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Molecular phylogenetics and evolution of host plant use in the Neotropical rolled leaf 'hispine' beetle genus *Cephaloleia* (Chevrolat) (Chrysomelidae: Cassidinae)

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Abstract

Here, we report the results of a species level phylogenetic study of *Cephaloleia* beetles designed to clarify relationships and patterns of host plant taxon and tissue use among species. Our study is based on up to 2088 bp of mtDNA sequence data. Maximum parsimony, maximum likelihood, and Bayesian methods of phylogenetic inference consistently recover a monophyletic *Cephaloleia* outside of a basal clade of primarily palm feeding species (the 'Arecaceae-feeding clade'), and *C. irregularis*. In all three analyses, the 'Arecaceae-feeding clade' includes *Cephaloleia* spp. with unusual morphological features, and a few species currently placed in other cassidine genera and tribes. All three analyses also recover a clade that includes all Zingiberales feeding *Cephaloleia* and most *Cephaloleia* species (the 'Zingiberales-feeding clade'). Two notable clades are found within the 'Zingiberales-feeding clade.' One is comprised of beetles that normally feed only on the young rolled leaves of plants in the families Heliconiaceae and Marantaceae (the 'Heliconiaceae & Marantaceae-feeding clade'). The other is comprised of relative host tissue generalist, primarily Zingiberales feeding species (the 'generalist-feeding clade'). A few species in the 'generalist-feeding clade' utilize Cyperaceae or Poaceae as hosts. Overall, relatively basal *Cephaloleia* (e.g., the 'Arecaceae clade') feed on relatively basal monocots (e.g., Cyclanthaceae and Arecaceae), and relatively derived *Cephaloleia* (e.g., the 'Zingiberales-feeding clade') feed on relatively derived monocots (mostly in the order Zingiberales). Zingiberales feeding and specialization on young rolled Zingiberales leaves have each apparently evolved just once in *Cephaloleia*.

Keywords: Cassidinae; Cephaloleia; Chrysomelidae; Hispinae; mtDNA; Zingiberales

1. Introduction

"The plant herbivore "interface" may be the major zone of interaction responsible for generating terrestrial organic diversity (Ehrlich and Raven, 1964)." Several ecological hypotheses have been proposed to explain how plant/insect interactions affect diversification (e.g., Berenbaum, 1983; Futuyma and Moreno, 1988; Thompson, 1994), for example, as a function of the physical environment, spatial distribution of resources, competition for resources, or limitations to dispersal. The advent of modern (especially molecular) phylogenetic studies has occasioned complementary, explicitly historical approaches to the study of plant/insect diversification (Farrell, 1998, 1999; Mitter et al., 1988; Page, 1994). Nevertheless, most such studies have focused on temperate insects, especially those feeding on conifers and dicots (Farrell et al., 2001; Jordal et al., 2000; Kelley and Farrell, 1998; Kelley et al., 2000; Normark et al., 1999; Sequeira et al., 2000; Sequeira and Farrell, 2001), with comparatively little study of tropical insect herbivores or monocot associates. Here, we address this deficiency by

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examining the phylogenetic relationships among monocot feeding Neotropical beetles in the genus *Cephaloleia* (Chevrolat) (Chrysomelidae: Cassidinae).

With more than 200 described species, *Cephaloleia* is one of the most species rich genera of monocot feeding leaf beetles (Staines, 1996, 2004). Members of the genus have been the subjects of ecological study for more than 25 years (Johnson, 2004; Seifert and Seifert, 1976; Strong, 1977a,b, 1982). *Cephaloleia* feed only on Monocotyledonae, especially the young rolled leaves of plants in the order Zingiberales, and the young folded leaves of various Arecaceae. Other *Cephaloleia* host plants include Bromeliaceae, Cyclanthaceae, Cyperaceae, Orchidaceae, and Poaceae (Table 1) (D. McKenna, unpublished data; D. Windsor pers. comm., 2002; Staines, 1996, 2004). Fossil evidence suggests that *Cephaloleia*-like beetles have maintained specialized interactions with their Zingiberales host plants for more than 66 Ma (Wilf et al., 2000). *Cephaloleia* are known from a diversity of tropical and subtropical New World plant communities from Mexico and Cuba to Argentina. All life stages and most behavior, including mating, take place on host plants, and for many Zingiberales feeders, almost entirely in rolled leaves (Auerbach and Strong, 1981; Morrison and Strong, 1981; Seifert and Seifert, 1976; Strong, 1977a,b, 1982).

Cephaloleia has traditionally been placed in the tribe Cephaloleiini Baly in the subfamily Hispinae (leaf-mining beetles) (Hincks, 1952), but recent studies have shown that the Hispinae are polyphyletic, and the division of the Cryptostoma into the subfamilies Cassidinae and Hispinae is unnatural (Borowiec, 1995). Here, the subfamily name Cassidinae is used following Staines (2002). The subfamily Cassidinae belongs to the exceptionally diverse family Chrysomelidae (leaf or plant beetles). Despite recent advances in subfamily-level taxonomy, the tribal-level taxonomy of former cassidoid Hispinae remains mostly contentious (Borowiec and Swietojanska, 2002; Staines, 2002; Staines and Staines, 1989, 1992).

Phylogenetic relationships among the more than 200 species of *Cephaloleia* are virtually unknown. Thus, the evolutionary history of host taxon and host tissue use in the genus remains uncertain. We do not know the number of origins, or the distribution of Zingiberales or leaf roll feeding in *Cephaloleia*, or whether they are derived or primitive traits. Here, we use maximum parsimony (MP), maximum likelihood (ML), and Bayesian methods of phylogenetic inference to examine relationships among *Cephaloleia* species based on partial mitochondrial DNA (mtDNA) sequences from cytochrome oxidase subunit I (COI), tRNA-leucine (tRNA-Leu), cytochrome oxidase subunit II (COII), and cytochrome *b* (Cyt *b*). In the context of these phylogenies, we then discuss patterns of host taxon and host tissue use in *Cephaloleia* (e.g., Zingiberales feeding, and specialization on young rolled leaves).

2. Materials and methods

2.1. Taxa

2.1.1. Taxon sampling and specimen identification

We sampled 105 taxa (98 ingroup, 7 outgroup) including 75 a priori designated *Cephaloleia* species for this study (Table 1). Central American *Cephaloleia* were sampled most thoroughly since they have been the subject of most interest in the genus, and because it was possible to identify many of them following Staines (1996). The omission of *Cephaloleia* spp. from the Atlantic coastal forests of South America is unfortunate. Efforts to obtain specimens from this region for DNA sequencing were unsuccessful. However, if morphology and host plant affiliations are reliable indicators of phylogeny, species endemic to this region are closely related to species we sampled elsewhere.

We used the descriptions and keys in Staines (1996) to identify Central American *Cephaloleia* species. Thus, the taxon sometimes called *Demotispa lata* Baly is here called *Cephaloleia lata* Baly (Staines, 1996). No comprehensive keys are available for South American *Cephaloleia*, so we primarily used museum specimens (including types), and Staines (1996) when possible, for identification. Based on field and morphological studies, several species in Staines (1996) were identified that included one or more potentially undescribed cryptic species. We included many of these a priori designated potentially unnamed taxa in this study, indicated by the Latin abbreviation cf. Species that are clearly over split are not explicitly indicated. Representatives of such taxa were included in this study (e.g., *C. championi* and *C. leucoxantha*). Several species are referred to by their respective DNA codes because they are undescribed, or because there are no reliable taxonomic revisions available to facilitate their identification (Table 1). Many of the specimens sequenced from the Arthropods of La Selva collection (ALAS) and from the Costa Rican National Biodiversity Institute (INBIO) were annotated by Staines (Edgewater, MD), an expert on *Cephaloleia*. Borowiec (University of Wroclaw) confirmed the identification of *Pseudostilpnaspis columbica* Borowiec, and McKenna made all other identifications. Voucher specimens are housed at the Harvard University Museum of Comparative Zoology (MCZ), Cambridge, Massachusetts, USA.

2.1.2. Outgroup choice

Phylogenetic relationships within and between cassidine tribes remain mostly unclear or unknown, so we selected outgroup taxa from several tribes: *Alurnus ornatus* Baly (Alurnini), *Chelobasis perplexa* Baly (Arescini), *Demotispa* sp. 175 (Cephaloleiini), *Imatidium* cf. *rufiventre* Boheman (Imatidiini), *Prosopodonta limbata* Baly (Prosopodontini), and *Pseudostilpnaspis columbica*

Table 1
Specimen information

Species	DNA code	COI[a]	tRNAleu[a]	COII[a]	Cyt *b*[b]	Plant collected from	Collector	Date	Locality
adusta Uhmann	057	1–1044	1045–1102	1103–1645	1–449	*Heliconia* sp. (Heliconiaceae)	DM	15 July 2002	Carate, Costa Rica
aequilata Uhmann	151	860–1044	1045–1102	1103–1645	0	*Calopterogyne ghiesbreghtiana* (Arecaceae)	DM	January 2004	OTS La Selva, Costa Rica
aequilata Uhmann	152	869–1044	1045–1102	1103–1645	0	*Calopterogyne ghiesbreghtiana*	DM	January 2004	OTS La Selva, Costa Rica
cf. *aequilata* Uhmann	108	1015–1044	1045–1102	1103–1645	46–449	*Chamaedorea* sp. (Arecaceae)	DW	24 August 1997	Lita, Ecuador
alternans Waterh.	082	277–1044	1045–1102	1103–1645	0	*Heliconia* sp.	DW	23 August 1997	Lita, Ecuador
antennalis Donckier	007	1–1044	1045–1102	1103–1645	1–449	*Renealmia* sp.	DM	12–21 Febraury 2003	La Tirimbina, Costa Rica
apicata Uhmann	042	1–1044	1045–1102	1103–1635	22–449	*Heliconia gracilis*	DM	16 July 2002	San Isidro, Costa Rica
bella Baly	017	1–1044	1045–1102	1103–1645	0	*Heliconia imbricata*	DM	16 July 2002	Corcovado NP, Costa Rica
belti Baly	095	1–1044	1045–1102	1103–1645	21–449	*Heliconia* sp.	DW	12 April 2003	La Fortuna, Panama
belti Baly	117	894–1044	1045–1102	1103–1645	0	*Heliconia* sp.	DM	23–30 June 2001	Braulio Carillo NP, Costa Rica
cf. *belti* Baly	134	859–1044	1045–1102	1103–1645	0	*Heliconia* sp.	DM	May 2002	Monteverde, Costa Rica
championi Baly	027	1–1044	1045–1102	1103–1645	22–49	*Heliconia* sp.	MB/RE/KN	29 September–14 October 2001	Rio Grande Orosi, Costa Rica
championi Baly	141	856–1044	1045–1102	1103–1645	0	*Heliconia secunda*	DM	January 2004	Braulio Carillo NP, Costa Rica
congener Baly	048	1–1044	1045–1102	1103–1645	1–449	*Heliconia imbricata*	DM	15 July 2002	Corcovado NP, Costa Rica
consanguinea Baly	025	1–1044	1045–1102	1103–1617	1–449	*Heliconia tortuosa*	DM	25 April 2002	Monteverde, Costa Rica
cyanea Staines	098	859–1044	1045–1102	1103–1645	25–381	Unknown	DW	10 April 2003	La Fortuna, Panama
deficiens Uhmann	001	1–1044	1045–1060	0	1–449	*Costus* sp. (Costaceae)	DM	2–6 April 2003	OTS La Selva, Costa Rica
deficiens Uhmann	150	859–1044	1045–1102	1103–1645	0	*Costus* sp.	DM	11 March 2003	La Virgen, Costa Rica
deficiens Uhmann	156	858–1044	1045–1102	1103–1645	0	*Costus* sp.	ALAS	8 April 2003	Southeast of La Virgen, Costa Rica
dilaticollis Baly	015	1–1044	1045–1102	1103–1645	1–449	*Calathea lutea* (Marantaceae)	DM	October 2002	Old Gamboa Rd., Panama
dilaticollis Baly	031	532–1044	1045–1102	1103–1645	0	*Calathea* sp.	DM	October 2002	Darien Prov., Panama
dilaticollis Baly	089	1–475, 1000–1044	1045–1102	1103–1645	25–381	*Calathea* sp.	DW	24 August 1997	Lita, Ecuador
cf. *dilaticollis* Baly	092	286–953, 1006–1044	1045–1102	1103–1645	0	*Renealmia* sp. (Zingiberaceae)	DW	16 August 1997	Yasuni NP, Ecuador
distincta Baly	053	1–1044	1045–1102	1103–1599	0	*Heliconia* sp.	DM	October 2002	Old Gamboa Rd., Panama
distincta Baly	110	1–1044	1045–1102	1103–1385	109–440	*Heliconia* sp.	DM	14 July 2003	Omar Torrijos NP, Panama
cf. *dorsalis* Baly	119	891–1044	1045–1102	1103–1645	0	*Renealmia* sp.	DW	7 August 1997	Ecuador
erichsonii Baly	008	1–1044	1045–1102	1103–1645	1–449	*Calathea gymnocarpa*	DM	2–6 April 2003	OTS La Selva, Costa Rica
erichsonii Baly	040	1–1044	1045–1102	1103–1645	1–449	*Calathea* sp.	DM	16 July 2002	Corcovado NP, Costa Rica
cf. *erichsonii* Baly	010	286–1044	1045–1102	1103–1645	1–449	*Heliconia* sp. or *Calathea* sp.	SR	5 January 2003	La Virginia, Risaralda, Colombia
cf. *erichsonii* Baly	028	1–1044	1045–1102	1103–1631	1–449	*Calathea* sp.	DM	October 2002	Old Gamboa Rd., Panama
exigua Uhmann	012	1–1044	1045–1102	1103–1645	1–449	*Cyclanthus bipartitus* (Cyclanthaceae)	DM	12–21 Febraury 2003	Gandoca–Manzanillo, Costa Rica
exigua Uhmann	023	274–1044	1045–1102	1103–1645	1–449	*Cyclanthus bipartitus*	DM	October 2002	Fort Sherman, Panama
fenestrata Weise	013	1–1044	1045–1102	1103–1645	1–449	*Pleiostachya pruinosa* (Marantaceae)	DM	12–21 Febraury 2003	Boca Tapada, Costa Rica
fenestrata Weise	021	1–1044	1045–1102	1103–1645	1–449	*Pleiostachya pruinosa*	DM	23 March–16 July 2002	OTS La Selva, Costa Rica
cf. *fenestrata* Weise	032	1–1044	1045–1102	1103–1645	1–449	*Pleiostachya pruinosa*	DM	15 July 2002	Puerto Jimenez–Carate Rd., Costa Rica

(*continued on next page*)

Table 1 (*continued*)

Species	DNA code	COI[a]	tRNAleu[a]	COII[a]	Cyt *b*[b]	Plant collected from	Collector	Date	Locality
cf. *fenestrata* Weise	2146	2589–1024	0	0	1–440	*Pleiostachya pruinosa*	DM	15 July 2002	Puerto Jimenez–Carate Rd., Costa Rica
flava Uhmann	065	10–1044	1045–1102	q	0	Unknown	DM	25 April 2002	Monteverde, Costa Rica
cf. *fulvicollis* Weise	049	1–1044	1045–1102	1103–1645	12–449	*Heliconia* sp.	DM	16 July 2002	Corcovado NP, Costa Rica
fulvolimbata Baly	126	1–1044	1045–1102	1103–1645	0	Unknown	ALAS	17 April 2003	Southeast of La Virgen, Costa Rica
gilvipes Uhmann	186	856–1044	1045–1102	1103–1637	0	Unknown	INBIO	22 March–9 April 2002	Vara Blanca, Costa Rica
gilvipes Uhmann	187	865–1044	1045–1102	1103–1635	0	Unknown	INBIO	Unknown	Costa Rica
gratiosa Baly	130	860–1044	1045–1102	1103–1645	0	*Heliconia lutea*	DM	14 July 2003	El Llano-Carti Rd., Panama
gratiosa Baly	131	45–203, 860–1044	1045–1102	1103–1645	8–449	*Heliconia spathocircinata*	DM	14 July 2003	El Llano-Carti Rd., Panama
heliconiae Uhmann	006	1–1044	1045–1102	1103–1641	1–386	*Calathea lutea*	DM	12–21 Febraury 2003	OTS La Selva, Costa Rica
heliconiae Uhmann	035	187–1044	1045–1102	1103–1645	12–449	*Calathea* sp.	MB/RE/KN	29 September–14 October 2001	Monteverde, Costa Rica
cf. *heliconiae* Uhmann	097	1–1044	1045–1102	1103–1615	0	*Calathea* sp.	DW	12 April 2003	La Fortuna, Panama
histrio Guérin	011	286–1044	1045–1102	1103–1645	53–449	*Heliconia* sp. or *Calathea* sp.	SR	5 January 2003	La Virginia, Risaralda, Colombia
histrionica Baly	070	1–1044	1045–1102	1103–1645	96–449	Unknown	DM	16 July 2002	Corcovado NP, Costa Rica
cf. *histrionica* Baly	003	1–1044	1045–1102	1103–1645	1–449	*Calathea* sp.	DM	12–21 Febraury 2003	OTS La Selva, Costa Rica
cf. *histrionica* Baly	068	1–1044	1045–1102	1103–1645	1–449	*Calathea* sp.	DM	16 July 2002	Corcovado NP, Costa Rica
immaculata Staines	132	274–1044	1045–1102	1103–1645	1–449	*Heliconia* sp.	DM	15 July 2002	Puerto Jimenez-Carate Rd., Costa Rica
instabilis Baly	2313	292–980	0	0	1–449	*Heliconia wagneriana*	DM	16 July 2002	Corcovado NP, Costa Rica
irregularis Uhmann	159	913–1044	1045–1102	1103–1645	0	Unknown	ALAS	13–21 Febraury 2002	Vara Blanca, Costa Rica
lata Baly	005	1–1044	1045–1102	1103–1645	1–449	*Chamaedorea tepejilote* (Arecaceae)	DM	2–6 April 2003	OTS La Selva, Costa Rica
lata Baly	067	1–1044	1045–1102	1103–1645	12–449	*Chamaedorea tepejilote*	DW	9 July 2002	OTS La Selva, Costa Rica
lepida Staines	128	860–1044	1045–1102	1103–1645	0	Unknown	DW	12 April 2003	La Fortuna, Panama
leucoxantha Baly	054	1–1044	1045–1102	1103–1645	12–440	*Heliconia* sp.	MB/RE/KN	29 September–14 October 2001	Rio Grande Orosi, Costa Rica
luctuosa Guérin	056	1–1044	1045–1102	1103–1645	12–398	*Heliconia* sp.	DM	Oct 2002	Old Gamboa Rd., Panama
marginella Uhmann	026	1–1044	1045–1102	1103–1645	1–449	*Heliconia tortuosa*	DM	25 April 2002	Monteverde, Costa Rica
marginella Uhmann	096	1–1044	1045–1102	1103–1645	0	*Heliconia* sp.	DW	12 April 2003	La Fortuna, Panama
mauliki Uhmann	064	1–1044	1045–1102	1103–1645	1–440	Unknown	DM	22 March 2002	OTS La Selva, Costa Rica
metallescens Baly	182	1006–1044	1045–1102	1103–1635	0	Unknown	INBIO	Unknown	Costa Rica
nigricornis (Fab.)	143	902–1044	1045–1102	1103–1645	0	Unknown	INBIO	17 April 2003	Southeast of La Virgen, Costa Rica
nigropicta Baly	2316	259–991	0	0	0	*Heliconia latispatha*	DM	1–10 August 2001	Pipeline Rd., Panama
ornatrix Donckier	366	914–1044	1045–1102	1103–1639	1–449	*Heliconia mariae*	DM	9 July 2002	OTS La Selva, Costa Rica
partita Weise	020	17–1044	1045–1102	1103–1645	0	*Heliconia latispatha*	DM	1–10 August 2001	Pipeline Rd., Panama
partita Weise	077	1–1044	1045–1102	1103–1605	0	*Heliconia* sp.	DM	October 2002	Old Gamboa Rd., Panama
placida Baly	055	1–1044	1045–1102	1103–1645	12–371	*Heliconia* sp. *or Calathea* sp.	DM	3 April 2003	Braulio Carillo NP, Costa Rica
pretiosa Baly	009	1–1044	1045–1102	1103–1645	1–449	*Heliconia* sp.	SR	5 January 2003	La Virginia, Risaralda, Colombia
cf. *pulchella* Baly	104	292–1044	1045–1102	1103–1645	109–440	*Calathea* sp.	DW	20 August 1997	Puerto Misahualli, Ecuador
quadrilineata Baly	039	1–1044	1045–1102	1103–1645	1–449	*Heliconia latispatha*	DM	16 July 2002	Rincon, Costa Rica
cf. *quadrilineata* Baly	036	1–1044	1045–1102	1103–1645	1–449	*Heliconia latispatha*	DM	15 July 2002	Corcovado NP, Costa Rica
reventazonica Uhmann	016	1–1044	1045–1102	1103–1645	1–449	*Heliconia latispatha*	DM	18 June 2002	Trinidad, Costa Rica

ruficollis Baly	149	860–1044	1045–1102	1103–1645	0	Unidentified Poaceae	ALAS	23 March 2003	Southeast of La Virgen, Costa Rica
sagittifera Uhmann	085	1–503, 1006–1044	1045–1102	1103–1645	0	*Calathea* sp.	DW	26 August 1997	Yasuni NP, Ecuador
sallei Baly	072	1–1044	1045–1102	1103–1645	1–449	*Heliconia irrasa*	DM	3 June 2002	OTS La Selva, Costa Rica
cf. *schmidti* Uhmann	107	1–1044	1045–1102	1103–1645	89–449	Unknown	DW	August 1997	Lita, Ecuador
semivittata Baly	177	1015–1044	1045–1102	1103–1645	0	Unknown	INBIO	Unknown	Costa Rica
sp. 060	060	854–1044	1045–1102	1103–1645	0	Unknown	DW	2001	Darien NP, Panama
sp. 113	113	875–1044	1045–1102	1103–1645	0	*Chamaedorea pinnatifida*	DW	17 August 1997	Yasuni NP, Ecuador
sp. 114	114	871–1044	1045–1102	1103–1624	0	Unknown	DW	19 August 1997	Rio Hollin, Ecuador
sp. 136	136	871–1044	1045–1102	1103–1645	0	*Heliconia* sp.	SR	14 July 2003	Volcan Tacana, Mexico
sp. 181	181	891–1044	1045–1102	1103–1645	0	Unknown	ALAS	13–21 Febraury 2002	Vara Blanca, Costa Rica
splendida Staines	094	1–1044	1045–1102	1103–1645	0	Unknown	DW	12 April 2003	La Fortuna, Panama
stenosoma Baly	043	1–1044	1045–1102	1103–1645	1–449	*Heliconia* sp.	DM	16 July 2002	Corcovado NP, Costa Rica
stevensi Baly	066	238–1044	1045–1102	1103–1645	1–449	*Calathea micans*	DM	22 March 2002	OTS La Selva, Costa Rica
suaveola Baly	122	860–1044	1045–1102	1103–1645	0	*Heliconia* sp.	SR	13 July 2003	El Asintal, Guatemala
cf. *suaveola* Baly	142	855–1044	1045–1102	1103–1645	0	*Heliconia* sp.	SR	15 July 2003	El Triunfo, Mexico
suturalis Baly	047	1–1044	1045–1102	1103–1645	1–449	*Costus* sp.	DM	12–21 Febraury 2003	La Virgen, Costa Rica
tenella Baly	004	1–1044	1045–1102	1103–1645	1–449	Unidentified Cyperaceae	DM	2–6 April 2003	OTS La Selva, Costa Rica
tetraspilota Guérin	071	1–1044	1045–1102	1103–1645	0	Unknown Zingiberaceae	DM	6 July 2002	OTS La Selva, Costa Rica
trimaculata Baly	029	1–1044	1045–1102	1103–1645	7–449	*Renealmia* sp.	DM	October 2002	Old Gamboa Rd., Panama
trivittata Baly	002	1–1044	1045–1102	1103–1645	46–413	*Calathea* sp.	DM	2–6 April 2003	OTS La Selva, Costa Rica
trivittata Baly	069	238–1044	1045–1102	1103–1645	0	*Calathea* sp.	DM	16 July 2002	Corcovado NP, Costa Rica
trivittata Baly	075	1–1044	1045–1102	1103–1645	1–449	*Pleiostachya pruinosa*	DM	23 March 2002	OTS La Selva, Costa Rica
uhmanii Staines	144	872–1044	1045–1102	1103–1645	0	Unidentified Poaceae	ALAS	20 April 2003	Southeast of La Virgen, Costa Rica
uhmanii Staines	147	859–1044	1045–1102	1103–1645	0	Unidentified Poaceae	ALAS	20 April 2003	Southeast of La Virgen, Costa Rica
unctula Weise	079	1–477, 1019–1044	1045–1102	1103–1645	0	*Stromanthe* sp. (Marantaceae)	DW	23 August 1997	Lita, Ecuador
Outgroups									
Alurnus ornatus Baly	165	44–686, 725–919	0	0	0	*Asterogyne martiana* (Arecaceae)	DM	January 2004	OTS La Selva, Costa Rica
Chelobasis perplexa Baly	176	838–1044	1045–1102	1103–1645	0	*Heliconia pogonantha*	DM	2002	OTS La Selva, Costa Rica
Crioceris duodecimpunctata (L.)	N/A	1–1044	1045–1102	1103–1645	12–449		GenBank AF467886		
Demotispa sp. 175	175	832–1044	1045–1102	1103–1645	0	*Asterogyne martiana*	DM	12–21 Febraury 2003	OTS La Selva, Costa Rica
Imatidium cf. *rufiventre* Boheman	172	842–1044	1045–1102	1103–1645	0	Unidentified Poaceae	DW	16 August 1997	Yasuni NP, Ecuador
Prosopodonta limbata Baly	155	863–1044	1045–1102	1103–1645	0	Unknown	ALAS	23 March 2003	Southeast of La Virgen, Costa Rica
Pseudostilpnaspis columbica Borowiec	100	1–492, 1009–1044	1045–1102	1103–1645	7–449	*Geonoma* sp. (Arecaceae)	DW	17 August 1997	Yasuni NP, Ecuador

Collectors: Arthropods of La Selva Project (ALAS), D. McKenna (DM), Costa Rican Institute for Biodiversity (INBIO), S. Ramirez, Harvard University Museum of Comparative Zoology (SR), M. Braby, R. Eastwood, and K. Nishida, Harvard University Museum of Comparative Zoology (MB/RE/KN), and D. Windsor, STRI (DW). Plant identifications were provided by the collector(s) or were made by DM.

[a] COI position 1 is located at position 1470 in the *Drosophila yakuba* mtDNA (Clary and Wolstenholme, 1985). We typically amplified a single fragment (s1859–a3661) containing consecutively, the last part of COI, the entire tRNAleu, and the first part of COII. Therefore, these gene regions are reported here using a single range of numbers 1–1645. The number of nucleotides reported for tRNA-Leu includes the six nucleotide ambiguously aligned region that was excluded from all analyses.

[b] Cyt *b* position 1 is located at position 10,923 in the *D. yakuba* mtDNA (Clary and Wolstenholme, 1985).

Borowiec (Imatidiini). All trees were rooted with *Crioceris duodecimpunctata* (L.) (Criocerinae: Criocerini) because we predicted that one or more of the other outgroup taxa may be paraphyletic with palm feeding *Cephaloleia* (predicted to be the pleisomorphic condition in *Cephaloleia*) (Farrell and Sequeira, 2004; Reid, 1995). Relationships between outgroups are beyond the scope of this paper.

2.2. Laboratory procedures

2.2.1. DNA extraction, amplification, and sequencing

Most specimens used in this study were collected in the field from host plants, but a few had been previously pinned and dried (Table 1). Total genomic DNA was extracted from the abdomen, legs (1–3), or the entire specimen, using a QIAquick DNeasy Tissue Kit (Qiagen, Valencia, CA). We used 50 μL volume PCRs comprised of 37.35 μL water, 5 μL of 5× buffer (Qiagen Inc.), 0.4 μL of 10 mM dNTP (Qiagen Inc.), 1 μL of each 10 mM primer, 3 μL of 2.5 mM $MgCl_2$ (Qiagen Inc.), 0.25 μL of *Taq* DNA Polymerase (Qiagen Inc.), and approximately 100 ng of genomic DNA template per 25 μL PCR volume.

To amplify COI, tRNA-Leu, and COII, we primarily used the following PCR program: (1) an initial denaturation of 5 min at 94 °C; (2) 30 s at 94 °C denaturation, 30 s at 49 °C annealing, and 1:30 min at 72 °C extension (40×); and (3) a final extension of 5 min at 72 °C. We occasionally used a program that differed from the above by having an initial denaturation of 1 min at 94 °C, and 30 s at 47 °C annealing. To amplify Cyt *b*, we primarily used the following program: (1) an initial denaturation of 5 min at 94 °C; (2) 30 s at 94 °C denaturation, 1:30 min at 58 °C annealing, and 1 min at 72 °C extension (2×); (3) 1 min each at 56 °C annealing and 72 °C extension (repeated twice at 2 °C annealing increments from 58–44 °C); (4) 1 min each at 42 °C annealing, and 72 °C extension (×18); and (5) a single final extension of 5 min at 72 °C. Amplified PCR products were cleaned with a QIAquick PCR Purification Kit (Qiagen Inc.), or were gel purified using a Qiagen QIAquick Gel Purification Kit.

Sequencing was performed using ABI PRISM BigDye Terminator Cycle Sequencing Kits (versions 3.0 and 3.1) (Applied Biosystems). The same primers were used for amplification and sequencing (Table 2). We designed some primers specifically for use in the genus *Cephaloleia* (using Oligo Primer Analysis Software version 4.05 (Long Lake, MN)), but in most cases the universal primers worked most reliably across taxa. All sequencing was performed on an ABI PRISM 3100 Genetic Analyzer.

2.2.2. DNA sequence data

We targeted an approximately 1800-base pair (bp) fragment (primers s1859–a3661) for amplification that included (1) part of the mtDNA COI gene, (2) the entire tRNA-Leu, and (3) a portion of the mtDNA COII gene (Table 2). We separately amplified an approximately 450 bp fragment of the mtDNA Cyt *b* gene (primers CB1, CytB B.1, CBdms, or CB1c, to CB2). We included taxa in our analyses even when they were represented by only a subset of the potential total sequence data (Table 1). This approach is supported by simulation studies which have shown that even highly incomplete taxa can be accurately placed in combined analyses with sufficient phylogenetically informative characters (Wiens, 1998, 2003a,b). Sequence for *Crioceris duodecimpunctata* (L.) was obtained from GenBank (Accession No. AF467886 (Stewart and Beckenbach, 2003)). All DNA sequences

Table 2
Oligonucleotide primers (5′ to 3′) used for amplification and sequencing

Name	Position[a]	Region	Sequence	Source
s1718	1693–1718	COI	GGA GGA TTT GGA AAT TGA TTA GTT CC	Farrell (2001)
s1859	1834–1859	COI	GGA ACI GGA TGA ACW GTT TAY CCI CC	Simon et al. (1994)
s2183	2161–2183	COI	CAA CAT TTA TTT TGA TTT TTT GG	Simon et al. (1994)
s2191	2191–2215	COI	GAA GTT TAT ATT TTA ATT TTA CCR G	Farrell (2001)
s2442	2410–2441	COI	CCA ACA GGA ATT AAA ATT TTT AGA TGA TTA GC	Modified from Simon et al., 1994
s2442B	2410–2441	COI	CCH ACW GGA ATT AAA ATT TTY AGA TGA YTA GC	Modified from Simon et al., 1994
s2798	2770–2798	COI	GGW ATA CCW CGA CGT TAY TCT GAY TAT CC	Dobler and Farrell (1999)
a2963	2993–2963	COI	AGG RAG TTC ATT ATA IGA ATG TTC	Normark et al. (1999)
s2993	2971–2994	COI	CWC CWG CWG AAC ATA GAT AAT CWG AAC TTC C	Dobler and Farrell (1999)
a3014	3038–3014	COI	TCC AAT GCA CTA ATC TGC CAT ATT A	Simon et al. (1994)
a3661	3684–3661	COII	CCA CAA ATT TCT GAA CAT TGA CCA	Simon et al. (1994)
CytB B.1	10,638–10,667	Cyt *b*	TTA ATT ATT CAA ATT GCA ACA GGA TTA TTT	Cryan et al. (2001)
CBdms	10,773–10,801	Cyt *b*	GGA GCW TCT TTM TTC TTT ATT TGT CTT TA	This study
CB1	10,908–10,933	Cyt *b*	TAT GTA CTA CCA TGA GGA CAA ATA TC	Crozier and Crozier (1992), Vogler and Welsh (1997)
CB1c	10,931–10,954	Cyt *b*	ATC ATT YTG AGG RGC NAC AGT ATT	This study
CB2	11,392–11,367	Cyt *b*	AAT ACA CCT CCT AAT TTA TTA GGA AT	Crozier and Crozier (1992), Vogler and Welsh (1997)

[a] Position relative to *Drosophila yakuba* mtDNA (Clary and Wolstenholme, 1985).

Table 3
Characteristics of the mtDNA regions sequenced, including the number of taxa in each analysis, the total number of characters (excluding the six nucleotide ambiguous region excluded from analyses), the number of parsimony informative and variable characters, and the number of equally parsimonious trees

Gene	Total number of taxa	Total number of characters	Parsimony informative characters	Variable characters	MP tree length	Number of equally parsimonious trees
COI	105	1044	473	539	4455	>50,000
tRNA-Leu	101	52	16	25	91	>50,000
COII	100	543	298	335	3418	27
Cyt *b*	57	449	233	247	1944	4
Combined	105	2088	1020	1156	10,400	162

were deposited in GenBank under Accession Nos. DQ026066–DQ026225.

2.2.3. Alignment of nucleotide sequences and preliminary sequence analysis

Protein coding sequences were aligned by eye using Sequencher 3.1.1 (GeneCodes Corporation, 1999) and viewed using MacClade 4.03 (Maddison and Maddison, 2001). A six-nucleotide region at the 3′ end of tRNA-Leu could not be unambiguously aligned and was excluded from subsequent analyses. Phylogenetic analyses were based on the remaining 2088 bp of aligned nucleotide data. Gaps were treated as missing data in all analyses.

We explored potential incongruence among the four mtDNA fragments using the incongruence length difference (ILD) test (Farris et al., 1994) (100 replications) implemented as the partition homogeneity test in PAUP*4.03b10 (PAUP) (Swofford, 2001). Uninformative sites were excluded from analysis, and we limited branch swapping to 1000 trees per replicate (Lee, 2001). The ILD test identified significant incongruence among the four data partitions ($P = 0.01$). However, we view this result with some skepticism due to differences in taxon sampling, missing data, and numbers of variable and phylogenetically informative characters among data partitions (Table 3) (Dowton and Austin, 2002). Further, empirical (Yoder et al., 2001) and simulated data (Barker and Lutzoni, 2002) have shown that the ILD test can fail to allow combination of data partitions when they should be combined. Finally, the regions sequenced form a single linkage group (mtDNA). Therefore, despite a significant ILD test, we combined the four mtDNA partitions and analyzed them together in subsequent analyses.

2.3. Phylogenetic analyses

Initial phylogenetic analyses were conducted using MP criteria in PAUP. Equally weighted heuristic tree searches were performed on the combined data using 1000 random sequence additions and tree bisection-reconnection (TBR) branch swapping. The parsimony ratchet procedure (Nixon, 1999) was then implemented in PAUP using 200 replicates and repeated with 10–25% weighted characters using batch files generated by PAUPRat version 1 (Sikes and Lewis, 2001). The MP tree(s) generated from the parsimony ratchet procedure were then used to start another equal weights heuristic tree search in PAUP. Nodal support was evaluated with 1000 non-parametric bootstrap pseudoreplicates, using a simple addition sequence of taxa and TBR branch swapping (Felsenstein, 1985; Hillis and Bull, 1993) in PAUP. Bremer support values were obtained using a command file of constraint trees generated by TreeRot version 2 (Sorenson, 1999).

Bayesian Markov Chain Monte Carlo methods were also used to estimate phylogeny in the program MrBayes version 3.0b4 (MrBayes) (Huelsenbeck and Ronquist, 2001; Larget and Simon, 1999; Rannala and Yang, 1996). The simplest best-fit substitution model was selected for each data partition with Modeltest version 3.5 (Posada and Crandall, 1998) using hierarchical likelihood ratio tests (LRT) and Akaike information criterion (AIC) (Posada and Crandall, 2001). Both methods selected the GTR+I+G model for COI. For tRNA-Leu, the LRT selected the F81+I+G model, and the AIC selected the TVMef+I+G model. For COII, both methods selected the GTR+I+G model, and for Cyt *b* the LRT selected the TVM+I+G model, and AIC selected the GTR+I+G model. For the combined data set, both methods selected the GTR+I+G model.

We ran four separate analyses in MrBayes using the GTR+I+G model, and starting with random trees generated by the program defaults. We allowed MrBayes to estimate parameter values separately for each data partition (COI, tRNA-Leu, COII, and Cyt *b*). Three heated and one cold chain was used in all analyses. We ran each analysis for 1.0×10^6 generations, sampling every 100 generations. A single additional analysis was run for 2.0×10^6 generations. We evaluated the log likelihood scores from each of the five runs to see if and when stationarity was reached and to evaluate convergence of log likelihood scores across runs. To avoid overrepresentation of trees from a single run, the trees obtained from the 2.0×10^6 generation analysis were used only for diagnosing convergence of log likelihood scores and stationarity across runs. We discarded all samples preceding stationarity as a "burn in." The post "burn in" trees saved from each of the four, 1.0×10^6 generation

runs were combined and used to generate a 50% majority rule consensus tree in PAUP, and a 95% credible set of trees.

Phylogeny was also estimated using ML as implemented in PAUP. We applied a successive approximations approach, similar to that of Lin et al. (2004), to search for the ML tree (Swofford et al., 1996). A heuristic search with TBR branch swapping was started using the Bayesian consensus as the starting tree, the GTR+I+G substitution model selected by Modeltest, and the initial parameter estimates obtained from the Bayesian consensus. The ML parameters were optimized for each of the iterations on the new tree, and the search was repeated with the optimized parameters fixed.

3. Results

3.1. Sequence alignment

The combined mtDNA data set comprised a total of 2094 nucleotide sites, including a maximum of 1044 sites from the 3′ end of COI (including a single three nucleotide insert at the 3′ end of COI at position 1033 in the alignment, present only in the outgroup *Chelobasis perplexa*), the complete tRNA-Leu (maximum 58 nucleotide sites total; 52 sites excluding those whose alignment was ambiguous), a maximum 543 nucleotide sites from the 5′ end of COII, and a maximum 449 nucleotide sites from near the middle of Cyt *b* (Table 1). Of the 2088 total nucleotide sites included in analyses, 1156 sites (55%) were variable and 1020 sites (49%) were parsimony informative (Table 3).

3.2. Maximum parsimony phylogenetic analyses

Maximum parsimony analysis yielded 162 trees of length 10,400 (Table 3). The shortest trees found by the parsimony ratchet were also of length 10,400. When the parsimony ratchet trees were used to start an MP heuristic search, the same 162 trees were recovered as when starting with random trees. Overall, relationships are well resolved in the MP strict consensus (Fig. 1). The MP tree recovers a monophyletic *Cephaloleia* but without robust bootstrap support (<50%), as long as *Demotispa* sp. 175, and *Pseudostilpnaspis columbica* are included. Most species relationships are resolved and supported by moderate (>75%) to high (>95%) bootstrap values. Higher-level relationships are generally supported by low bootstrap (<50%) and moderate Bremer support values. Summary statistics resulting from separate MP analysis of the four regions sequenced, and from the combined data set are provided in Table 3. *Cephaloleia belti*, *C. deficiens*, *C.* cf. *erichsonii*, *C. gilvipes*, and *C. trivittata* are polyphyletic in the MP tree. *Cephaloleia dilaticollis* and *C. erichsonii* are paraphyletic. The unexpected placement of *C. semivittata* in a clade bounded by *C. reventazonica* and *C. pulchella* is not well supported (<50% bootstrap value). *Demotispa* sp. 175 and *Pseudostilpnaspis columbica* are recovered in a basal clade with several unique *Cephaloleia* spp. *Cephaloleia irregularis* is resolved in a position that is basal to all other *Cephaloleia*.

3.3. Model selection and Bayesian inference of phylogeny

The best-fit substitution model for the combined data set selected with the LRT and AIC in Modeltest was the GTR+I+G (log likelihood = 43610.348). The parameter values estimated by Modeltest were A⇔C: 0.29, A⇔G: 8.88, A⇔T: 0.40, C⇔G: 1.81, C⇔T: 4.76, and G⇔T: 1.0. Estimated base composition was A = 0.39, C = 0.16, G = 0.04, T = 0.41, the proportion of invariable sites = 0.38, and the α values of the γ shape distribution = 0.49. The log likelihood scores from each run converged on approximately the same value and were stable after approximately 3.0×10^5 generations. The first 5000 trees from each run were discarded as a conservative "burn in." The remaining 5000 trees from each of the four 1.0×10^6 generation runs were pooled for a total of 20,000 trees and used to generate a 50% majority rule consensus tree (Fig. 2). The 95% credible set of post "burn in" trees contained 10,075 trees.

The Bayesian majority rule consensus tree recovers most of the same clades as the MP tree (Fig. 2). Forty-four nodes have $\geqslant$70% bootstrap support and $\geqslant$0.95 BPP. Several taxa differ in placement in the two trees, most notably, *C. dilaticollis*, *C.* cf. *dilaticollis*, *C. irregularis*, *C.* cf. *pulchella*, *C. semivittata*, and *Imatidium* cf. *rufiventre*. Bootstrap values for most of these placements are low, and Bayesian posterior probabilities (BPP) mostly non-significant (<0.95). The MP and Bayesian trees otherwise differ primarily in resolution and the arrangement of tips within major clades. The Bayesian tree recovered the same polyphyletic and paraphyletic taxa as the MP tree.

3.4. ML phylogenetic analyses implemented in PAUP

One tree with a log likelihood of 43188.323 resulted from the ML analysis of the combined data set in PAUP (applying the GTR+I+G model) after branch swapping with exceedingly little improvement (<0.0005%) of log likelihood score from 43209.203 (Bayesian consensus tree) (Fig. 3). The parameter values estimated for our final ML tree were A⇔C: 0.35, A⇔G: 8.97, A⇔T: 0.48, C⇔G: 1.77, C⇔T: 5.83, and G⇔T: 1.0. Estimated base composition was A = 0.40, C = 0.15, G = 0.04, T = 0.41, the proportion of invariable sites = 0.37, and the α values of the γ shape distribution = 0.46.

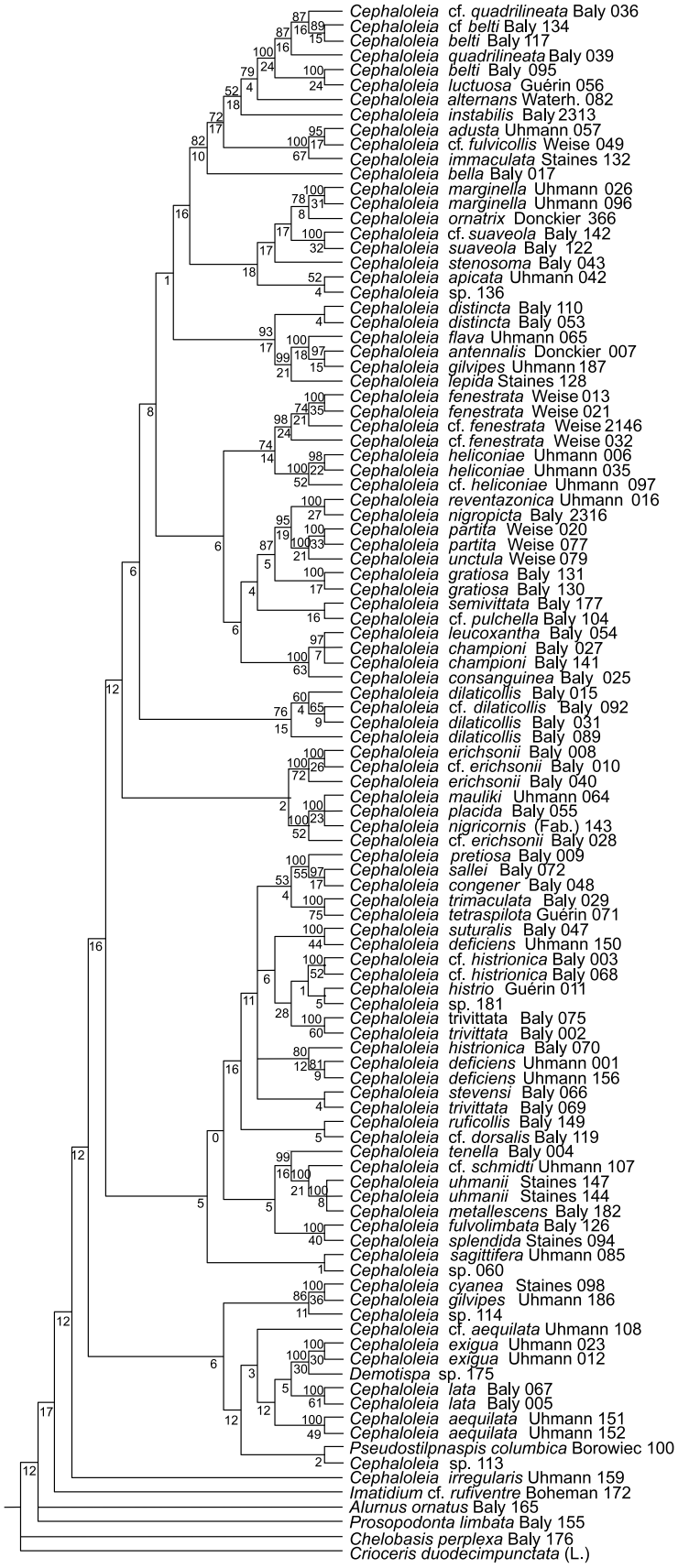

Fig. 1. Strict consensus of 162 equally parsimonious trees of length 10,400 steps generated by PAUP from the combined data set with equal weights. Bootstrap and Bremer support values are shown above and below branches, respectively. Bootstrap values <50% are not shown.

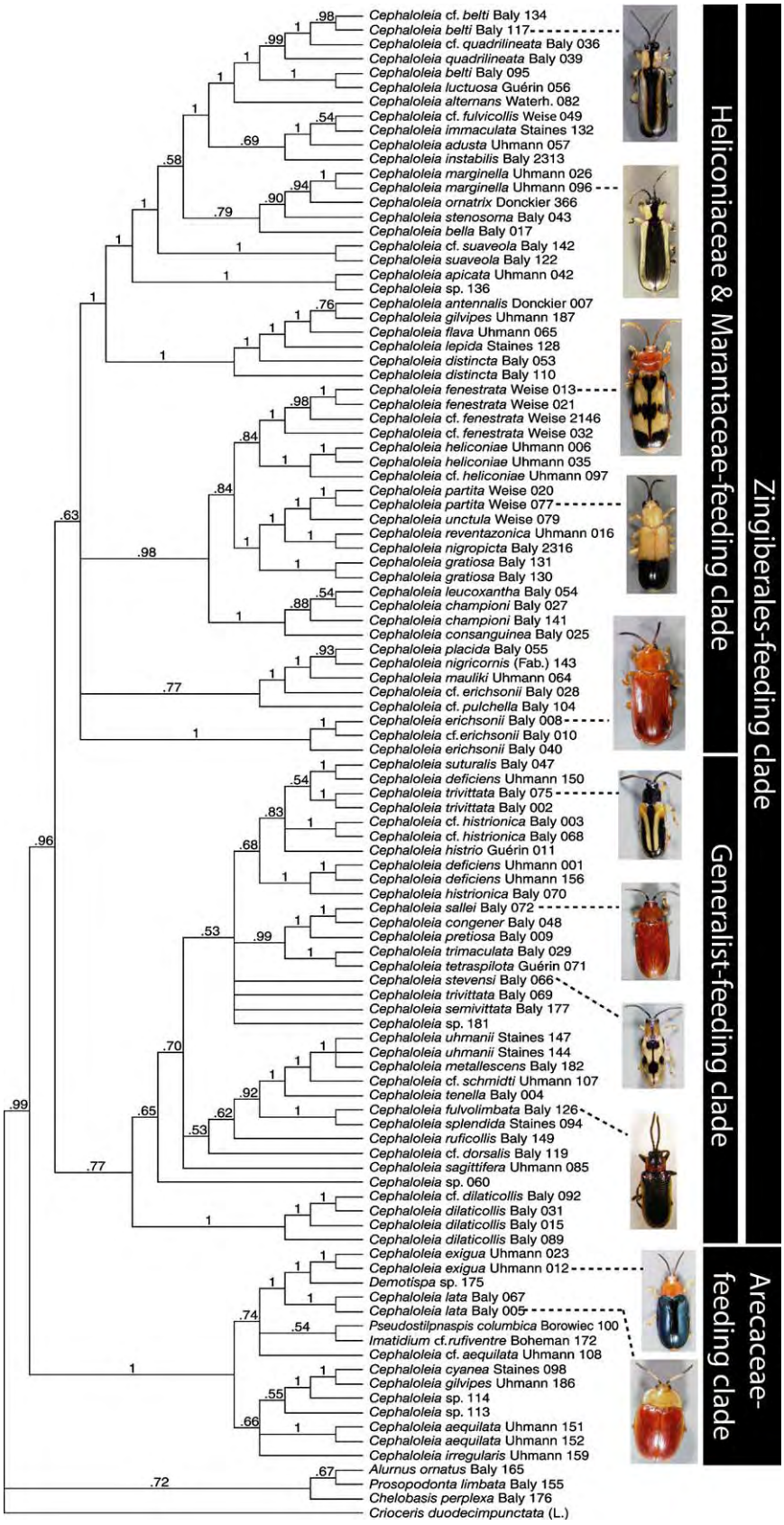

Fig. 2. Fifty percent majority rule consensus tree generated from the 20,000 trees retained from the four separate 1.0×10^6 generation Bayesian analyses of the combined data set based on the GTR+I+G substitution model in MrBayes. Numbers above branches are posterior probability values of the nodes (e.g., 1 = 100%, .95 = 95%, etc.). Values <0.5 are not shown.

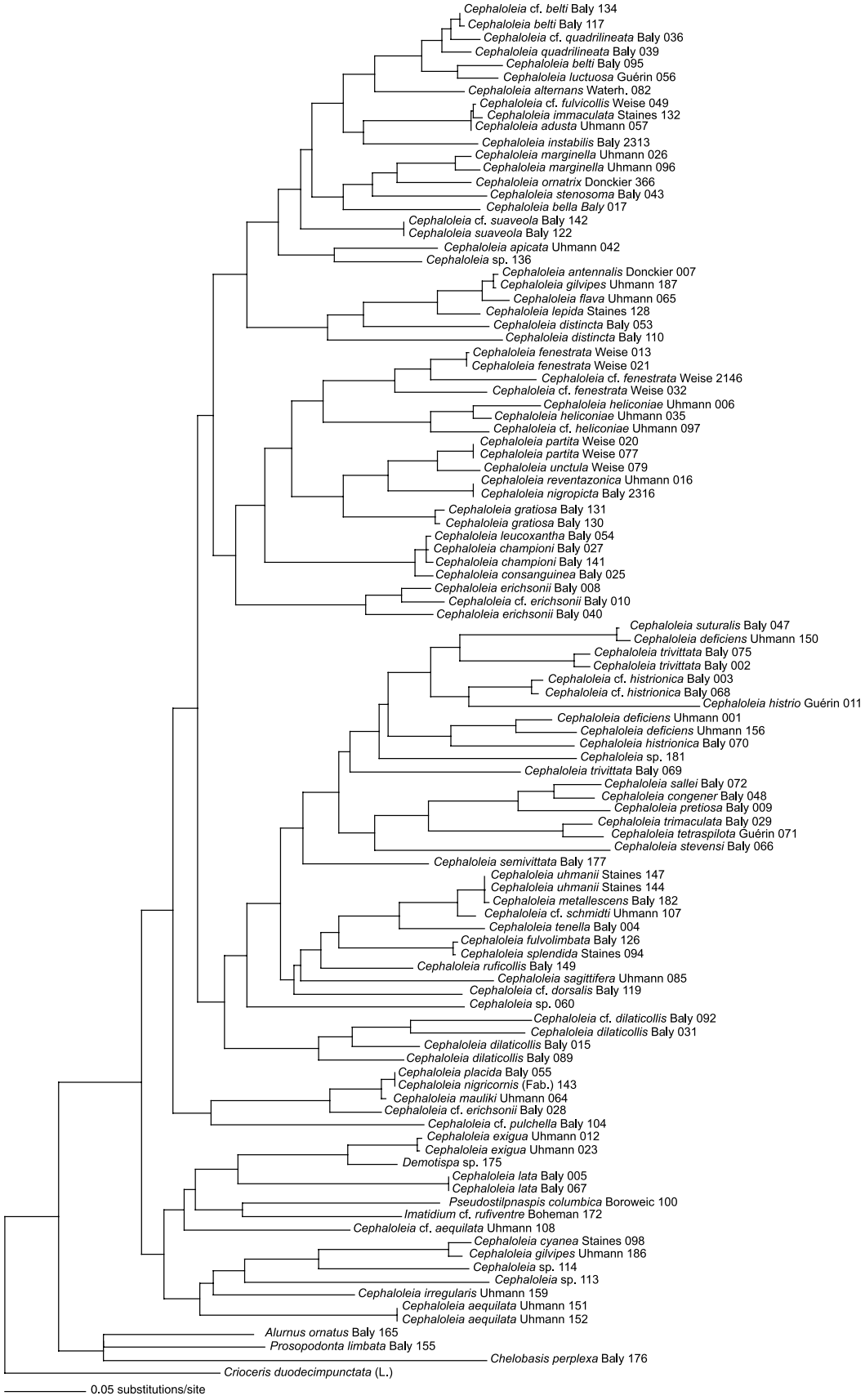

Fig. 3. Maximum likelihood phylogram (log likelihood 43188.323) generated in PAUP from analysis of the combined data set based on the GTR+I+G substitution model.

The ML tree, like the Bayesian consensus, recovers a monophyletic *Cephaloleia* as long as *Demotispa* sp. 175, *Imatidium* cf. *rufiventre*, and *Pseudostilpnaspis columbica* are included (Fig. 3). The ML tree recovered the same polyphyletic and paraphyletic taxa as the MP tree and the Bayesian consensus. Differences between the ML tree and the MP tree are mostly the same as those for the Bayesian tree and the MP tree (see above), except for the placement of the clade containing *C.* cf. *erichsonii*, *C. mauliki*, *C. nigricornis*, *C. placida*, and *C.* cf. *pulchella*.

3.5. Patterns of host tissue and host taxon usage

In all three analyses, all Zingiberales feeders and most *Cephaloleia* species form a single large derived clade (<50% bootstrap, 0.96 BPP) (the 'Zingiberales-feeding clade'). Notable within the 'Zingiberales-feeding clade' is a clade of beetles that normally feed as adults only on the leaf rolls of plants in the tropical families Heliconiaceae and Marantaceae (the 'Heliconiaceae & Marantaceae-feeding clade'). Bootstrap support for the 'Heliconiaceae & Marantaceae-feeding clade' is low (<50%), and BPP non-significant (0.63). Sister to the Heliconiaceae & Marantaceae-feeding clade' is a clade of relative generalist Zingiberales feeders (the 'generalist-feeding clade') with low bootstrap support (<50%), and non-significant BPP (0.79) (Fig. 2). Most species in the 'generalist-feeding clade' feed on Zingiberales in the families Costaceae, Heliconiaceae, Marantaceae, and Zingiberaceae. *Cephaloleia ruficollis*, *C. tenella*, and *C. uhmanii* feed on Cyperaceae or Poaceae. A basal clade we refer to as the 'Arecaceae-feeding clade' (bootstrap value < 50%, BPP = 1.0) includes all Arecaceae feeding *Cephaloleia* and *C. exigua* (a Cyclanthaceae feeder). None of these species are known to feed on Zingiberales. The 'Arecaceae-feeding clade' includes several unusual *Cephaloleia* spp., and species currently placed in other cassidine genera in the tribes Cephaloleiini (*Demotispa* sp. 175) and Imatidiini (*Imatidium* cf. *rufiventre* and *Pseudostilpnaspis columbica*).

4. Discussion

4.1. Cephaloleia phylogeny

None of the 162 MP trees, the ML tree, or the 20,000 trees used to generate the Bayesian consensus tree, recovers a monophyletic *Cephaloleia*. We are not surprised that *Cephaloleia* is monophyletic only with the inclusion of *Demotispa* sp. 175 (Cephaloleiini), *Pseudostilpnapsis columbica* (Imatidiini), and *Imatidium* cf. *rufiventre* (Imatidiini) (resolved within *Cephaloleia* only in the MP and Bayesian consensus trees), because a large number of *Demotispa* (and members of several other genera of Cephaloleiini), and at least a few *Pseudostilpnaspis* (and other Imatidiini) feed on palms, much like most *Cephaloleia* spp. placed in the 'Arecaceae-feeding clade' in our analyses. However, relationships between basal Cephaloleiini and Imatidiini remain little known. We are surprised that *Chelobasis perplexa* (Arescini) may be only distantly related to *Cephaloleia*. *Chelobasis* spp. (and other very closely related Arescini in the genera *Arescus, Nympharescus*, and *Xenarescus*) often co-occur with *Cephaloleia* spp. in the leaf rolls of plants in the family Heliconiaceae.

All three methods of analysis identify the same polyphyletic taxa (*C. belti*, *C. deficiens*, *C.* cf. *erichsonii*, *C. gilvipes*, and *C. trivittata*), and the same paraphyletic taxa (*C. dilaticollis* and *C. erichsonii*). All polyphyletic taxa are the result of cryptic species. The two paraphyletic taxa require further study. Moderate to high-bootstrap values and significant BPPs occur primarily near the tips of the tree. Where topologies differ, bootstrap values are generally low, and BPPs non-significant. A few deep nodes in the Bayesian consensus tree have relatively high BPPs (e.g., the 'Zingiberales-feeding clade'), but lack robust bootstrap support. Bayesian posterior probabilities should be interpreted cautiously in such situations. Suzuki et al. (2002) showed that when analyzing concatenated DNA sequences, BPPs could be "excessively liberal." Further, it should be noted that the Bayesian consensus and the ML tree are not entirely independent estimates of phylogeny, especially given our approach to the ML analysis (see Section 2 for details).

The conflicting placement of the clade containing *C.* cf. *erichsonii*, *C. mauliki*, *C. nigricornis*, *C. placida*, and *C.* cf. *pulchella* in the ML versus the MP and Bayesian trees is intriguing. All of the above species with known host plants feed on Zingiberales leaf rolls, suggesting that the most likely of potential affiliations is with the 'Heliconiaceae and Marantaceae-feeding clade' as in the MP and Bayesian consensus trees. The positions of *C. dilaticollis*, and *C.* cf. *dilaticollis*, are likewise perplexing. Placement at the base of the 'generalist-feeding clade' as in the ML and Bayesian trees seems to make the most sense in light of the fact that both species are host tissue generalists on Zingiberales (especially Marantaceae).

4.2. Evolution of host taxon and host tissue use

Overall, the evolution of host taxon usage in *Cephaloleia* is remarkably consistent with current concepts of monocot phylogeny (Janssen and Bremer, 2004). For example, relatively basal *Cephaloleia* (members of the 'Arecaceae-feeding clade') feed on the relatively most basal host plant families (e.g., Cyclanthaceae and Arecaceae). Zingiberales are considered derived with respect to the aforementioned plant families, and this is reflected in the *Cephaloleia* phylogeny by placement of the 'Zingiberales-feeding clade' in a derived position relative to the 'Arecaceae-feeding clade.' Host taxon usage in the 'Heliconiaceae & Marantaceae-feeding clade' is limited to plants in the families Heliconiaceae and Marantaceae.

The 'Heliconiaceae & Marantaceae-feeding clade' contains the only *Cephaloleia* species that feed exclusively in the rolled leaves of Zingiberales. While patterns of host plant taxa and tissue use are not definitively known for all species included in our study, 100% of species in the 'Heliconiaceae & Marantaceae-feeding clade' with known hosts feed exclusively in the rolled leaves of Heliconiaceae and Marantaceae as adults. The vast majority of larvae are also limited to leaf rolls. Many species in the 'generalist-feeding clade' feed on Zingiberales; however, in contrast to the tissue specialized 'Heliconiaceae & Marantaceae-feeding clade,' the larvae and adults of Zingiberales feeding species in the 'generalist-feeding clade' utilize multiple host plant parts, including inflorescence bracts, petioles, and leaf rolls. None of the included species feed exclusively on leaf rolls. Most beetles in the 'Arecaceae-feeding clade' feed primarily on the immature folded leaves of their hosts, none of which are Zingiberales. Thus, association with Zingiberales, and specialization on Zingiberales leaf rolls have each apparently evolved just once in *Cephaloleia*.

4.3. Phylogenetic relationships among Cephaloleia species and implications for taxonomy

Whereas the 'Arecaceae-feeding clade' is clearly divergent from other *Cephaloleia*, the relationships among members of the 'Arecaceae-feeding clade,' and between members of the 'Arecaceae-feeding clade' and putative outgroups, remain somewhat unclear. Resolution is also limited within the 'Zingiberales-feeding clade.' Ongoing research, including sequencing of nuclear protein coding genes, reducing the amount of missing data, and additional taxon sampling (including *Cephaloleia*-like taxa in other genera such as *Aslamidium*) may help clarify some of these issues, and may provide more resolution and better nodal support, especially for deeper divergences.

Given the relative concordance of morphological and molecular genetic concepts of the genus *Cephaloleia*, we forego making any formal taxonomic changes until sequence data from additional taxa and from the nuclear genome are available to corroborate polyphyly of especially, the 'Arecaceae-feeding clade.' Nevertheless, the results of our work demonstrate novel relationships among *Cephaloleia* spp., and between *Cephaloleia* and select other Cassidinae, and provide a glimpse into the evolution of patterns of host plant taxon and tissue use in the genus.

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