one for each codon position of COI. Analyses incorporated the GTR+I+Γ model of evolution, as this model best fit the data of each partition as determined by the Akaike Information Criterion (AIC) implemented in Modeltest (Posada and Crandall 1998). Parameter values for the model of evolution were allowed to vary between partitions. The Markov Chain Monte Carlo (MCMC) process was set so that four chains ran simultaneously for 5,000,000 generations each, sampling trees every 1000 generations. Two independent Bayesian runs were performed in tandem under these conditions until the average deviation of split frequencies between the last 75% of trees saved during the two runs was less than 0.01. Tracer v.1.3 (Rambaut and Drummond 2005) was used to confirm that a burn-in of one million generations was sufficient to attain convergence of likelihood scores and parameter values, and then those trees were deleted from further analysis. The remaining 8000 trees (4000 from each run) were combined into one treefile in PAUP*4.0d81 and posterior probabilities were summarized in a majority rule consensus tree. Branch lengths of the consensus tree were inferred in MrBayes 3.1.2.

Diagnostic Morphological Characters

Diagnostic morphological characters of adults were sought for well-supported clades inferred from the molecular data by visually comparing adult morphology of DNA voucher specimens after organizing them into groups consistent with those clades (Table 3).

RESULTS

Trees resulting from parsimony and Bayesian analyses of the matrices of single genes are presented in Figs. 1 and 2, and trees resulting from parsimony and Bayesian analyses of the concatenated matrix (both genes) are presented in Fig. 3. Results are summarized in Fig. 4. All trees resulting from parsimony and Bayesian analyses of the matrices of single genes and the concatenated matrix contain the same three suprageneric clades (Figs. 1–4): the *Ozaena* group,

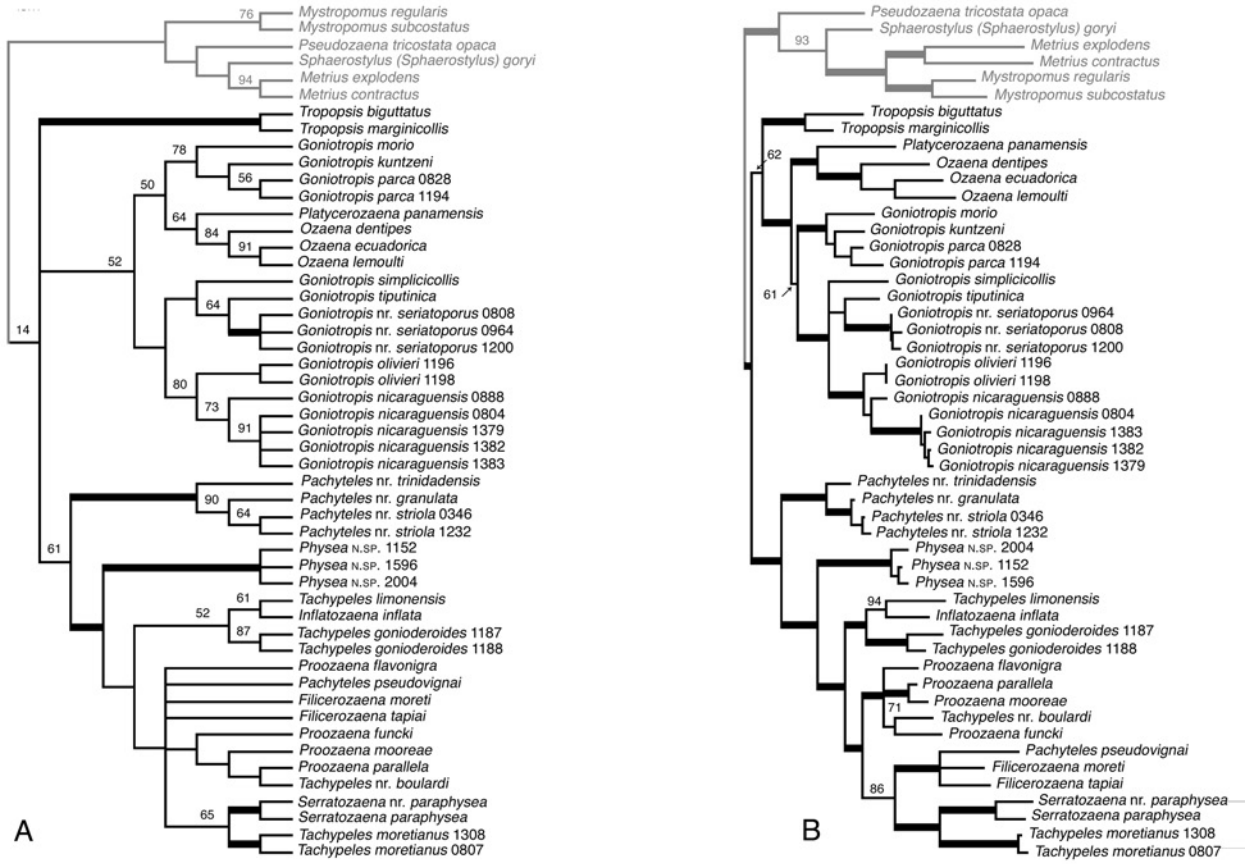

Fig. 2.—Optimal trees found for the COI dataset. Bootstrap values and posterior probability values are provided above each branch for values greater than 50 and less than 95; values greater than or equal to 95 are indicated by thick branches. **A**, strict consensus of the 58 most parsimonious trees (TL = 1650; CI = 0.31; RI = 0.62); **B**, Bayesian consensus tree.

including *Goniotropis, Ozaena* and *Platycerozaena*; the *Tropopsis* group, including *Tropopsis* and *Entomoantyx*; and the *Pachyteles* group, which includes *Pachyteles*, *Physea*, *Tachypeles*, *Inflatozaena*, *Filicerozaena*, *Proozaena*, *Serratozaena.* While the relationship among these three clades varies among data sets and analyses, their monophyletic status is consistently supported by all molecular data sets with high bootstrap and posterior probability values. This is a most surprising and unexpected result, given that there is not a single identified morphological apomorphy defining *Goniotropis*, *Tropopsis*, or *Pachyteles (sensu lato).* Based on our knowledge of morphology alone, one would synonymize these genera (see Ball and McCleve 1990). Nevertheless, there is now strong molecular evidence that *Pachyteles* (*sensu lato*), *Tropopsis*, and *Goniotropis* are not sister groups, but rather each of these genera is a member of a well-defined clade that includes other morphologically distinctive genera. In fact, according to the inferred phylogeny of the New World Ozaenini, combining *Goniotropis* and *Tropopsis* with *Pachyteles* would require combining every New World ozaenine genus into one highly varied genus, *Ozaena* (see Figs. 1–3 for individual analyses and Fig. 4 for the summary).

Ozaena genus group

Based on molecular evidence the *Ozaena* group includes *Goniotropis* and the easily recognized genera, *Ozaena* and *Platycerozaena*. Although this is one of the best-supported clades in every analysis of the molecular sequence data (Fig. 4), there is not an identified morphological apomorphy defining this group. Fortunately all members of the *Ozaena* group have a suite of characters, which allow members of this group to be clearly distinguished from members of the *Pachyteles* group. Although these characters are surely plesiomorphic, and therefore do not provide additional support for the monophyly of the *Ozaena* group, they are useful for distinguishing members of *Goniotropis* from the *Pachyteles* group. These characters include a well-defined mesopleurosternal suture, which separates the mesosternum from the mesepisternum (Fig. 5B), and a straight meta-katepisternal sulcus (Fig. 5D) that is well-defined throughout its length and reaches the metepimeron (Fig. 5F). All members of this group also have well-developed intercoxal processes that completely separate the middle coxae (Fig. 5B). However, this last character does not unambiguously separate the members of the *Ozaena*

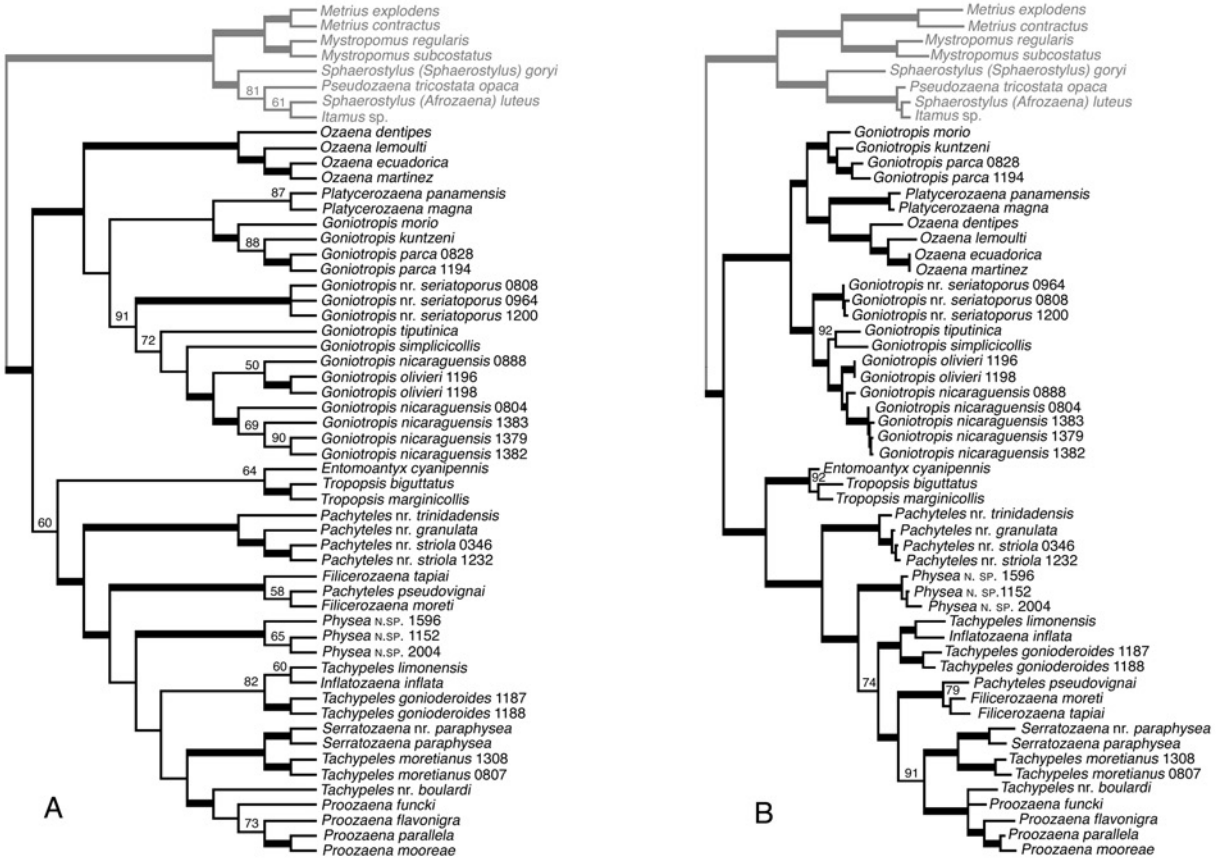

Fig. 3.—Optimal trees found for the 28S rDNA + COI dataset. Bootstrap values and posterior probability values are provided above each branch for values greater than 50 and less than 95; values greater than or equal to 95 are indicated by thick branches. **A**, strict consensus of the two most parsimonious trees (TL = 3394; CI = 0.43; RI = 0.76); **B**, Bayesian consensus tree.

group from all members of the *Pachyteles* group, since some members of the *Pachyteles* group (e.g., *Proozaena*) also have this character.

Goniotropis appears polyphyletic in most analyses (Figs. 1A–B, 2A, 3A–B) and appears monophyletic only in the Bayesian analysis of COI with a posterior probability of 61 (Fig. 2B). The genus *Scythropasus* (Chaudoir) was combined with *Goniotropis* by Bänninger in 1927. However, this synonymy is not recognized by all authors (see Deuve 2001, 2004). The type species, *Scythropasus elongatus* (Chaudoir) can be placed within a well-supported molecular clade consisting of *Goniotropis nicaraguensis* (Bates) and *Goniotropis olivieri* (Chaudoir). All members of this clade are long and narrow with striate elytra. Females have extremely long and narrow gonocoxae and males have adhesive setae on tarsomeres 1-3 (all other *Goniotropis* males have adhesive setae only on tarsomeres 1-2). Decisions as to whether the name *Goniotropis* belongs to all species labeled as such or should be restricted to a subset of those taxa, and whether the name *Scythropasus* should be removed from synonymy, must await determination of the boundaries of the genus *Goniotropis*, which appears polyphyletic in most trees presented here.

Tropopsis genus group

The *Tropopsis* group includes *Tropopsis* and *Entomoantyx*. Species in this group are distinguished from *Pachyteles* (*sensu lato*) and *Goniotropis* species by the shape of the forefemur. Members of the *Tropopsis* group have a smooth forefemur (see Ball and McCleve 1990, fig. 58), whereas members of *Pachyteles* (*sensu lato*) and *Goniotropis* have a ventral spine-like projection on the forefemur (see Ball and McCleve 1990, figs. 59–60). Members of *Entomoantyx* are the only Western Hemisphere Ozaenini with a single, long seta in the mandibular scrobe. This is a plesiotypic character within the Paussinae that predicts a basal position of *Entomoantyx* within the Ozaenina. Based on the molecular data available at this time, *Entomoantyx* is the sister group of the genus *Tropopsis*. In the future this relationship will be tested with additional molecular data, once a specimen of *Entomoantyx* is preserved adequately for molecular study. The results presented here are based on a small fragment of one gene (28S) amplified from a dried museum specimen of *Entomoantyx*.

Pachyteles genus group

The *Pachyteles* group includes *Pachyteles*, *Physea*, *Tachypeles*, *Inflatozaena*, *Filicerozaena*, *Proozaena*, and *Serratozaena.* This group is defined by a completely fused mesosternum and mesepisternum, without a meso-pleurosternal suture (Fig. 5A); and a sinuate meta-katepisternal sulcus (Fig. 5C) that completely fades before reaching the metepimeron (Fig. 5E). Most members of this group also have reduced intercoxal processes that result in contiguous middle coxae (Fig. 5A); however members of *Proozaena* are exceptions.

Results also provide evidence for polyphyly of the genera *Pachyteles* and *Tachypeles* as they are currently defined. The presence of a close relative of the type species of *Pachyteles*, *Pachyteles striola* Perty, in the taxon sampling allows us to recognize which clade should bear the name *Pachyteles* (Fig. 4). Unfortunately the type species of *Tachypeles*, *Tachypeles arechavaletae* (Chaudoir), was not included in the taxon sampling and it cannot be placed on the tree based on morphology without study of the type specimen. The genus *Tachypeles* is defined as having long, tubular gonocoxae with an apical setal organ (see Deuve 2001, fig. 21). The gonocoxae of DNA voucher specimens were dissected and many subtle differences in the shape of gonocoxae and positioning of setal organ were found among those specimens identified as belonging to *Tachypeles,* which attach to different places in the molecular trees. Gonocoxae with a long, tubular shape and an apical setal organ may have evolved multiple times. According to the results of the molecular analysis this appears to be a homoplasious character, which should be given less weight in future taxonomic work and more attention should be paid to the subtle characteristics of the gonocoxal shape, especially at the apex. For example, although the specimen identified as *Tachypeles* nr. *boulardi* (voucher DNA1378) has relatively long, narrow gonocoxae, attributes of the apex, setal organ and general shape other than length show close affinity to other specimens identified as *Proozaena*. Additional morphological work, in combination with molecular-based phylogenies with greater taxon sampling, will help determine those characters indicative of natural groups (Deuve and Moore, in prep).

One interesting aspect of the tree topology within the *Pachyteles* group is the sister-group relationship between *Serratozaena* species and *Tachypeles moretianus* Deuve (Fig. 4). As mentioned in the introduction to this paper, these three species are associated with *Atta*, the Neotropical leaf cutting ants, as are all *Physea* species. That the *Serratozaena* species, *Tachypeles moretianus,* and *Physea setosa* do not form a clade in any analysis (Figs. 1–3) indicates that Western Hemisphere ozaenines could have adapted to life with ants two or three times in evolutionary history, and/or the possibility that other members of the *Pachyteles* group are also associated with ants.

DISCUSSION

These results provide an example of the broad impact that the field of molecular phylogenetics is having on the field of systematics, for it was the result of the analyses of molecular data that guided the discovery of morphological characters which define the *Pachyteles* group and which allow one to distinguish members of *Pachyteles* and *Goniotropis*. This is testament to the potential power of molecular phylogenetics, not only to contribute to our knowledge of the phylogeny of a group, but also to contribute by guiding the discovery of additional morphological characters that we can use to identify and classify organisms. In turn, that the molecular clades reported here are consistent with some apparently strong morphological characters indicates that 28S and COI are good genes to infer the evolutionary history of Western Hemisphere Ozaenini, which has proven difficult to resolve based on morphology alone.

Definitive nomenclatural decisions within the three suprageneric clades are beyond the scope of this paper and must await careful comparisons of DNA voucher specimens and type specimens, as well as increased taxon sampling within select clades. Eventually, a combined molecular and morphological approach will be used to postulate a more resolved phylogeny of the genera within the suprageneric clades and to determine generic boundaries and additional diagnostic characters that will be used in keys to the genera (Deuve and Moore, in prep).

In the spirit of the continuing quest toward attaining knowledge of this challenging group, I borrow an eloquent line from Dr. George E. Ball (Ball and McCleve 1990:107): "I hope that those who use this publication find in the wealth of implied questions about ozaenines adequate recompense for the lack of answers that we have been able to provide."

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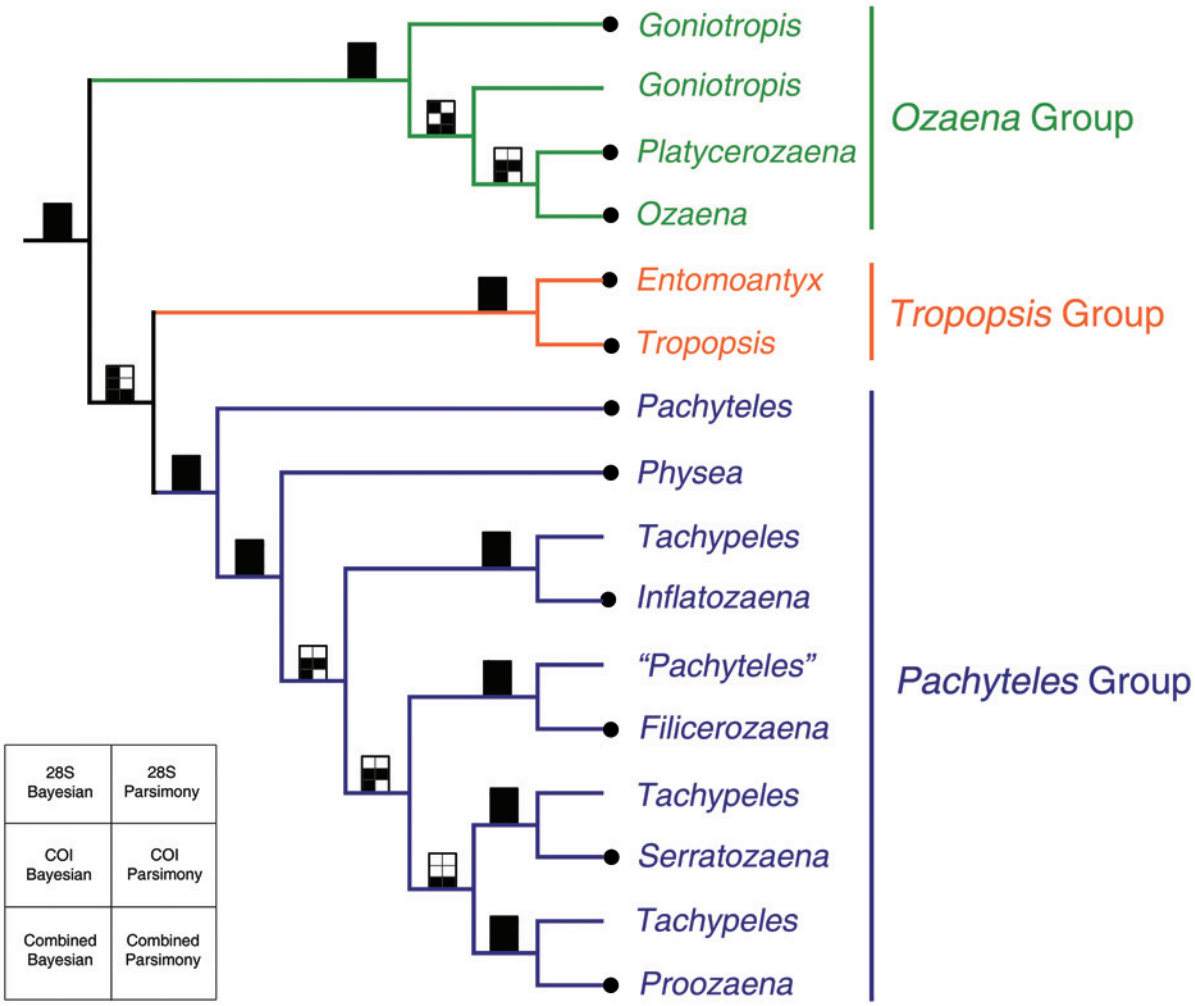

Fig. 4.—Summary tree depicting clades consistently supported by various datasets and analysis algorithms. Three well-supported suprageneric clades are color coded and labeled. Black dots at the end of terminal branches indicate the type species of the genus can confidently be placed on the tree either based upon strong morphological evidence or because the type species of the genus was included in the taxon sampling.

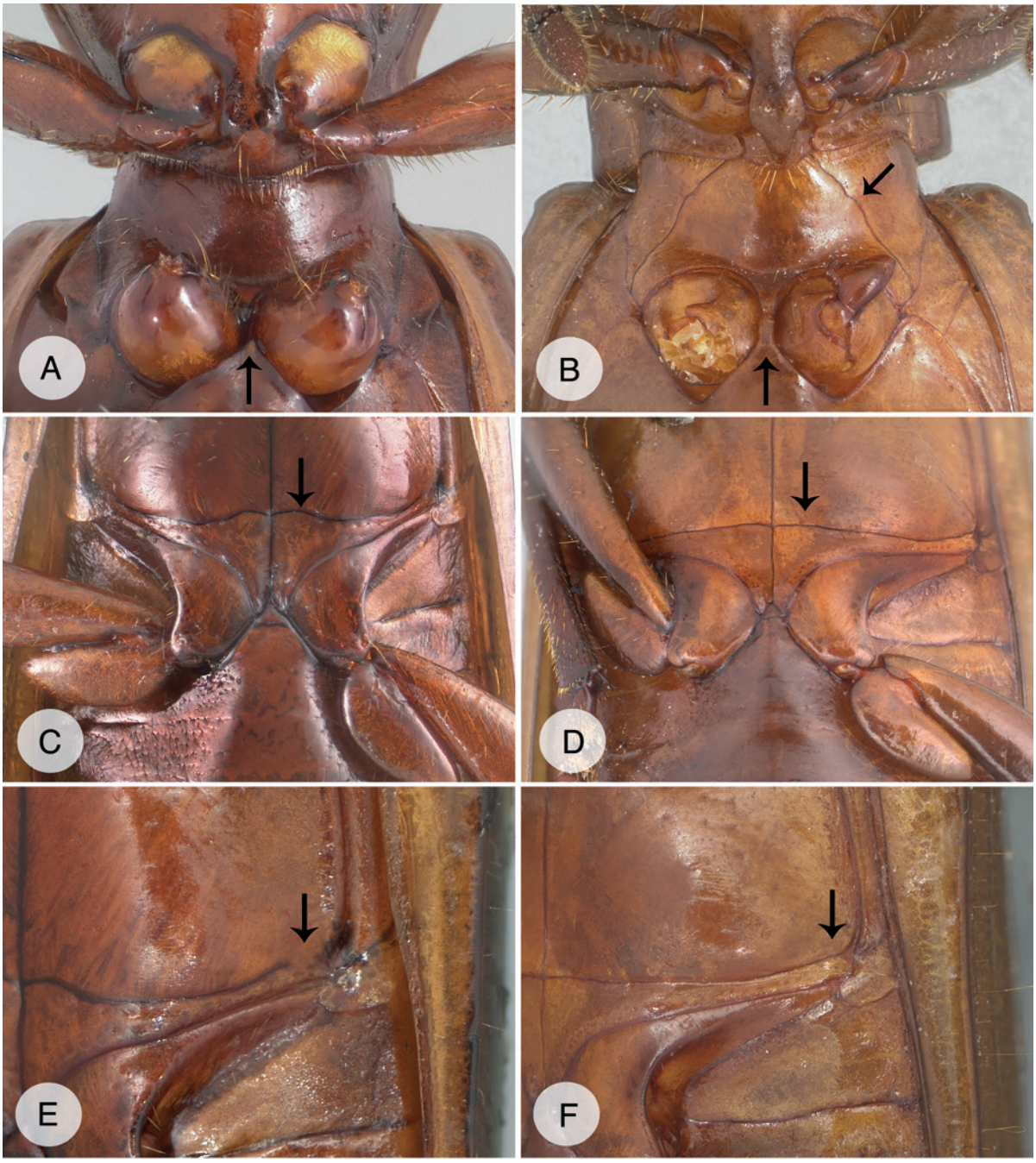

Fig. 5.—Diagnostic character states of select well-supported clades. **A, C, E**: *Pachyteles (sensu lato)*; **B, D, F**: *Goniotropis*. **A**, mesosternum and mesepisternum completely fused, and intercoxal processes of mesosternum and metasternum reduced such that the middle coxae touch medially; **B**, mesosternum and mesepisternum clearly separated by a distinct mesopleurosternal suture, and intercoxal processes of mesosternum and metasternum loosely articulated but completely separating the middle coxae such that they do not touch medially; **C**, metasternum with the metakatepisternal sulcus sinuate near the discriminal line; **D**, metasternum with the metakatepisternal sulcus straight near the discriminal line; **E**, right side of metasternum with metakatepisternal sulcus obliterated near metepimeron; **F**, right side of metaseternum with metakatepisternal sulcus well-defined throughout its length and reaching metepimeron.

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