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A molecular phylogenetic analysis of the pleasing fungus beetles (Coleoptera: Erotylidae): evolution of colour patterns, gregariousness and mycophagy

JAMES A. ROBERTSON[1], JOSEPH V. MCHUGH[2] and MICHAEL F. WHITING[1]

[1]Department of Integrative Biology, Brigham Young University, Provo, Utah, U.S.A. and
[2]Department of Entomology, University of Georgia, Athens, Georgia, U.S.A.

Abstract. Phylogenetic relationships of Erotylidae (pleasing fungus beetles) were inferred based on DNA sequence data. Relationships of clades within Erotylidae were examined, as was the relationship of the entire family to Languriidae (lizard beetles). 18S and 28S ribosomal DNA were sequenced for sixty-one taxa representing major erotylid lineages and outgroups. Phylogenetic analyses under varying parameter settings using standard parsimony and likelihood techniques were performed. These data indicate a paraphyletic Erotylidae and Languriidae. Encaustinae (including *Coptengis*), Megalodacninae and Erotylinae are supported as monophyletic, whereas Dacninae and Tritominae are paraphyletic. Taxonomic and biological implications are discussed. Gregariousness has arisen at least three times in Erotylidae. The erotylid clade has experienced at least one evolutionary transition from mycophagy (on Aphyllophorales) to phytophagy, three transitions from Aphyllophorales hosts to Euagarics, and one transition from Euagarics hosts to Mucorales (Zygomycetes). There are no recognizable phylogenetic trends in coloration across higher-level erotylid lineages.

Introduction

The cosmopolitan family Erotylidae (pleasing fungus beetles) currently includes approximately 125 genera and 2500 described species (McHugh, 2001). There are five currently recognized erotylid subfamilies: Dacninae, Megalodacninae, Eucaustinae, Tritominae and Erotylinae (Lawrence & Newton, 1995). Erotylidae are mycophagous, feeding on basidiomycete fungi as larvae and adults. Most species are striking in appearance, exhibiting bright colours including red, yellow, orange, pink and purple, frequently in combination with contrasting black to form conspicuous patterns of stripes, zigzags, bands, speckles, spots or rings.

Some erotylid species exhibit parental care or gregariousness in immature stages (O'Toole & Preston-Mafham, 1985; Preston-Mafham & Preston-Mafham, 1993; Leschen, 1994). It remains unclear whether these behaviours originated within Erotylidae and, if so, whether there was a single origin for each within the family. It is also unknown if these two behaviours are evolutionarily linked in some way.

Erotylidae is one of thirty-two families belonging to Cucujoidea (Lawrence *et al*., 2000), a superfamily of primarily detritus and fungus-associated beetles. Phylogenetic relationships among the cucujoid families are poorly known and the monophyly of many constituent families remains dubious. Such is the situation for the families Erotylidae and Languriidae.

Historically there has been disagreement about the validity of both of these families with respect to the other. Many taxonomists have supported the recognition of Languriidae as a separate family (e.g. Lewis, 1884; Arrow, 1925; Boyle, 1956; Sen Gupta & Crowson, 1971). Others have included them as a subgroup within Erotylidae (e.g. Crotch, 1873; Chapuis, 1876; Gorham, 1899; Fowler, 1908; Roberts, 1939, 1958). In addition, there is a great deal of instability in the recognition of subfamilies, genera and species within these lineages and no clear consensus of phylogenetic relationships has emerged. Indeed, the five subfamilies of Languriidae have at times been placed within Erotylidae

Correspondence: James A. Robertson, Department of Entomology, University of Georgia, 413 Biological Sciences Building, Athens, GA 30602, U.S.A. E-mail: jrobertson@bugs.ent.uga.edu

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and/or Cryptophagidae (Leschen & Wegrzynowicz, 1998). The only published phylogeny for Erotylidae to date (Boyle, 1956) is entirely intuitive, considers only a small sampling of taxa, and lacks a character matrix or formal analysis to support hypothesized relationships.

The aposematic colour patterns in Erotylidae are thought to warn potential predators of chemical defences or are mimetic of other beetle groups with such defences (e.g. Donisthorpe, 1901; Arrow, 1925). Although erotylids often are assumed to be chemically defended themselves, support is little more than anecdotal (e.g. Leschen, 1994; McHugh *et al*., 1997). New studies (McHugh & Pitts, unpublished data) confirm that some erotylid species produce chemicals that are known to be defensive in other insect groups.

The phylogenetic pattern of erotylid aposematism has never been elucidated, and thus the origin of a specific colour pattern may result either from a single evolutionary event, or certain colour patterns may have evolved multiple times within Erotylidae. Similarly, repeated colour pattern progressions, such as a transition from banded to spotted patterns, have been investigated within certain genera (e.g. Skelley, 1998), but it has yet to be determined if such progressions exist across higher-level erotylid taxa.

The purpose of this research is to establish a robust phylogenetic hypothesis for Erotylidae based on molecular data. Specifically we examine the placement of Languriidae relative to Erotylidae to gain insight about higher-level relationships within Erotylidae. Additionally, we attempt to examine whether larval gregariousness and parental care are phylogenetically linked, or if they have evolved independently in separate lineages. We investigate phylogenetic patterns of host utilization. Finally, we test whether there is any phylogenetic signal in the coloration patterns that are so conspicuous in most Erotylidae.

Materials and methods

Eighteen outgroup exemplars were selected from the beetle superfamilies Scarabaeoidea (Scarabaeidae, 1 exemplar), Tenebrionoidea (Zopheridae 1; Ciidae 1; and Tenebrionidae, 3) and Cucujoidea (Coccinellidae 1; Endomychidae, 3; Nitidulidae, 2; Cucujidae, 1; Silvanidae, 1; and Languriidae, 4). Languriid exemplars consist of four taxa representing two (Languriinae and Toraminae) of the five currently recognized subfamilies. Ingroup taxa consist of forty-three erotylid exemplars representing the five recognized subfamilies, seventeen genera, and thirty-seven species (Table 1).

DNA was extracted from EtOH preserved specimens using the Qiagen DNeasy tissue kit (Valencia, CA) following standard protocols. Muscle tissue was dissected from the thorax and voucher specimens are deposited at Brigham Young University. The nuclear genes 18S ribosomal DNA (18S rDNA) and 28S ribosomal DNA (28S rDNA) were amplified via the polymerase chain reaction (PCR) using primers and protocols published elsewhere (Whiting, 2002). Negative controls in which no DNA was added to the PCR reaction were used to monitor contamination. PCR products were visualized via gel electrophoresis, purified with Geneclean III DNA Purification Kits (Bio 101, Vista, CA), sequenced using d-Rhodamine chemistry, and fractionated on an ABI 3100 automated sequencer (ABI, Foster City, CA). Assembly of contig sequences and editing of nucleotide fragments were performed using Sequencher 3.1.1 (Genecodes, 1999). The resulting length ranges for the 18S and 28S fragments obtained were 1839–1922 base pairs (bp) and 2156–2559 bp, respectively.

The 18S and 28S rDNA sequences were aligned initially via Sequencher 3.1.1 (Genecodes, 1999). For the 18S gene, approximately 1840 bp were used for phylogenetic analysis, together with approximately 2260 base pairs from the 28S data. A highly autapomorphic insert (*c*. 255 bp in length) in the 28S sequence of *Toramus* sp. 2, occurring at approximately residue 1950, was excluded from the alignment. Each gene was partitioned into conserved and variable domains, resulting in seven regions for 18S and fourteen regions for 28S. Each region underwent more rigorous analysis via Optimization Alignment (OA) (Wheeler, 1996) using the computer program POY (Gladstein & Wheeler, 1997) as implemented in parallel on an IBM SP2 supercomputer. Optimization alignment allows for simultaneous alignment and phylogenetic analysis, permitting a given set of analytical parameters to be applied uniformly throughout the alignment and tree reconstruction process. POY analyses were executed initially using the following search strategy: 'fitchtrees -parallel -noleading -norandomize-outgroup -impliedalignment -sprmaxtrees 1 -tbrmaxtrees 1 -maxtrees 5 -holdmaxtrees 100 -slop 5 -checkslop 10 -buildspr -buildmaxtrees 2 -random 25 -stopat 25 -multirandom -treefuse -fuselimit 10 -fusemingroup 5 -fusemaxtrees 100 -numdriftchanges 30 -driftspr -numdriftspr 10 -drifttbr -numdrifttbr 10 -slop 10 -checkslop 10 -molecularmatrix 111.txt -seed −1'. Multiple parameters for optimization alignment were employed using this search strategy to explore the sensitivity of the resulting topologies to certain analytical parameters. The goal of sensitivity analysis is not to determine the 'true' analytical parameters per se, as these are unknown and unknowable, but rather to test the sensitivity of the phylogenetic conclusions to a wide range of analytical parameters. We varied the cost ratios for gap insertion, transversion and transition from identity to treating gaps and transversions as four times the cost of transitions (Table 2) (Wheeler *et al*., 2001). Using the Incongruence Length Difference metric (ILD) (Mickevich & Farris, 1981), the topology from the parameter set that maximized congruence was retained as the best phylogenetic estimate (Wheeler *et al*., 2001), and this underwent a more exhaustive search. Additionally, a consensus tree was constructed for the topologies from parameter sets with ILD values adjacent to the optimal ILD value. Trees were reconstructed for 18S, 28S and combined data sets. In all analyses trees were rooted to *Phyllophaga* sp.

The implied alignment obtained from POY was analysed also using standard parsimony and likelihood techniques as implemented in PAUP* 4.0 (Swofford, 2000). For parsimony, 1000 random addition sequences with TBR branch swapping

Table 1. Taxa used in this analysis with GenBank accession numbers.

Family	Subfamily	Taxon	18S	28S
Scarabaeidae		*Phyllophaga* sp.	AY310601	AY310662
Zopheridae		*Bitoma* sp.	AF423768	AY310661
Ciidae		*Cis* sp.	AY310605	AY310666
Tenebrionidae		Coelometopinae sp.	AY310606	AY310667
Tenebrionidae		Coelometopinae sp.	AY310607	AY310668
Tenebrionidae		Diaperinae sp.	AY310610	AY310671
Cucujidae		*Cucujus clavipes* Fabricus	AF423767	AY310660
Silvanidae		*Uleiota* sp.	AY310604	AY310665
Nitidulidae		*Carpophilus* sp.	AY310603	AY310664
Nitidulidae		Nitidulinae sp.	AY310652	AY310713
Coccinellidae		*Olla v-nigrum* Mulsant	AY310602	AY310663
Endomychidae		*Encymon bipustulatus* Gorham	AY310600	AY310659
Endomychidae		*Encymon bipustulatus* Gorham	AY310608	AY310669
Endomychidae		*Chondria armipes* Strohecker	AY310609	AY310670
Languriidae	Languriinae	*Caenolanguria* sp.	AY310611	AY310672
Languriidae	Languriinae	*Languria mozardi* Latreille	AY310599	AY310658
Languriidae	Toraminae	*Toramus pulchellus* (LeConte)	AY310598	AY310657
Languriidae	Toraminae	*Toramus* sp.	AY310615	AY310676
Erotylidae	Dacninae	*Dacne californica* (Horn)	AY310634	AY310695
Erotylidae	Dacninae	*Coptengis* sp.	AY310643	AY310704
Erotylidae	Megalodacninae	*Megalodacne fasciata* (Fabricius)	AY310635	AY310696
Erotylidae	Megalodacninae	*Megalodacne heros* (Say)	AY310636	AY310697
Erotylidae	Megalodacninae	*Episcaphula* sp. 2	AY310646	AY310707
Erotylidae	Encaustinae	*Encaustes verticalis* MacLeay	AY310641	AY310702
Erotylidae	Encaustinae	*Encaustes cruenta* MacLeay	AY310642	AY310703
Erotylidae	Encaustinae	*Aulacochilus f. flavocinctus* Arrow	AY310644	AY310705
Erotylidae	Encaustinae	*Aulacochilus papuanus* Csiki	AY310647	AY310708
Erotylidae	Tritominae	*Triplax thoracica* Say	AY310637	AY310698
Erotylidae	Tritominae	*Spondotriplax antennalis* Arrow	AY310645	AY310706
Erotylidae	Tritominae	*Tritoma unicolor* Say	AY310639	AY310700
Erotylidae	Tritominae	*Tritoma pulchra* Say	AY310638	AY310699
Erotylidae	Tritominae	*Tritoma erythrocephala* Lacordaire	AY310633	AY310694
Erotylidae	Tritominae	*Mycotretus scitulus* Lacordaire	AY310618	AY310679
Erotylidae	Tritominae	*Mycotretus scitulus* Lacordaire	AY310621	AY310682
Erotylidae	Tritominae	*Mycotretus* sp. 1	AY310617	AY310678
Erotylidae	Tritominae	*Mycotretus* sp. 2	AY310630	AY310691
Erotylidae	Tritominae	*Pselaphacus puncticollis* Guerin-Meneville	AY310651	AY310712
Erotylidae	Tritominae	*Pselaphacus signatus* Guerin-Meneville	AY310623	AY310684
Erotylidae	Tritominae	*Pselaphacus vitticollis* Crotch	AY310625	AY310686
Erotylidae	Tritominae	*Lybas* sp. 1	AY310622	AY310683
Erotylidae	Tritominae	*Lybas* sp. 2	AY310632	AY310693
Erotylidae	Erotylinae	*Iphiclus* sp. 3	AY310656	AY310717
Erotylidae	Erotylinae	*Iphiclus (Saccomorphus)* sp.	AY310650	AY310711
Erotylidae	Erotylinae	*Iphiclus (Iphiclus) sedecimmaculatus* Buquet	AY310613	AY310674
Erotylidae	Erotylinae	*Iphiclus (Habrodactylus)* sp. 4	AY310620	AY310681
Erotylidae	Erotylinae	*Iphiclus* (*Habrodactylus*) sp. 5	AY310616	AY310677
Erotylidae	Erotylinae	*Iphiclus (Habrodactylus)* sp. 10b	AY310640	AY310701
Erotylidae	Erotylinae	*Iphiclus* (*Habrodactylus*) *conspicillatus* (Gorham)	AY310655	AY310716
Erotylidae	Erotylinae	*Iphiclus (Megaprotus)* nr. *pulcher*	AY310612	AY310673
Erotylidae	Erotylinae	*Iphiclus (Megaprotus)* nr. *pulcher*	AY310614	AY310675
Erotylidae	Erotylinae	*Iphiclus* (*Megaprotus*) nr. *pulcher*	AY310624	AY310685
Erotylidae	Erotylinae	*Iphiclus (Megaprotus) delineatus* (Lacordaire)	AY310626	AY310687
Erotylidae	Erotylinae	*Iphiclus (Barytopus)* sp. 2	AY310627	AY310688
Erotylidae	Erotylinae	*Aegithus cardinalis* Chevrolat	AY310648	AY310709
Erotylidae	Erotylinae	*Aegithus meridionalis* Crotch	AY310631	AY310692
Erotylidae	Erotylinae	*Prepopharus xanthomelas* (Crotch)	AY310628	AY310689
Erotylidae	Erotylinae	*Prepopharus xanthomelas* (Crotch)	AY310653	AY310714
Erotylidae	Erotylinae	*Erotylina jaspidea* Erichson	AY310649	AY310710
Erotylidae	Erotylinae	*Ellipticus* nr. *testaceus*	AY310619	AY310680
Erotylidae	Erotylinae	*Ellipticus* nr. *testaceus*	AY310629	AY310690
Erotylidae	Erotylinae	*Ellipticus* nr. *testaceus*	AY310654	AY310715

Table 2. Parameter settings explored in this study with respective ILD metric values.

Gap: Transversion: Transition cost ratio	1:1:1	1:2:1	1:3:1	1:4:1	2:1:1	2:2:1	2:3:1	2:4:1	3:1:1	3:2:1	3:3:1	3:4:1	4:1:1	4:2:1	4:3:1	4:4:1
18S length	691	815	894	900	798	981	1111	1217	883	1078	1248	1389	960	1162	1349	1512
28S length	3166	3742	4061	4055	3760	4642	5351	5874	4229	5200	6066	6783	4685	5697	6626	7470
Combined length	3872	4583	4987	4996	4585	5654	6515	7138	5179	6339	7382	8246	5761	6946	8042	9086
ILD metric	0.00387	0.00567	0.00642	0.00821	0.01025	0.00548	0.00813	0.00658	0.01294	0.00962	0.0092	0.00897	0.02014	0.01253	0.00833	0.01145

was performed with gaps treated as missing or as a fifth state. TreeRot (Sorenson, 1999) was used with PAUP to calculate partitioned Bremer (Baker & DeSalle, 1997) support values for each gene. Nonparametric bootstraps were calculated using 1000 replicates with ten random additions per replicate. Likelihood analysis was performed by using Modeltest (Posada & Crandall, 1998) to select a 'justified' model of evolution. Likelihood analysis was executed using seventeen random addition replicates with TBR branch swapping, and executed on the IBM SP2 supercomputer.

Characters associated with gregariousness, host preference and aposematic coloration (see below) were treated as unordered and optimized using MacClade 4.0 (Maddison & Maddison, 1994) (Table 3). Characters for gregarious behaviour and host preference were scored based on personal observations (J.V.M.) and specimen data, although these data are unavailable for some taxa and were treated as missing. Characters for aposematic coloration are difficult to code, but we attempted to characterize general classes of coloration (Fig. 6).

Characters

1. *Elytral fasciae*: (0) absent; (1) present.
2. *Iridescence*: (0) absent; (1) present.
3. *Pronotal/elytral colour*: (0) monochromic; (1) multichromic.
4. *Black pronotal spots*: (0) absent; (1) present.
5. *Black pronotum*: (0) absent; (1) present.
6. *Circular or subcircular rings*: (0) absent; (1) present.
7. *Larval feeding behaviour*: (0) independent; (1) gregarious.
8. *Host preference*: (0) Aphyllophorales fungi; (1) Euagarics fungi; (2) plants; (3) Zygomycetes.

Results

Weighting gaps, transitions and transversions as identical resulted in the minimal ILD value (0.00387; Table 2) for all parameter combinations explored. This became the parameter combination used in all subsequent analyses. Separate OA analysis of the 18S data resulted in 199 most parsimonious trees, the strict consensus of which is partially unresolved, recovering only clades 1–3, 12, 15, 18, 29 and 30 of the combined tree (described below) (Fig. 1). Separate OA analysis of 28S resulted in two most parsimonious trees, the strict consensus of which is congruent with the combined 18S + 28S tree, although less resolved. Clades present in the combined tree (described below) but absent in the 28S tree include 6, 11, 13, 16, 21, 25, 28, 42 and 43 (Fig. 1). OA analysis of the combined data set resulted in a single topology (length = 3869). Using the implied alignment from POY and treating gaps as a fifth state in PAUP, resulted in a topology one step shorter (L = 3868; RI = 0.697; CI = 0.548; Fig. 1), and is our estimation of the best supported topology. The difference in lengths is probably due to limitations in

Table 3. Character data scored for coloration (chs. 1–6), gregariousness (ch. 7) and host preference (ch. 8).

Taxa	1	2	3	4	5	6	7	8
Outgroup	?	?	?	?	?	0	0	?
Dacne californica	0	0	1	0	0	0	0	0/1
Languria mozardi	0	1	1	0	0	0	0	2
Caenolanguria sp.	0	1	1	0	0	0	0	2
Encaustes verticalis	0	0	1	?	1	0	?	0
Encaustes cruenta	?	0	1	0	0	0	?	0
Coptengis sp.	0	0	0	?	1	0	?	?
Aulacochilus flavocinctus	?	1	1	0	0	0	?	0
Aulacochilus papuanus	?	1	1	0	0	0	?	0
Episcaphula sp. 2	1	0	1	?	1	0	?	0
Megalodacne fasciata	1	0	1	?	1	0	0	0
Megalodacne heros	1	0	1	?	1	0	0	0
Triplax thoracica	0	0	1	0	0	0	0	1
Toramus pulchellus	1	0	1	0	0	0	0	3
Toramus sp.	1	0	1	0	0	0	0	?
Spondotriplax antennalis	0	1	0	0	0	0	?	?
Tritoma erythrocephala	0	0	0	?	1	0	0	1
Tritoma unicolor	0	0	0	?	1	0	0	1
Mycotretus sp. 2	0	0	0	0	0	0	0	1
Mycotretus scitulus	?	0	1	1	0	0	0	1
Pselaphacus pucticollis	1	0	1	1	0	0	1	0
Pselaphacus signatus	0	0	1	?	1	0	1	0
Pselaphacus vitticollis	1	0	1	1	0	0	1	0
Mycotretus sp. 1	0	0	0	0	0	0	0	0
Tritoma pulchra	0	0	1	?	1	0	0	0
Lybas sp. 1	0	0	0	0	0	0	0	0
Lybas sp. 2	0	0	0	0	0	0	?	0
Aegithus cardinalis	0	0	1	0	0	0	0	0
Aegithus meridionalis	0	0	0	0	0	0	0	0
Iphiclus (*Habrodactylus*) *conspicillatus*	0	0	1	0	0	1	1	0
Prepopharus xanthomelas	1	0	1	0	0	0	?	0
Iphiclus (*Barytopus*) sp. 2	?	0	1	?	1	0	?	0
Iphiclus (*Megaprotus*) *delineatus*	?	0	1	0	0	0	0	0
Iphiclus (*Megaprotus*) nr. *pulcher*	?	0	1	0	0	0	0	0
Iphiclus (*Habrodactylus*) sp. 5	0	0	0	0	0	0	?	0
Iphiclus (*Iphiclus*) *sedecimmaculatus*	0	0	1	1	0	1	0	0
Erotylina jaspidea	0	0	1	?	1	0	?	0
Iphiclus (*Saccomorphus*) sp.	0	0	1	?	1	0	?	?
Iphiclus (*Habrodactylus*) sp. 4	0	0	1	0	0	1	?	0
Ellipticus nr. *testaceus*	0	0	1	0	0	0	1	0
Iphiclus (*Habrodactylus*) sp. 10b	0	0	0	0	0	0	0	0
Iphiclus sp. 3	0	0	0	0	0	0	?	?

computation and search strategy, as has been observed elsewhere (Cognato & Vogler, 2001). The parsimony topology with gaps treated as a fifth state is identical to the OA topology except clade 25 is not recovered in the OA topology (Fig. 1). Treating gaps as missing data results in three most parsimonious trees, the strict consensus of which is entirely congruent with the 'fifth state' tree, although less resolved within Erotylinae. As the PAUP 'fifth state' topology is the most parsimonious estimate, it was retained as the best phylogenetic hypothesis for the Erotylidae and was used to optimize gregariousness, host preference and coloration characters.

The 18S data provide moderate nodal support at the familial level but appear to be more erratic at the subfamilial and generic levels. On the subfamilial and generic levels, 18S offers high support for some of these lineages, whereas others are only poorly supported, and in the case of some generic and intergeneric relationships, nodes are unsupported or support conflicting relationships (Table 4). Most of the phylogenetic signal comes from the 28S data, providing 81% of the total Bremer support. The 28S partitioned Bremer support values generally are high throughout the topology, with strong support at the familial and subfamilial levels and offering moderate to high support for most generic clades. The low Bremer support values among some

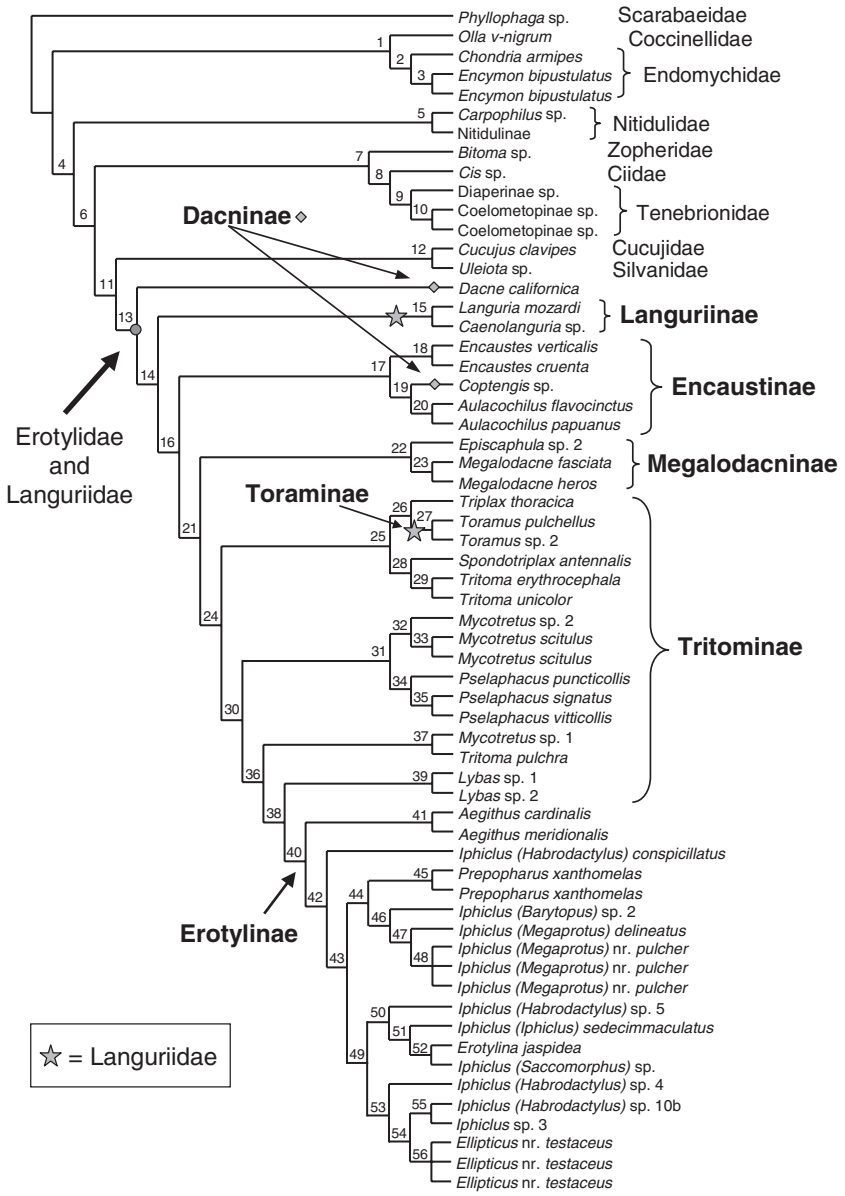

Fig. 1. Single most parsimonious tree for combined 18S and 28S data (Parsimony 'fifth state' tree); our best estimation of erotylid phylogeny. This analysis produces a single most parsimonious tree (L = 3868; RI = 0.697; CI = 0.548) when all transformations are treated equally. Nodes are numbered, and corresponding Bremer and bootstrap support values are given in Table 2.

generic clades (e.g. clades 25, 31, 43, 49, 53 and 54) suggest that these genes alone are insufficient to decipher robust relationships at this phylogenetic level for these taxa. Bootstrap values for the combined 18S and 28S topology overall were fairly high across the topology (Table 4).

When the combined data set was executed through Modeltest, the Tamura Nei + Invariable site + Gama distribution (TrN + I + G) model (Tamura & Nei, 1993) was selected as most appropriate for these data with the following parameter settings: Base frequencies: freqA = 0.2645; freqC = 0.2192; freqG = 0.2557; freqT = 0.2606; Substitution model: Rate matrix R(a) [A-C] = 1.0000; R(b) [A-G] = 2.6616; R(c) [A-T] = 1.0000; R(d) [C-G] = 1.0000; R(e) [C-T] = 5.7626; R(f) [G-T] = 1.0000. Performing seventeen random addition sequences with TBR branch swapping using the above parameters produced a topology (L = 22108.568) that is quite similar to the MP tree. The ML tree differs from the MP tree in that clades 4, 6, 37, 42, 43, 49, 53 and 54 (Fig. 1) are not recovered in the ML tree.

The parameter combinations 1 : 2 : 1 (gap : transversion : transition), 1 : 3 : 1, 2 : 2 : 1 and 2 : 4 : 1 produced ILD values that were adjacent to the optimal ILD value (Table 2). A sensitivity analysis tree was calculated by taking a majority rule consensus of the set of trees originating from each of these parameter values, including the optimal (1 : 1 : 1) parameter value (Fig. 2). The topology is almost entirely congruent with the 'fifth state' topology, recovering *Dacne* as the basal most erotylid, and Megalodacninae, Encaustinae (including *Coptengis*) and Erotylinae as monophyletic lineages, suggesting that these data are fairly

Table 4. Nodal support for the combined 18S and 28S topology (Fig. 1).

Node	Bootstrap support	Bremer support	Partitioned Bremer 18S	Partitioned Bremer 28S	Node	Bootstrap support	Bremer support	Partitioned Bremer 18S	Partitioned Bremer 28S
1	100				29	100	37	4	33
2	100	21	2	19	30	99	10	3	7
3	100	19	7	12	31	<50	1	−1	2
4	100	23	0	23	32	67	4	1.3	2.7
5	100	25	6	19	33	100	37	9	28
6	99	11	2	9	34	100	12	0	12
7	100	26	4	22	35	96	3	1	2
8	100	17	0	17	36	66	3	−3	6
9	100	39	3	36	37	<50	1	−1.5	2.5
10	100	31	5	26	38	55	2	0.4	1.6
11	100	12	3	9	39	100	18	1.8	16.2
12	100	50	14	36	40	100	11	6	5
13	93	10	5	5	41	100	29	8.2	20.8
14	100	22	0	22	42	79	11	6	5
15	100	18	9	9	43	<50	1	−0.8	1.8
16	97	9	3	6	44	94	6	1	5
17	100	34	6	28	45	100	16	8	8
18	100	41	11.5	29.5	46	86	3	−0.5	3.5
19	100	12	1	11	47	97	5	−1	6
20	99	10	4	6	48	100	5	1	4
21	91	5	1	4	49	<50	1	−1	2
22	100	15	1	14	50	99	11	−1	12
23	100	16	2	14	51	86	5	−1.6	6.6
24	86	4	0	4	52	62	2	0	2
25	63	1	0	1	53	<50	1	−1	2
26	100	18	4	14	54	<50	1	−1	2
27	100	191	51	140	55	<50	12	−0.8	12.8
28	76	3	−1	4	56	100	28	3	25
Total partitioned Bremer support								183	776
Percent of total Bremer support								19	81

insensitive to parameter values. In all analyses, Languriidae was paraphyletic.

Discussion

Taxonomic implications

Our results demonstrate that Languriidae is a paraphyletic assemblage nested, at least in part, within Erotylidae. Languriinae is placed at the node between *Dacne* and the remaining ingroup taxa, and this placement is relatively well supported via bootstrap (93) and Bremer (10) values. These data support the supposition of earlier coleopterists (Crotch, 1873; Chapuis, 1876; Gorham, 1899; Fowler, 1908; Roberts, 1939, 1958), that Languriinae is subordinate within Erotylidae, but provide a specific placement as a basal lineage. What is more surprising is that these data support the placement of the languriid genus *Toramus* (Toraminae) within Tritominae, as the sister taxon in this analysis to *Triplax* with strong bootstrap (100) and Bremer support (18) values. The deeply nested placement of *Toramus* in the middle of the erotylid clade has not been suggested previously and no morphological characters have been proposed that would support this finding. The host utilization analysis (see below) makes the phylogenetic placement of this genus even more curious.

Although this analysis lacks some key languriid and basal erotylid taxa, it is unlikely that Erotylidae and Languriidae are monophyletic. Inclusion of additional dacnine taxa such as *Combocerus, Cnecosa*, *Episcapha*, *Microsternus* and *Thallis* would help to resolve basal erotylid relationships and the exact position of Languriinae. This analysis lacks exemplars for three of the five languriid subfamilies, including Xenoscelinae. The xenosceline genus *Pharaxanotha* has been hypothesized to be a 'missing link' between the Languriidae and Erotylidae (Sen Gupta & Crowson, 1971; Roberts, 1939). Although the addition of these taxa may provide further insight into erotylid relationships with respect to Languriidae, it is clear from the current analyses that both Erotylidae and Languriidae are paraphyletic groups.

Dacninae, as currently delimited, including *Dacne* and *Coptengis* clearly is paraphyletic. In all analyses, *Coptengis*

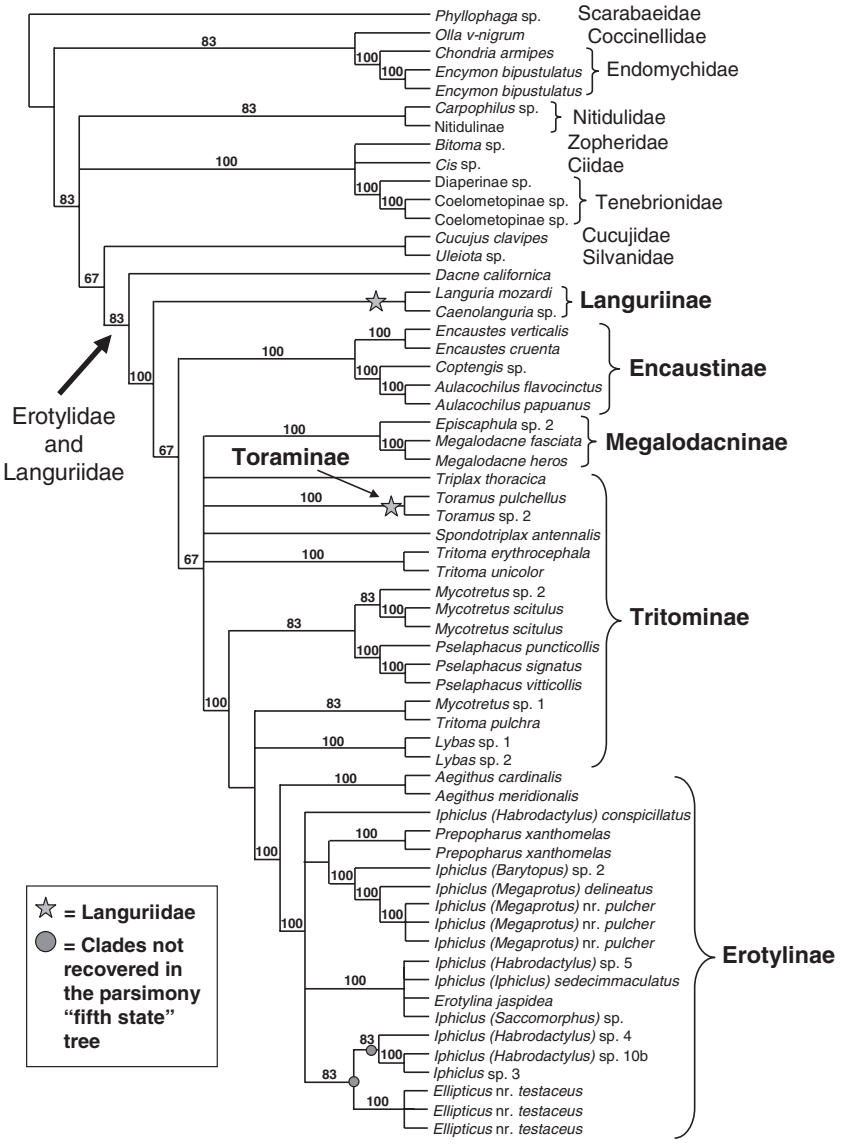

Fig. 2. Sensitivity analysis tree. This topology was derived by taking a majority rule consensus of the set of trees (six trees) originating from each of the parameter values [1 : 2 : 1 (gap : transversion : transition), 1 : 3 : 1, 2 : 2 : 1 and 2 : 4 : 1] that produced ILD values adjacent to the optimal ILD value, including the optimal (1 : 1 : 1) parameter value. Clades incongruent with the 'fifth state' topology are denoted with a circle.

was recovered within the Encaustinae as the sister to *Aulacochilus*, suggesting that *Coptengis* should be placed within Encaustinae. These data support the recognition of the subfamily Megalodacninae, as the clade *Megalodacne* + *Episcaphula* was recovered in all our analyses.

Tritominae appears in multiple lineages between the megalodacnines and the erotylines, in a grossly paraphyletic group indicated also by morphological evidence (McHugh, unpublished data). The genus *Tritoma* is not recovered as monophyletic, rather *Tritoma pulchra* groups with *Mycotretus* sp.1 and is separate from the clade *Tritoma erythrocephala* + *Tritoma unicolor*. In his revision of Erotylidae, Boyle (1956) proposes two species groups within *Tritoma*: the Humeralis Species Group including *T. erythrocephala* and *T. unicolor*, and the Sanguinipennis Species Group including *T. pulchra*. Our data suggest these species groups should be recognized as independent genera. Similarly, *Mycotretus* is not a monophyletic group and should be divided to form natural genera.

The monophyly of Erotylinae, as recovered in our analysis, received strong support from morphological, behavioural and biogeographical evidence. Erotylinae are restricted to the New World and almost exclusively to the Neotropics. Several synapomorphies based on larval morphology also support this group including: Antennomere II elongated to more than four times the length of Antennomere I, Mesothoracic spiracles with side chambers highly reduced or absent; Abdominal terga divided longitudinally by a broad membranous ecdysial suture (McHugh, unpublished data). Within the Erotylinae, however, the genus *Iphiclus* is grossly paraphyletic. *Iphiclus* currently includes ten recognized subgenera (Alvarenga, 1994). Our results, however, support the recognition of at least some of the *Iphiclus* subgenera at the generic level.

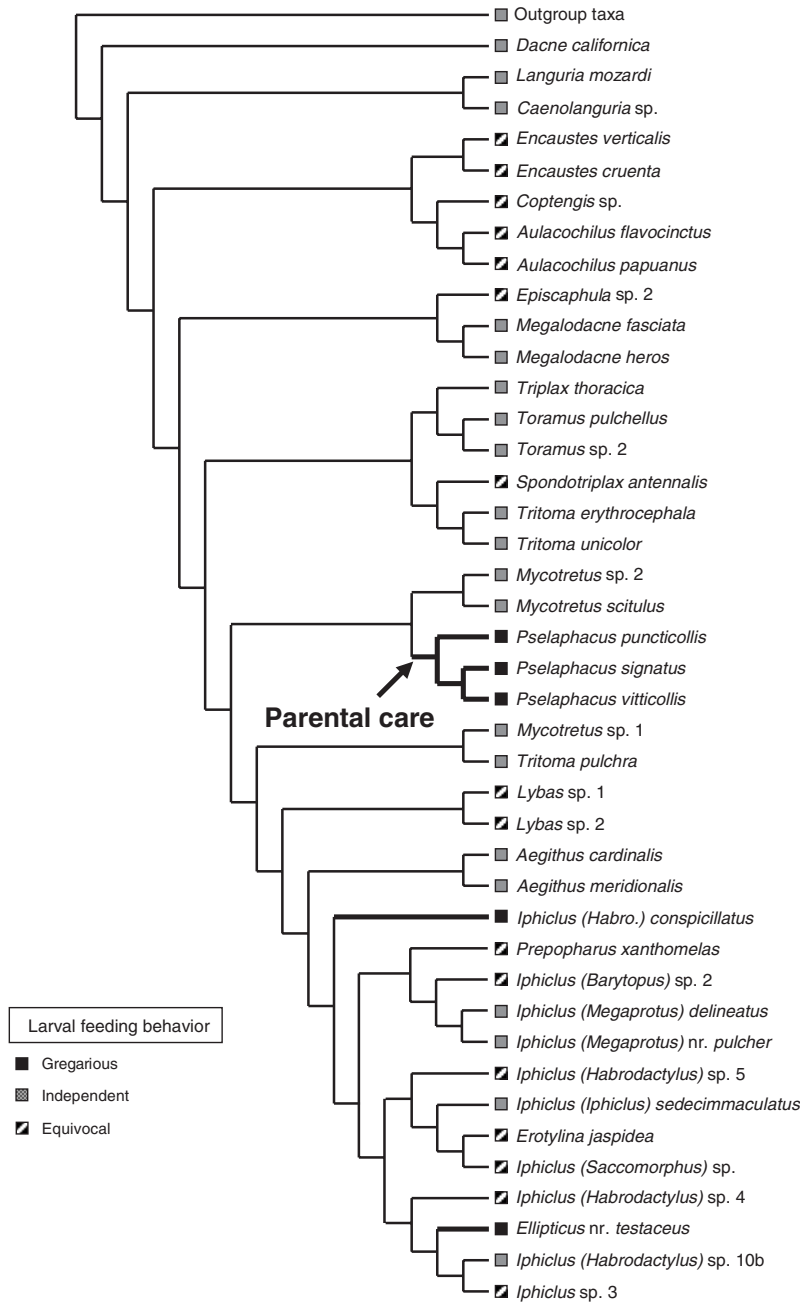

Fig. 3. Larval feeding behaviour optimized via Fitch parsimony on the combined parsimony fifth state tree (Fig. 1). Nodes where conspecific taxa are present are represented by one exemplar only.

Currently we are undertaking an analysis that includes a more extensive sampling of erotylid and languriid taxa, and thus defer making formal recommendations for reclassification of the problematic subfamilies and genera in Erotylidae until that second analysis is completed.

Biological implications

Gregariousness. With a well-supported topology in place, these data permit the exploration of the origin of larval gregariousness and its potential relationship to parental care in *Pselaphacus*. Here we define gregarious behaviour as collective feeding in a tight, coordinated association, which occurs in multiple erotylid taxa. For instance, at least some species of the erotyline genera *Iphiclus* (subgenus *Habrodactylus*) and *Ellipticus* are gregarious as larvae, whereas some species of *Prepopharus* (Erotylinae) (e.g. *Prepopharus americanus*) engage in larval and pupal gregariousness. Species of *Pselaphacus* (Tritominae) exhibit not only larval gregariousness, but parental care of larval aggregations, as adult females herd masses of

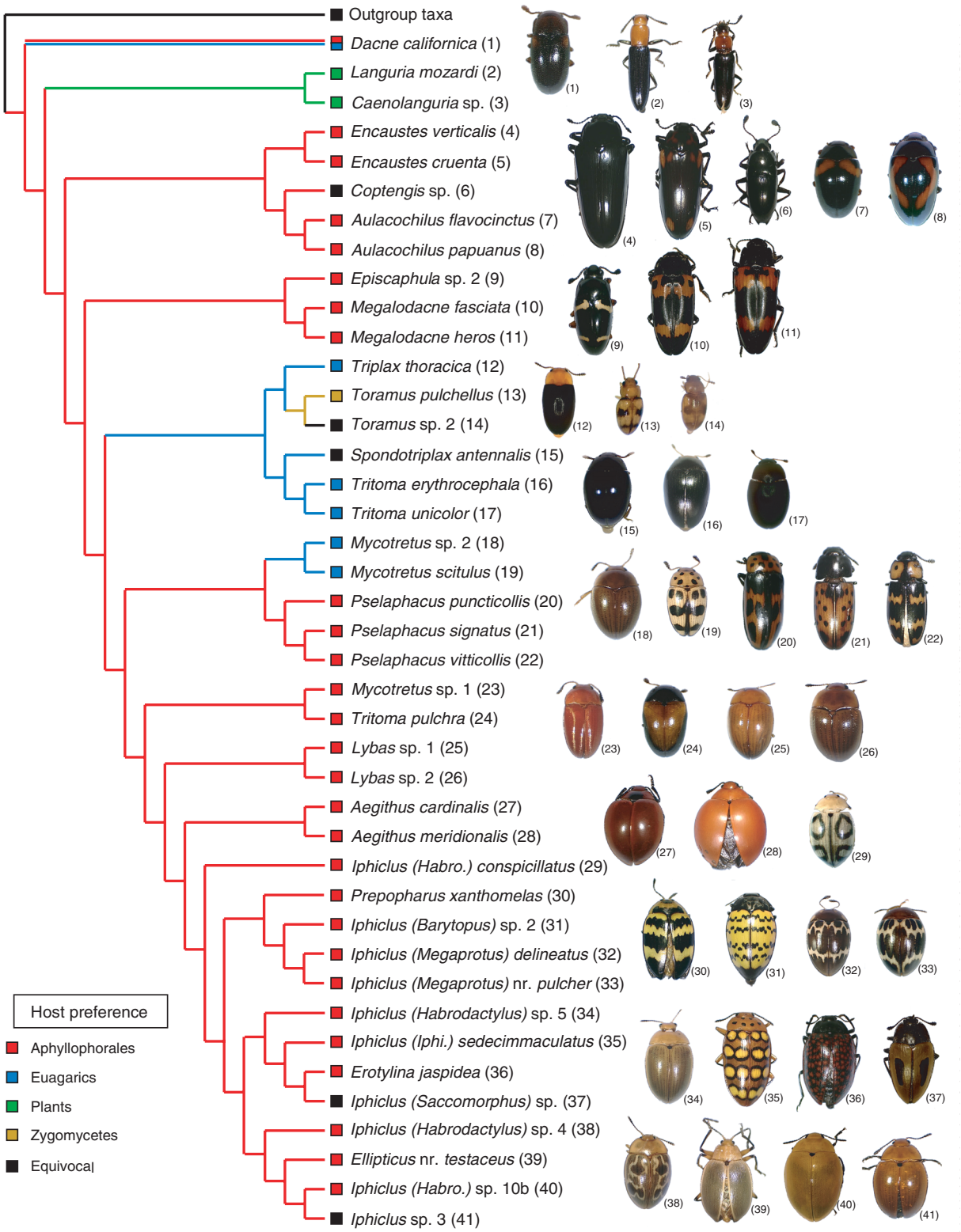

Fig. 4. Host preference optimized via Fitch parsimony on the combined parsimony fifth state tree (Fig. 1), with photo exemplars for all erotylid taxa in this analysis, depicting colour pattern diversity found within this beetle lineage. Numbers associated with taxon names correspond with numbers located to the bottom right of each photograph. Nodes where conspecific taxa are present are represented by one exemplar only.

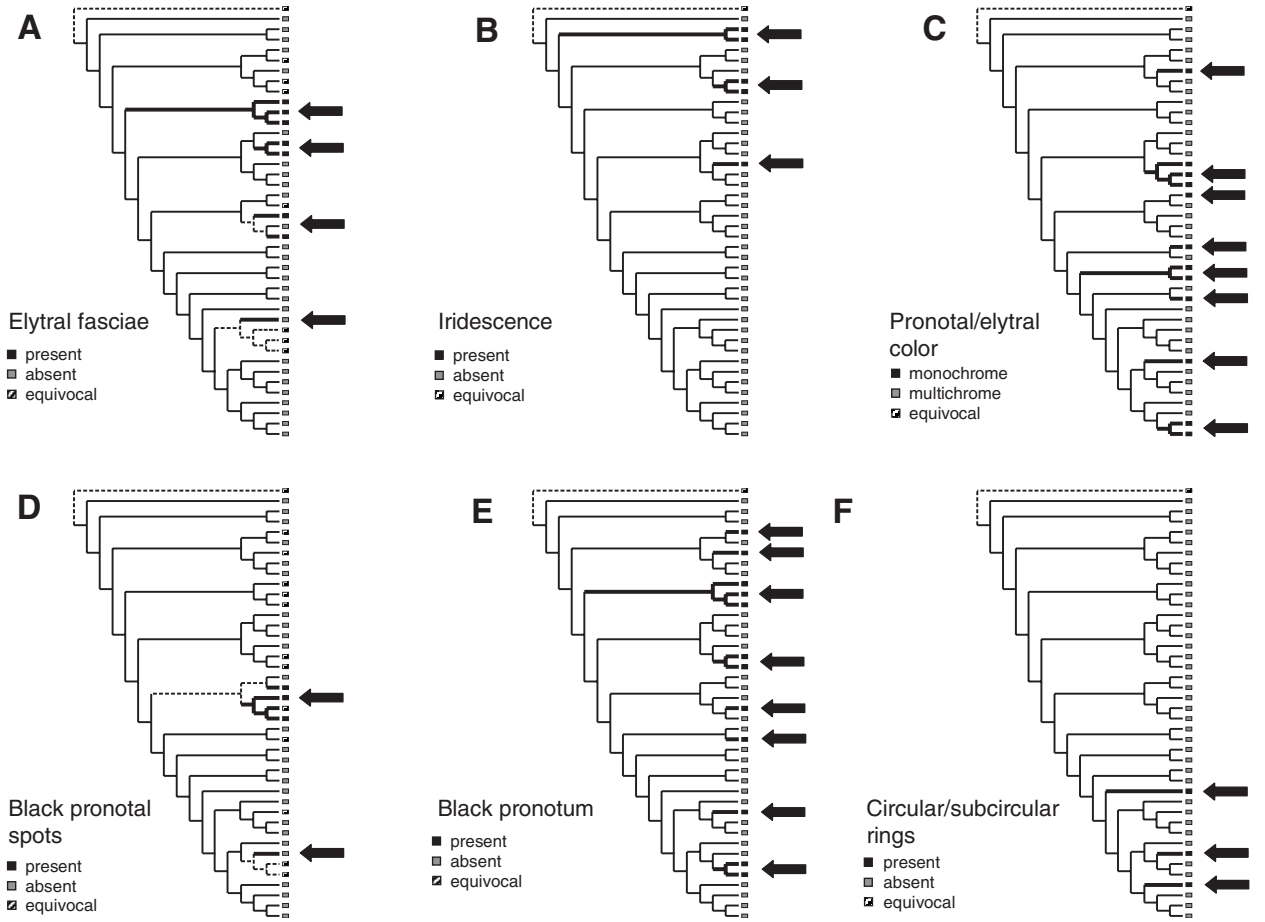

Fig. 5. Coloration characters optimized via Fitch parsimony on the combined parsimony fifth state tree (Fig. 1). Topology corresponds to Fig. 1 with names excluded. Nodes where conspecific taxa are present are represented by one exemplar only. A, Elytral fasciae; B, Iridescence; C, Pronotal/elytral colour; D, Black pronotal spots; E, Black pronotum; F, Circular/subcircular rings.

entangled larvae along fruitings of their fungal host. It is unknown whether gregariousness in its different forms evolved independently in multiple lineages, or if there was a single origin of gregariousness with a subsequent transition to parental care. Larval gregariousness and parental care were optimized on the topology using Fitch parsimony. Regardless of optimization method, these data suggest that there were at least three independent origins of larval gregariousness (Fig. 3). Gregariousness unambiguously originated once within the *Pselaphacus* clade and again in *Iphiclus* (*H.*) *conspicillatus* and *Ellipticus* nr. *testaceus*. As gregariousness data are unavailable for many of the erotyline taxa in this analysis, these data cannot refute the premise that gregariousness arose once within Erotylinae and was subsequently lost in multiple lineages. Nonetheless, these data support the hypothesis that larval gregariousness evolved multiple times within the erotylid beetles and that parental care does not appear to be a necessary prerequisite for larval gregariousness.

Host preference. The effort to optimize host preference character transformations on the topology using Fitch parsimony (Fig. 4) was complicated by recent volatility in the classification of the Basidiomycetes. The classification of fungi is undergoing major changes in light of results from recent phylogenetic studies (e.g. Hibbett *et al*., 1997; Wu *et al*., 2001; Binder & Hibbett, 2002; Moncalvo *et al*., 2002). Most fungus hosts in this study would have been classified easily as Aphyllophorales or Agaricales in the past; however, this traditional division is no longer satisfactory because neither group is thought to be monophyletic. Whereas the hosts for the mushroom-eating exemplars all fall into the newly recognized Euagarics clade (see Moncalvo *et al*., 2002), many of the Aphyllophorales hosts cannot be placed confidently into a currently recognized group because they were not included in recent phylogenetic studies. With this caveat, Aphyllophorales was used as a category for the host utilization analysis despite its known paraphyletic nature.

The host utilization tree suggests that the plesiotypic host for Erotylidae was a member of Aphyllophorales. From that state there has been one transition to phytophagy (in Languriinae) and three to Euagarics-feeding within the erotylid clade. Two clades of Tritominae have switched from Aphyllophorales to Euagarics. The *Mycotretus scitulus* + *Mycotretus* sp.2 clade represents the more distal transition. A second transition occurred in the clade comprising *Spondotriplax* sp. + *Tritoma erythrocephala* + *T. unicolor* + *T. thoracica* + *Toramus pulchellus* + *Toramus* sp.2. This clade experienced a subsequent host transition for *T. pulchellus*, which feeds on Mucorales (Zygomycetes). The third transition to Euagarics-feeding occurs in the genus *Dacne* near the basal node for the family. *Dacne californica* feeds on both Euagarics and Aphyllophorales.

Host shifts probably played an important role in erotylid evolution, possibly triggering bouts of diversification similar to those associated with host shifts in other beetle lineages (e.g. Farrell, 1998). As the classification of the fungus hosts becomes clearer, the host utilization tree should be revisited to recognize additional transitions between monophyletic subgroups of Aphyllophorales. Denser sampling of Dacninae, Languriidae and related families will help to clarify further the specific plesiomorphic host condition for Erotylidae.

Coloration. An example of the diversity of colour patterns among pleasing fungus beetles is provided in Fig. 4. We acknowledge that this analysis does not include some remarkable erotylid coloration patterns, but believe that our sampling is sufficient to begin to address some questions about the evolution of colour patterns in Erotylidae. Historically, the use of coloration as a diagnostic trait has contributed to the taxonomic disarray in Erotylidae as there appears to be much plasticity in colour patterns in some lineages (e.g. Arrow, 1925; Skelley, 1998). Nonetheless, it is possible that a chemically defended group displaying a wide range of aposematic coloration may contain among its lineages some degree of phylogenetic conservation for specific elements of overall colour patterns. Our goal is to determine if any such phylogenetic conservation of colour patterning is discernible in Erotylidae.

There seem to be some general trends in colour patterning that are conserved across the topology. Despite our best efforts, for any particular character that we code (Fig. 6), certain taxa can at best be assigned an ambiguous state. Moreover, every colour pattern we define requires multiple origins and/or losses across the topology, so there is no single unique colour pattern defining any monophyletic group in this analysis.

For instance, a classic pattern description in erotylid systematics is the presence or absence of elytral banding, or fasciae, but there are several difficulties in using this banding pattern in a phylogenetic context. First, it is unclear how to code this character in certain taxa. *Aegithus* species lack any sort of elytral banding, whereas almost all species of *Megalodacne* have banding. It is unclear, however, if the pattern found in *Iphiclus* (*Megaprotus*) species or *Mycotretus scitulus* is interrupted banding or simply dense spotting, and if *Iphiclus sedecimmaculatus* is coded for elytral fasciae as present, then should *Iphiclus* (*Barytopus*) sp.2 also be coded for possessing elytral fasciae? Secondly, it is unclear what the plesiomorphic state should be for some characters. For instance, beetles can be coded for having the pronotum and elytra the same colour (monochromic) or having multiple colours with patterning (multichromic). However, because outgroups possess both monochromic and multichromic forms, the node subtending the erotylids becomes optimized differently depending on the selection of outgroup taxa, such that this node is best considered to be ambiguously optimized. If multichromic is the pleisomorphic condition in erotylids, then monochrome pronotal/elytral coloration has evolved independently in at least eight different erotylid lineages, distributed fairly evenly across the topology (Fig. 5C). If monochromic is the plesiomorphic condition, then multichromic coloration has evolved once with eight subsequent reversions to monochromic state during erotylid evolution. Furthermore, among erotylid clades, no recurring colour pattern progressions (e.g. transitions from elytral spots to bands) were identified across the topology. It is likely that colour pattern progressions exist within certain genera, and while this may be clarified with more extensive taxon sampling, the current data suggest that a common transition pattern appears unlikely. One general conclusion of this study is that regardless of how we code characters, there appear to be multiple independent origins for colour patterning in erotylids, suggesting that colour patterning is phylogenetically labile in erotylids.

Although coloration patterns provide little phylogenetic signal, a question remains: is it still possible to correlate certain colour patterns with host preference and/or feeding habits? For instance, it may be reasonable to hypothesize that fungus beetles that feed within the body of their host may tend to be monochromic and exhibit no striking colour patterns, because aposematic coloration may be of limited utility for an internal feeder. Only in four lineages are multiple taxa with monochrome coloration contained in a monophyletic assemblage. Interestingly, those taxa exhibiting monochrome coloration do not all share the same host preference or feeding mode. For instance, *Spondotriplax* sp., *Tritoma erythrocephala* and *T. unicolor* feed on Euagarics mushrooms, on which they often are concealed while feeding among the gills and inside the soft tissues. *Aegithus* species, however, feed on Aphyllophorales fungi, grazing externally on the surface of encrusting and shelf-like forms.

According to these data, there are no apparent general trends of colour pattern across higher-level erotylid lineages with respect to host preference or feeding habits. Other biological aspects of these beetles surely complicate interpretation of this colour pattern question. For example, beetles that feed while concealed within the soft tissues of fleshy mushrooms might be exposed frequently to predators as they move around looking for new food due to the more ephemeral nature of their hosts.

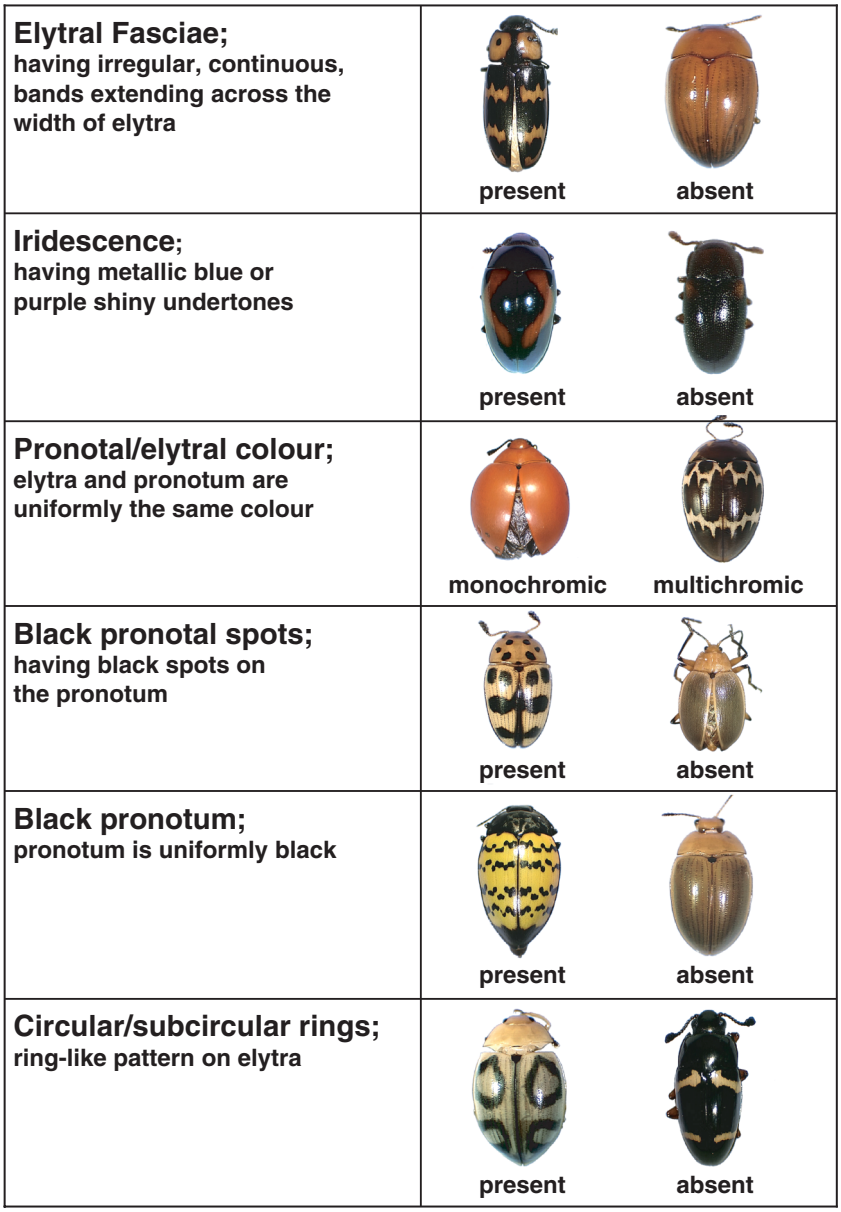

Fig. 6. Coloration characters used in this study.

This analysis provides the first quantitative estimate of erotylid phylogeny. These data support the paraphyly of the families Erotylidae and Languriidae and suggest that these families should be combined into a single group. They further indicate that additional nomenclatorial changes are needed within this beetle clade. Our data suggest that although host preference defines some major erotylid clades, there is little phylogenetic signal in coloration patterns at that level. A more extensive analysis that includes a greater sampling of taxa and combines morphological data from larvae and adults is underway. The inclusion of exemplars from all major languriid and erotylid lineages, as well as the addition of morphological data and new sequence data, should provide the basis for a more stable and natural classification for these problematic lineages, making these fungus beetles even more pleasing.

Endnote

When this manuscript was completed there were two papers in press that included phylogenetic studies of Erotylidae based on morphological data of the adult stage. These are Wegrzynowicz (2002) and Leschen (2003).

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