Molecular Phylogenetics and Evolution 60 (2011) 137–151

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Phylogeny, classification and evolution of ladybird beetles (Coleoptera: Coccinellidae) based on simultaneous analysis of molecular and morphological data

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ARTICLE INFO

Article history:
Received 6 November 2010
Revised 24 February 2011
Accepted 12 March 2011
Available online 21 March 2011

Keywords:
Coccinellidae
Cucujoidea
Phylogeny
Radiation
Diversification

ABSTRACT

Ladybird beetles (family Coccinellidae) are a species-rich, ecologically diverse group of substantial agricultural significance, yet have been consistently problematic to classify, with evolutionary relationships poorly understood. In order to identify major clades within Coccinellidae, evaluate the current classification system, and identify likely drivers of diversification in this polyphagous group, we conducted the first simultaneous Bayesian analysis of morphological and multi-locus molecular data for any beetle family. Addition of morphological data significantly improved phylogenetic resolution and support for early diverging lineages, thereby better resolving evolutionary relationships than either data type alone. On the basis of these results, we formally recognize the subfamilies Microweisinae and Coccinellinae *sensu* Ślipiński (2007). No significant support was found for the subfamilies Coccidulinae, Scymninae, Sticholotidinae, or Ortaliinae. Our phylogenetic results suggest that the evolutionary success of Coccinellidae is in large part attributable to the exploitation of ant-tended sternorrhynchan insects as a food source, enabled by the key innovation of unusual defense mechanisms in larvae.

1. Introduction

The extraordinary species richness of beetles reflects one of biology's greatest evolutionary radiations, and as such has been the longstanding subject of an ongoing search for explanation (e.g. Erwin, 1982; Farrell, 1998). Although it is clear that the tremendous diversity of this order cannot be fully attributed to any single factor, a recurring theme is the origin of specialist phytophagy on angiosperm plants (Farrell, 1998; Janz et al., 2006; McKenna et al., 2009). According to this line of reasoning, the species richness of phytophagous beetles can be explained by adaptive radiation following the rapid diversification of the angiosperms approximately 100 million years ago. This "angiosperm explosion" hypothesis has also been cited as a key factor in the diversification of Lepidoptera and Hymenoptera (e.g. Labandeira et al., 1994; Moreau et al., 2006; Danforth et al., 2006). However, angiosperm association cannot explain all beetle diversity – indeed, the majority of beetle species are non-phytophagous, and the ancestral condition for the order is most likely saprophagy or fungivory (Crowson, 1960; Lawrence and Newton, 1982). How, then, do non-phytophagous lineages diversify? What ecological or morphological correlates of diversification exist in these groups?

An ideal candidate group with which to approach these questions is the ladybird beetles (Coleoptera: Coccinellidae), an almost entirely predatory group unambiguously derived from within the Cerylonid Series, a predominantly fungivorous clade of relatively small (>1500 spp) families (Robertson et al., 2008). Ladybird beetles are ecologically and morphologically diverse, comprising roughly 6000 species that range in size from 0.8 mm (*Carinodulinka* Ślipiński and Tomaszewska) to 18 mm (*Megalocaria* Crotch). This family also exhibits a broad trophic diversity that encompasses herbivory, pollenophagy, fungivory, and highly specialized predation on aphids, whiteflies, and other invertebrates. These predatory habits have popularized certain coccinellid species as biocontrol agents in agricultural systems, as well as urban gardens. Coccinellids are also a focus of interest for studies of chemical ecology as many species possess aposematic coloration and exude noxious alkaloid-based compounds when disturbed.

Despite a long history of taxonomic attention, attempts to delineate a "natural" classification for this group below the family level have been largely unsuccessful. Since the establishment of the family name by Latreille (1807), dozens of systematic studies have proposed subfamily and tribe-level classifications (e.g. Mulsant, 1846, 1850; Crotch, 1874; Chapuis, 1876; Ganglbauer, 1899; Casey, 1899; Sasaji, 1968, 1971; Gordon, 1994; Kovář,

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doi:10.1016/j.ympev.2011.03.015

1996). Although most of these authors agree on the validity of the subfamily Coccinellinae, the infra- and interrelationships of every other proposed subfamily are fraught with contradiction and lacking in support from morphological characters.

Within the last several years, the increasing feasibility of large-scale molecular sequencing and computationally intensive phylogenetic analysis has resulted in a surge of molecular systematic studies in Coleoptera, particularly in Cucujoidea (Robertson et al., 2008) and Coccinellidae (Giorgi et al., 2009; Magro et al., 2010). The latter two authors used DNA sequence data to infer the phylogeny of Coccinellidae, with particular reference to testing the monophyly of previously proposed subfamilies and reconstructing the evolution of feeding habits and host preferences. All studies found robust support for the monophyly of Coccinellinae but disagree significantly on deeper relationships. The lack of supported relationships and short internodes along the "backbone" of both phylogenies suggest that a rapid diversification early in the history of Coccinellidae contributes to the occlusion of distinct natural lineages at a subfamily level.

Although Robertson et al. (2008) Giorgi et al. (2009) and Magro et al. (2010) have confirmed the monophyly of Coccinellidae, none employ taxon sampling that is adequate to test the currently accepted classification scheme (both of the latter two studies included all subfamilies, but only 24 and 13 tribes respectively) or to identify the factors that drive diversification in a phylogenetic framework. In order to evaluate morphological and molecular support for the current classification scheme of Coccinellidae, as well as to identify potential morphological and ecological correlates of diversification, we have conducted the first Bayesian analysis to incorporate morphological and molecular data in inferring the phylogeny of any beetle family. In light of our phylogenetic results, we present a revised subfamily and tribal classification scheme for Coccinellidae, and discuss the evolution of the group, as well as the utility of the morphology-based character systems that have traditionally been used to classify ladybird beetles.

2. Materials and methods

2.1. Taxon sampling

We chose taxa to represent all proposed tribes and subfamilies of Coccinellidae, with outgroups from the closely-related Cerylonid Series families Corylophidae and Endomychidae (Table 1; Crowson, 1960; Lawrence and Newton, 1982; Hunt et al., 2007; Robertson et al., 2008).

The 116 sampled taxa represented all seven proposed subfamilies and 42 proposed tribes outlined by Ślipiński and Tomaszewska (2010), Fürsch (1990), and Kovář (1996). For large, cosmopolitan genera of questionable monophyly (e.g. *Diomus* Mulsant, *Rhyzobius* Stephens), we sampled multiple species. Several of the genera included in this study were described subsequent to the publication or most recent electronic updating of Fürsch's classification scheme; in Table 1 and Fig. 4, these genera (*Guillermo* Ślipiński, *Poorani* Ślipiński, *Chaetolotis* Ślipiński, and *Roger* Ślipiński) are shown in the subfamilies where they were tentatively placed in the original descriptions. One genus and numerous species used in this study have not yet been formally described; we refer to them by their tribe followed by *gen. sp.* (or *sp.* for undescribed species that can be confidently associated with known genera).

2.2. Morphology coding

We scored 112 multistate characters for 116 terminal taxa in DELTA (Dallwitz et al., 1999) and subsequently exported these data into Nexus format for phylogenetic analysis. This matrix (Appendix S1) is available electronically at TreeBASE (treebase.org; matrix at http://purl.org/phylo/treebase/phylows/matrix/TB2:MXXXX). Where possible, morphological characters were scored using the same specimens used in our molecular phylogenetic analysis (see Fig. 1).

2.3. Extraction, PCR, sequencing and alignment

DNA was extracted from 95% ethanol-preserved specimens using a Qiagen DNEasy extraction kit (Qiagen, Valencia, CA). In order to preserve morphological vouchers, small-bodied specimens were split between the pro- and mesothorax but not otherwise damaged; this treatment results in well-cleared vouchers and has been no less effective than maceration. For larger beetles, a single leg was removed and split along both the femur and tibia. Morphological vouchers for all extractions were labeled with ANIC extraction numbers and slide-mounted in glycerine for further dissection and identification; these voucher specimens will be permanently deposited in wet storage at the Australian National Insect Collection.

We amplified portions of the 28S large ribosomal subunit and the mitochondrial protein-coding genes cytochrome oxidase I and II (COI and COII). Two different PCR techniques were used over the course of this study: (1) a recipe of 0.3 μl HotMaster taq polymerase (Eppendorf), 1 μl of 10 μM dNTP mix, 5 μl HotMaster taq buffer, 2.5 μl each 5-pmol/μl primer, 35.7–32.7 μl water and 3–5 μl template DNA per reaction and (2) a recipe of 12.5 μl GoTaq Master Mix (including DNTPs, buffer, taq and dye; Promega Corporation, Madison, WI), 1.25 μl 10 μM forward and reverse primer, 9.0 μl water, and 1.0 μl template DNA. GoTaq Master Mix was found to be more robust to variations in concentration of template DNA and PCR conditions. Primers used in this study are listed in Table 2.

PCR products were purified with Exosap-IT (US Biochemical Corporation, Cleveland, OH). Sequencing was performed at the JCMSR DNA Sequencing Facility (John Curtin School of Medical Research, Australian National University), using an Applied Biosystems 96-capillary 3730 DNA Analyzer. Sequence files were checked for chromatogram quality in FinchTV v. 1.3.1 (Geospiza Inc, Seattle, Washington); contigs were assembled and edited in Geneious Pro v. 4.6.4 (Biomatters Ltd.). All sequences were initially aligned using the MUSCLE webserver (Edgar, 2004, 2009), then visually inspected and adjusted in MacClade v. 4.08 (Maddison and Maddison, 2005). The 28S data were manually aligned into putative stems and loops corresponding to conserved and variably-alignable regions; regions that could not be homologized with confidence were then excluded from the phylogenetic analysis (full alignment available from authors and from Treebase) For sequences with coding regions (COI, COII), we used MacClade to confirm the correct reading frame and the absence of stop codons.

2.4. Phylogenetic analyses

The morphological dataset was analyzed using both maximum parsimony (MP) and Bayesian inference (BA). Given the size of the data set, we implemented the parsimony ratchet heuristic search strategy (Nixon, 1999) in PAUP*4.0b10 (Swofford, 2000) using scripts generated in PAUPrat (Sikes and Lewis, 2001). We performed an initial heuristic search using 100 random-addition replicates, TBR branch swapping, and holding 10 trees per replicate. Using this initial starting tree, we performed a 200-iteration parsimony ratchet weighting 5% of the characters, holding 10 trees per iteration. We repeated this analysis weighting 10% of the characters. Furthermore, we assessed clade support using 500 parsimony bootstrap pseudoreplicates with 10 random addition

Table 1
Specimens used in this study, their localities, voucher numbers, and GenBank accession numbers.

Subfamily	Tribe	Genus	Species	Locality	Voucher code	COI	COII	28S
Chilocorinae	Chilocorini	Anisorcus	sp.	Fiji: Vanua Levu	(morph)			
Chilocorinae	Chilocorini	Axion	sp.	USA: New Mexico	COC123	JF763622	JF763700	JF763533
Chilocorinae	Chilocorini	Brumoides	suturalis	India: Bangalore	COC098	JF763624	JF763701	JF763535
Chilocorinae	Chilocorini	Chilocorus	sp	Mexico	COC118	JF763629	–	JF763541
Chilocorinae	Chilocorini	Exochomus	sp.	Ethiopia: Welega Prov.	COC211	JF763646	JF763724	JF763559
Chilocorinae	Chilocorini	Exochomus	4-pustulatus	Czech Republic: Moravia	COC214	JF763647	JF763725	–
Chilocorinae	Chilocorini	Exochomus	4-pustulatus	Poland: Przedborski NP	(morph)			
Chilocorinae	Chilocorini	Halmus	sp.	Australia: Queensland	BYU-CO467	JF763651	JF763728	JF763563
Chilocorinae	Chilocorini	Orcus	sp.	Australia: Western Australia	CO587	JF763665	JF763742	JF763580
Chilocorinae	Chilocorini	Telsimia	sp	Australia: Queensland	COC173	JF763694	JF763771	JF763615
Chilocorinae	Chilocorini	Trichorcus	sp.	Australia: Queensland	COC087	–	–	JF763618
Chilocorinae	Platynaspidini	Crypticolus	sp.	Madagascar: Prov. d'Antsiranana	(morph)			
Chilocorinae	Platynaspidini	Platynaspis	luteorubra	Poland: Warsaw	(morph)			
Chilocorinae	Platynaspidini	Platynaspis	sp.	South Africa: KwaZulu-Natal	COC082	JF763668	JF763745	JF763585
Chilocorinae	Sticholotidini	Chaetolotis	amae	Australia: Queensland	COC069	–	JF763707	JF763539
Coccidulinae	Azyini	Bucolus	fourneti	Australia: ACT	COC056	–	JF763703	–
Coccidulinae	Azyini	Pseudoazya	sp.	Venezuela: Aragua	COC227	–	JF763749	JF763590
Coccidulinae	incertae sedis	Hypoceras	sp.	Australia: Queensland	COC074	JF763655	JF763732	JF763568
Coccidulinae	Coccidulini	Apolinus	sp.	Papua New Guinea: Varirata N. P.	COC239	–	JF763699	JF763531
Coccidulinae	Coccidulini	Coccidula	scutellata	Poland: Rudka	COC223	JF763633	JF763711	JF763545
Coccidulinae	Coccidulini	Cryptolaemus	sp.	Papua New Guinea: Supa	COC238	JF763637	–	JF763549
Coccidulinae	Coccidulini	Cryptolaemus	montrouzieri	Australia: ACT	AG05	JF763638	JF763715	JF763550
Coccidulinae	Coccidulini	Erithionyx	sp.	Australia: Queensland	COC072	JF763645	JF763723	JF763558
Coccidulinae	Coccidulini	Microrhyzobius	sp.	South Africa: Western Cape	COC166	JF763660	JF763738	–
Coccidulinae	Coccidulini	Rhynchortalia	sp.	Papua New Guinea: Goroka	COC241	JF763674	JF763751	JF763592
Coccidulinae	Coccidulini	Rhyzobius	sp.	Australia: ACT	COC075	–	–	JF763593
Coccidulinae	Coccidulini	Rhyzobius	sp	Australia: Queensland	COC176	–	JF763753	JF763595
Coccidulinae	Coccidulini	Rhyzobius	sp.	Poland: Przedborski NP	COC219	JF763676	JF763754	JF763596
Coccidulinae	Coccidulini	Rhyzobius	chrysomeloides	Poland: Sutno	COC222	–	JF763755	JF763597
Coccidulinae	Coccidulini	Rhyzobius	sp	Australia: Victoria	COC102	JF763675	JF763752	JF763594
Coccidulinae	Coccidulini	Rodatus	sp.	Australia: Queensland	(morph)			
Coccidulinae	Coccidulini	Roger	boothi	Australia: Queensland	COC164	–	JF763757	JF763599
Coccidulinae	Coccidulini	Scymnodes	sp.	Papua New Guinea: Crater Mtn.	COC237	JF763682	JF763761	JF763602
Coccidulinae	Cranophorini	Cranophorus	sp.	Panama: Barro Colorado Island	AG06	JF763635	JF763713	JF763547
Coccidulinae	Exoplectrini	Ambrocharis	sp.	Madagascar: Toliara Prov.	(morph)			
Coccidulinae	Exoplectrini	Chnoodes	sp.	Venezuela: Tachita PN	COC231	JF763630	–	JF763542
Coccidulinae	Exoplectrini	Exoplectra	sp.	Brazil	(morph)			
Coccidulinae	Exoplectrini	Sumnius	sp.	Thailand: Loei	COC161	JF763692	JF763769	JF763613
Coccidulinae	Monocorynini	Mimolithophilus	sp.	South Africa: Transvaal	(morph)			
Coccidulinae	Monocorynini	Monocoryna	sp.	Malaysia: Panang	AG01	JF763662	JF763740	JF763576
Coccidulinae	Oryssomini	Oryssomus	sp.	Panama	(morph)			
Coccidulinae	Poriini	Poria	sp.	(no data)	BYU-CO720	JF763671	JF763748	JF763588
Coccidulinae	Tetrabrachini	Tetrabrachys	tauricus	Ukraine: Crimea	COC249	JF763696	JF763773	JF763617
Coccinellinae	Coccinellini	Bulaea	anceps	(no data)	AG04	JF763625	JF763704	JF763537
Coccinellinae	Coccinellini	Coccinella	sp.	India: Maharashtra	BYU-CO571	JF763634	JF763712	JF763546
Coccinellinae	Coccinellini	Halyzia	straminea	China: Yunnan Prov.	COC-041	JF763652	JF763729	JF763564
Coccinellinae	Coccinellini	Hippodamia	sp.	(no data)	BYU-CO627	JF763653	JF763730	JF763565
Coccinellinae	Coccinellini	Illeis	cincta	China	COC-046	JF763656	JF763733	JF763569
Coccinellinae	Discotomini	Seladia	sp.	Costa Rica	COC224	JF763686	JF763764	JF763607
Coccinellinae	Singhikalini	Singhikalia	latemarginata	Papua New Guinea: Morobe Prov.	(morph)			
Coccinellinae	Tytthaspidini	Tytthaspis	sp.	Poland: Poznan	(morph)			
Epilachninae	Epilachnini	Chnootriba	sp.	Ethiopia	COC210	JF763631	JF763709	JF763543
Epilachninae	Epilachnini	Epilachna	sp.	Australia: NSW, Mt Warning NP	COC052	JF763644	JF763722	JF763557
Epilachninae	Epilachnini	Epiverta	sp.	Tibet	(morph)			
Epilachninae	Epilachnini	Subcoccinella	24-punct.	Poland: Sutno	COC221	JF763691	JF763768	JF763612
Epilachninae	Eremochilini	Eremochilus	sp.	(no data)	(morph)	–	–	–
Epilachninae	Madaini	Lorma	sp.	Venezuela: Aragua	COC113	JF763658	JF763735	JF763572
Epilachninae	Pentiliini	Curticornis	sp.	Venezuela Aragua, Facultad de Agronoonic UCV, Campo experimented INIA	COC228	JF763639	JF763716	JF763551
Ortaliinae	Noviini	Rodolia	sp	Madagascar: Amoron'i Mania Prov.	COC174	JF763678	JF763756	JF763598
Ortaliinae	Noviini	Rodolia	sp.	Madagascar: Prov. d'Antsiranana	COC172	JF763677	–	–
Ortaliinae	Ortaliini	Amida	sp.	China: Guangdong	COC079	JF763619	–	JF763529
Coccinellinae	Coccinellini	Nadina	mirabjlis	China	COC049		–	–
Ortaliinae	Ortaliini	Ortalia	sp.	Thailand: Chaiyaphum	COC060	JF763666	JF763743	–
Scymninae	Aspidimerini	Acarinus	sp.	Java: Cibodas	(morph)			
Scymninae	Aspidimerini	Aspidimerus	sp.	Sumatra: Barat	COC134	JF763621	–	JF763532
Scymninae	Brachiacanthidini	Cyra	sp.	Venezuela: Tachita PN.	COC232	JF763640	JF763718	JF763553
Scymninae	Brachiacanthidini	Tenuisvalvae	sp	Panama: Colon Prov.	COC130	JF763695	JF763772	JF763616

(*continued on next page*)

Table 1 (*continued*)

Subfamily	Tribe	Genus	Species	Locality	Voucher code	COI	COII	28S
Scymninae	Cryptognathini	Cryptognatha	sp.	(no data)	BYU-CO727	JF763636	–	JF763548
Scymninae	Diomini	Diomus	sp.	New Zealand: Coromandel	COC165	JF763642	JF763720	JF763555
Scymninae	Diomini	Diomus	sp	Australia: WA Leeuwin-Naturaliste N.P.	COC175	JF763643	JF763721	JF763556
Scymninae	Hyperaspidini	Hyperaspis	campestris	Poland: Rudka at Oleszno	COC224	JF763654	JF763731	JF763567
Scymninae	Madaini	Cynegetis	sp.	Poland: Snieznik Mtn.	COC226	–	JF763717	JF763552
Scymninae	Pentiliini	Pentilia	sp	Costa Rica: Puntarenas	COC126	–	–	JF763582
Scymninae	Scymnillini	Zilus	sp.	Mexico	(morph)			
Scymninae	Scymnini	Brachiacantha	sp	Costa Rica: Puntarenas	COC124	JF763623	–	JF763534
Scymninae	Scymnini	Horniolus	sp.	Cameroon, SW Prov. Butu	COC135	–	–	JF763566
Scymninae	Scymnini	Nephus	sp.	Morocco: Marrakesh pref.	COC215	JF763663	JF763741	JF763577
Scymninae	Scymnini	Sasajiscymnus	sp.	Japan: Osaka Pref.	COC137	–	–	–
Scymninae	Scymnini	Scymnodes	sp. (larva)	Australia: ACT	COC155	JF763681	JF763760	JF763603
Scymninae	Scymnini	Scymnus	sp	Panama: Coclé Prov.	COC129	JF763684	–	JF763605
Scymninae	Scymnini	Scymnus	sp.	Ethiopia (S)	COC216	JF763685	JF763763	JF763606
Scymninae	Selvadiini	Selvadius	sp.	(no data)	BYU-CO822	JF763687	JF763765	JF763608
Scymninae	Stethorini	Stethorus	sp	Bolivia: Santa Cruz Dist.	COC107	JF763689	–	JF763610
Sticholotidinae	Argentipilosini	Argentipilosa	sp.	Brazil	(morph)			
Sticholotidinae	Carinodulini	Carinodulina	burakowskii	Thailand: Doi Inthanon	(morph)			
Sticholotidinae	Carinodulini	Carinodulinka	sp.	USA: California	COC109	JF763626	JF763705	–
Sticholotidinae	Cephaloscymnini	Cephaloscymnus	sp.	Venezuela: Aragua	COC111	JF763627	JF763706	JF763538
Sticholotidinae	Limnichopharini	Limnichopharus	sp.	Indonesia: Sulawesi	(morph)			
Sticholotidinae	Microweisini	Coccidophilus	sp.	(no data)	AGNM05	JF763632	JF763710	JF763544
Sticholotidinae	Microweisini	Microweisea	sp.	USA: New Mexico	COC121	JF763661	JF763739	JF763575
Sticholotidinae	Microweisini	Sarapidius	sp.	Chile: Curico	AG09	JF763679	JF763758	JF763600
Sticholotidinae	Plotinini	Plotina	sp.	China: Zhuizhou	COC143	JF763669	JF763746	JF763586
Sticholotidinae	Serangiini	Delphastus	sp.	Bolivia: Santa Cruz	COC242	JF763641	JF763719	JF763554
Sticholotidinae	Serangiini	Serangium	sp.	South Africa: KwaZulu-Natal	COC140	JF763688	JF763766	JF763609
Sticholotidinae	Shirozuellini	Ghanius	karachiensis	Pakistan	COC116	JF763648	JF763726	JF763560
Sticholotidinae	Shirozuellini	Guillermo	sp.	Australia: Queensland	COC070	JF763650	–	JF763562
Sticholotidinae	Shirozuellini	Poorani	sp.	Australia: Queensland	COC088	JF763670	JF763747	JF763587
Sticholotidinae	Shirozuellini	Sasajiella	sp.	Indonesia, W Jawa Rancabal	COC142	JF763680	JF763759	JF763601
Sticholotidinae	Sticholotidini	Bucollelus	sp.	Australia: Queensland	COC144	–	JF763702	JF763536
Sticholotidinae	Sticholotidini	Glomerella	sp.	Venezuela: Aragua	COC112	JF763649	–	JF763561
Sticholotidinae	Sticholotidini	Habrolotis	sp.	Madagascar: Toliara Prov.	COC170	–	JF763727	–
Sticholotidinae	Sticholotidini	Jauravia	sp.	India: Kerala	COC145	–	JF763734	JF763571
Sticholotidinae	Sticholotidini	Lotis	sp.	South Africa: KwaZulu-Natal	COC146	JF763659	JF763736	JF763573
Sticholotidinae	Sticholotidini	Lotis	sp.	South Africa: KwaZulu-Natal	COC147	–	JF763737	JF763574
Sticholotidinae	Sticholotidini	Nesolotis	sp.	Sumatra: Utara	COC245	–	–	JF763578
Sticholotidinae	Sticholotidini	Nexophallus	sp.	Venezuela: Aragua	COC247	JF763664	–	JF763579
Sticholotidinae	Sticholotidini	Prodilis	sp.	Bolivia: Santa Cruz Dist.	COC106	JF763672	–	JF763589
Sticholotidinae	Sticholotidini	Sticholotidini gen. sp.		New Caledonia: Lower Comboul (Xwe Bwi) river	(morph)			
Sticholotidinae	Sticholotidini	Sticholotis	sp.	Australia: Queensland	COC148	JF763690	JF763767	JF763611
Sticholotidinae	Sticholotidini	Synonychimorpha	sp.	India: Calicut	COC100	JF763693	JF763770	JF763614
Sticholotidinae	Sukunahikonini	Paraphellus	rostratus	Australia: Queensland	COC139	JF763667	JF763744	JF763581
Sticholotidinae	Sukunahikonini	Scymnomorpha	sp.	Australia: Queensland	COC178	JF763683	JF763762	JF763604
	(Corylophidae)	Periptyctus	sp.	Australia: Victoria	COC233	–	–	JF763583
	(Corylophidae)	Periptyctus	sp.	Australia: New South Wales	BYU-CO940	–	–	JF763584
	(Endomychidae)	Amphix	sp.	Panama: Canal Zone, BCI	BYU-CO318	JF763620	JF763698	JF763530

sequence replicates per pseudoreplicate. Given computational constraints, we did not employ the parsimony ratchet in the bootstrap analysis.

We performed Bayesian phylogenetic analyses of the morphology, individual loci, and combined data sets using parallel MrBayes v3.1.2 (Altekar et al., 2004; Huelsenbeck and Ronquist, 2001). Each analysis consisted of 10^8 generations sampled every 10^5 generation, appropriate models of evolution (see below), and default priors with the exception that we employed eight MCMC chains and assumed an exponential branch length prior with lambda = 100 (see Marshall et al., 2006).

For the Bayesian analyses of the morphological data, we used the Mk1 model (Lewis, 2001) modeling rate heterogeneity among characters using a gamma distribution. For the analyses of DNA, we applied different nucleotide substitution models to the first, second, and third codon positions of the COI and COII protein-coding genes (six partitions), and the 28S gene (one partition). This partitioning strategy was selected because it has been repeatedly demonstrated that incorporating the heterogenous characteristics of DNA evolution both within and among genes dramatically outperforms analyses assuming a single model for the combined data (e.g. Winterton et al., 2007; Brandley et al., 2005; Fyler et al., 2005). For each data partition, we estimated the appropriate model of sequence evolution using the Akaike information criterion (AIC), implemented in MrModeltest (Nylander, 2002). The GTR+I+G model was selected for five of these; the exceptions were GTR+G for COI and COII third positions, and F81+I+G for COI second position. Convergence was assessed using the program Tracer v.1.5 (Rambaut and Drummond, 2009). The combined Bayesian analysis was repeated four times

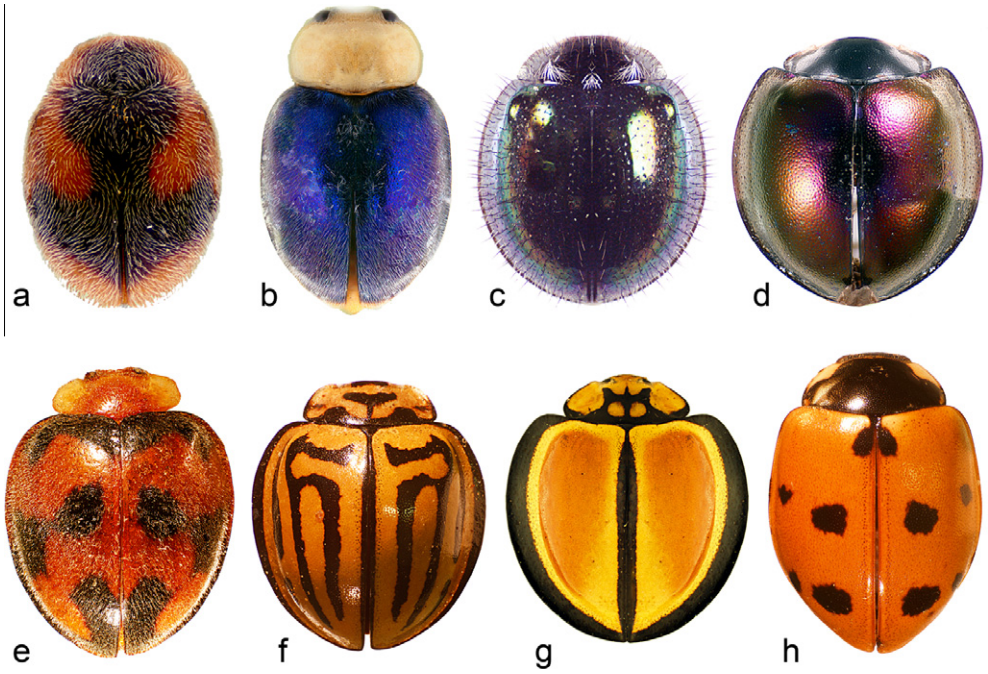

Fig. 1. Morphological diversity of Coccinellidae, habitus view of adults. (a) *Scymnus* (*Pullus*) *mitior* Blackburn; sp.; (b) *Rhynchortalia australis* Poorani and Ślipiński.; (c) *Chaetolotis amy* Ślipiński; (d) *Halmus cupripennis* Weise; (e) *Epilachna mjoebergi* Weise; (f) *Archegleis delta* (Weise); (g) *Australoneda bourgeoisi* (Kerville); (h) *Coccinella undecimpunctata* Linnaeus.

Table 2
Primer names and sequences used in this study.

Gene	Primer	Sequence	Reference
COI	TL2-N-3014	5′ TCC AAT GCA CTA ATC TGC CAT ATT A 3′	Simon et al. (1994)
	C1-J-2183	5′ GGA ACI GGA TGA ACA GTT TAC CCI CC 3′	Simon et al. (1994)
	C1-J-1718	5′ GGA TCA CCT GAT ATA GCA TTC CC 3′	Simon et al. (1994)
	LCO-1490	5′ TGA TTT TTT GGT CAC CCT GAA GTT CA	Folmer et al. (1994)
	C1-J-2195	5′ TTG ATT TTT TGG TCA TCC AGA AGT 3′	Simon et al. (1994)
COII	TL2-J-3037	5′ ATG GCA GAT TAG TGC AAT GG 3′	Simon et al. (1994)
	C1-J-2441	5′ CCA ACA GGA ATT AAA ATT TTT AGA TGA TTA GC 3′	Simon et al. (1994)
	Rlys-3772	5′ GTT TAA GAG ACC AGT ACT TG 3′	Simon et al. (1994)
28S	D1	5′ GGG AGG AAA AGA AAC TAA C 3′	Will and Gill (2008)
	D3	5′ GCA TAG TTC ACC ATC TTT C 3′	Will and Gill (2008)
	NLF184	5′ ACC CGC TGA AYT TAA GCA TAT 3′	Wild and Maddison (2008)
	D3aR	5′ TCC GTG TTT CAA GAC GGG TC 3′	Wild and Maddison (2008)

from random starting trees to ensure adequate sampling of the posterior distribution of parameters.

In order to examine the data for topological incongruence, Bayesian consensus trees from each single-gene analysis were visually inspected for strongly-supported differences in topology. Posterior probabilities (PP) greater than 0.95 were considered strongly supported (Huelsenbeck and Rannala, 2004).

3. Results

3.1. Morphology

The morphology dataset comprised 112 characters, 106 of which were parsimony informative. An initial heuristic search followed by two 200-iteration searches using the parsimony ratchet (with pct settings of 5 and 10, respectively) identified an island of most parsimonious trees (MPTs) of 1079 steps. A subsequent heuristic search within this island found 20,000 + MPTs, the majority-rule consensus of which is shown in Fig. 2; clade support is indicated by bootstrap proportions.

Bayesian analysis of the morphology dataset achieved stationarity after two million generations; the 50% majority-rule consensus tree of the post-burn-in posterior distribution is shown in Fig. S2. Besides supporting Coccinellidae and Coccinellinae monophyly both analyses failed to resolve any of the deeper relationships within Coccinellidae.

3.2. Molecular data

The combined molecular dataset comprised a total of 2253 characters: 688 from COI, 689 from COII, and 862 from 28S; of these, 1923 were variable and 1671 were parsimony informative. Independent analyses of each gene revealed no significantly-supported conflict between the various datasets (Supplementary material, Figs. A1–A3).

Bayesian analysis of the combined molecular dataset (Fig. 3) supported a moderately resolved tree, with *Microrhyzobius* sister to remaining coccinellids (PP = 1.0) but without significant support for any other nodes along the backbone. Significant support was found only toward the tips of the topology, mainly among

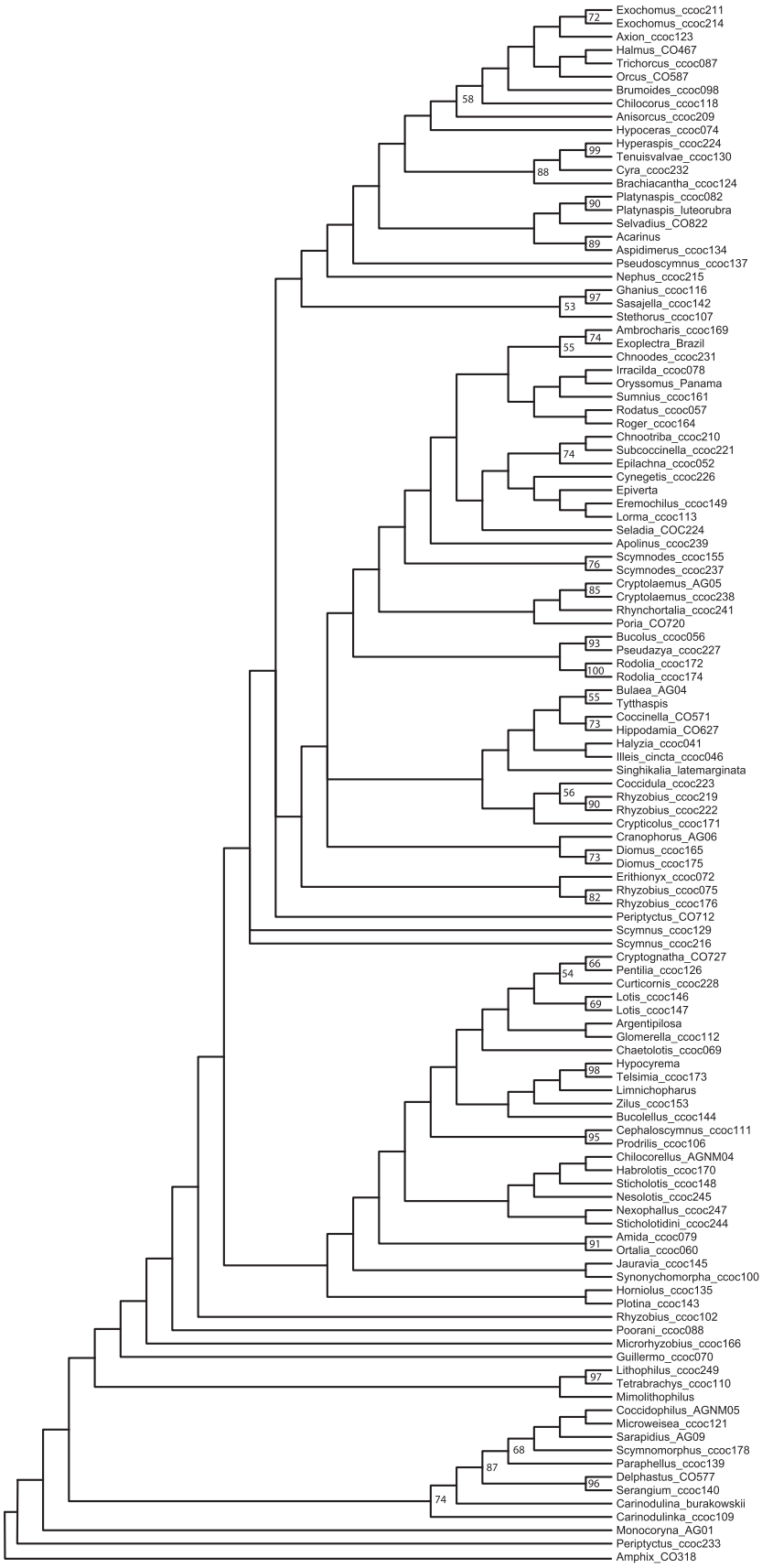

Fig. 2. Results of parsimony analysis of morphological data; 50% majority-rule consensus of most parsimonious trees, bootstrap proportions at right of nodes.

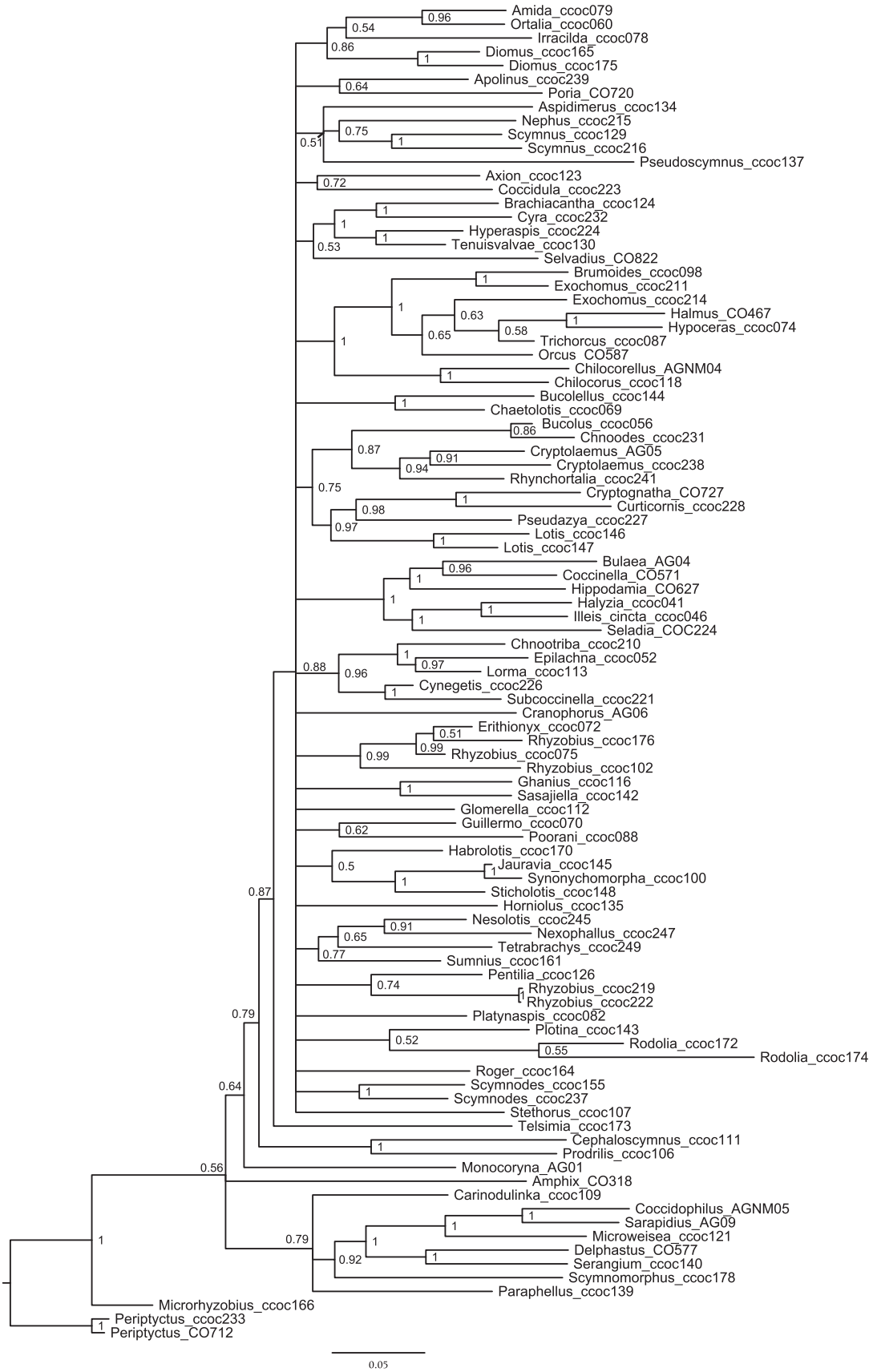

Fig. 3. Results of partitioned Bayesian analysis of combined molecular data, majority-rule consensus of post-burnin trees; posterior probabilities at right of nodes.

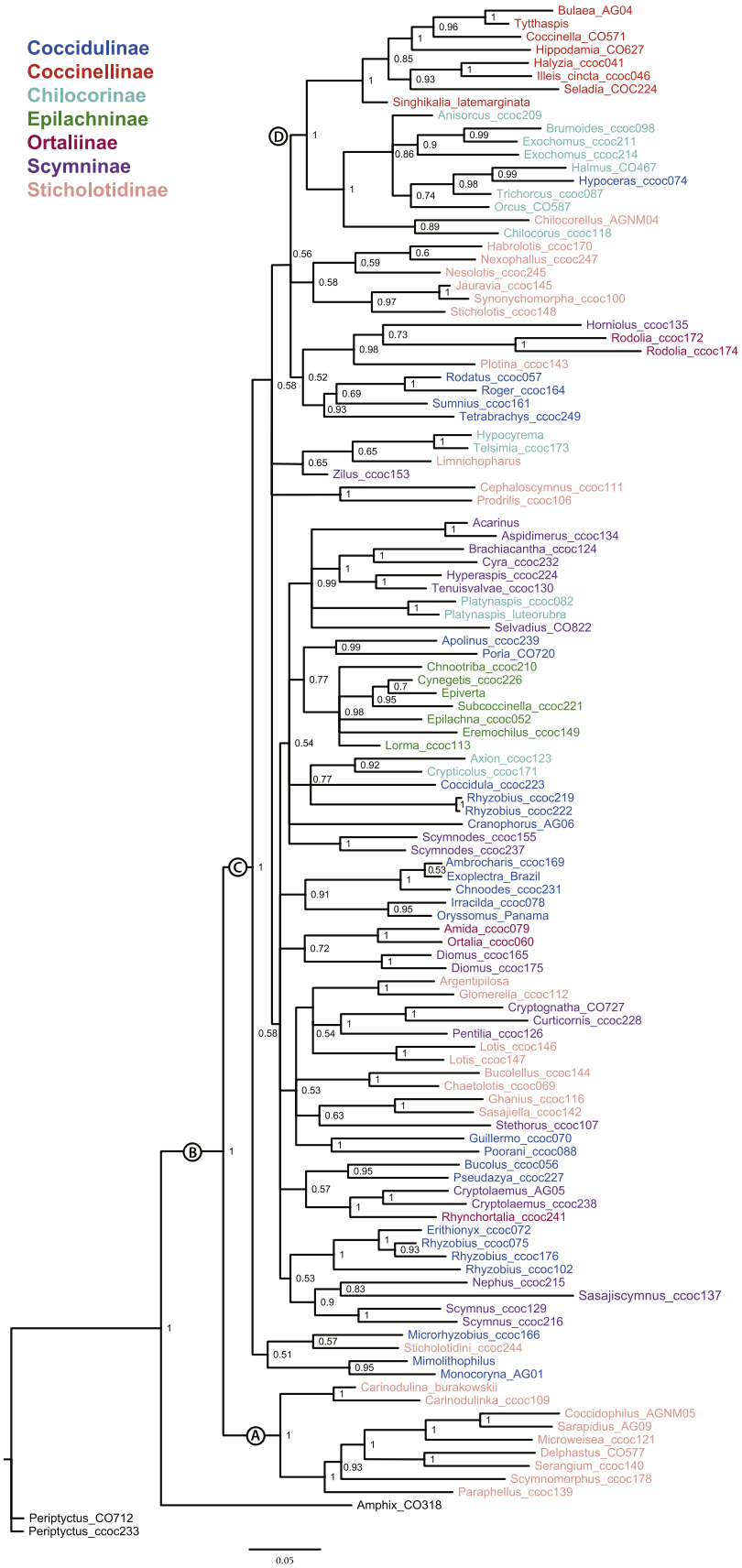

Fig. 4. Results of partitioned Bayesian analysis of combined molecular and morphological dataset, majority-rule consensus of post-burnin trees; posterior probabilities shown to right of nodes. Letters indicate key clades referred to in text.

sister-group pairs of genera (most with PP = 1.0). Coccinellinae, Epilachninae, and a group corresponding to Chilocorinae + *Hypoceras* were all significantly supported (PP > 0.95), but the molecular dataset alone was inadequate to resolve any relationships between these or other lineages.

3.3. Combined dataset

Bayesian analyses of the combined morphological and molecular dataset (Fig. 4) resulted in a better-resolved phylogeny than did the analyses of molecular or morphological data alone. These analyses supported the monophyly of Coccinellidae (PP = 1.0) as well as clades comprising Coccinellinae (PP = 1.0) and Epilachninae (PP = 0.98) in the traditional sense. The remaining subfamilies were all split into two or more well-separated clades: Ortaliinae in two clades, Chilocorinae in three clades (including the non-chilocorines *Hypoceras* and *Chilocorellus*), Scymninae in nine clades, Sticholotidinae in four clades (one including the scymnines *Cryptognatha*, *Curticornis*, and *Pentilia*), and Coccidulinae in eight clades as well as several taxa interspersed with other groups (*Hypoceras*, *Microrhyobius*, *Cranophorus*, and *Coccidula*). However, many of the currently recognized tribes were supported, including Noviini, Aspidimerini, Brachiacanthidini, Telsimiini, Cephaloscymnini, Carinodulini, Microweisini, Serangiini, and Tytthaspidini.

Four additional major clades within Coccinellidae were significantly supported by the combined dataset: a clade (A) comprising the members of "Microweisinae" *sensu* Ślipiński (2007); the sister relationship (B) between (A) and the remainder of Coccinellidae; the sister relationship (C) between a grade comprising ((*Mimolithophilus* + *Monocoryna*)(*Microrhyzobius* + *Sticholotidini gen.* sp.)) and the remainder of Coccinellidae; and the sister-group relationship (D) between Coccinellinae and a clade composed primarily of Chilocorinae.

4. Discussion

This study represents the first simultaneous Bayesian analysis of combined molecular and morphological data for any beetle family. Our results have considerable implications for both the classification and evolution of Coccinellidae, indicating a need for substantial taxonomic changes and illuminating dramatic asymmetry in clade size and species richness throughout the family. Our phylogenetic results (Fig. 4) are consistent with the classification proposed by Ślipiński (2007), but show absolutely no evidence of morphological or molecular support for the traditional classification as described by Ślipiński and Tomaszewska (2010).

We formally revise the subfamily and tribe classification of Coccinellidae based on the results of our analysis (Fig. 4) and use this phylogenetic framework to identify correlates of diversification in this ecologically unusual group.

4.1. Classification and morphological apomorphies

Below, we discuss each subfamily and tribe in the current classification system, the relevant implications of our topology, and describe the diagnostic morphological characters for each group. Characters of various instars are denoted as follows: A – adult characters, L – larval characters, P – pupal characters.

Family Coccinellidae. Monophyly confirmed (PP = 1.0, Clade B). The adult mandible with a well-developed molar part (but devoid of grinding surfaces) and the complex tegmen of male genitalia combined with a simple but rigid penis are unambiguous apomorphies for this family. All three potential sister taxa (Alexiidae, Anamorphinae Endomychidae and Corylophidae) are fungus feeders and have mandibles with well-developed grinding molar surfaces (secondarily reduced in some Corylophidae (Ślipiński et al., 2010)).

Subfamily Microweiseinae. Monophyly confirmed (PP = 1.0, Clade A). Diagnostic morphological characters include: (A) antennal insertions well in front of eyes and close together; (A) tegmen with phallobase, parameres and penis guide asymmetrical and often rotated; (A) spermatheca bi- or multicameral; (L) without dorsal glands; (L) tibiotarsus with paired spatulate setae apically; (P) without urogomphi.

This subfamily includes four tribes of very small and poorly known beetles distributed in tropics and subtropics worldwide. Each group bears some distinguishing characters but according to our results only Carinodulini are unquestionably monophyletic and sister to remaining Microweiseinae. Three remaining tribes may need to be combined in Microweiseini, as the monophyly of Sukunahikonini (*Paraphellus* and *Scymnomorphus*) was not supported by either the molecular or combined analyses. A comprehensive review of the morphology and phylogeny of this group is currently in progress (Escalona and Ślipiński, unpublished data).

Microweiseini. Monophyly confirmed (PP = 1.0). Diagnostic morphological characters include: (A) broad and complete prosternal process; (A) head rostrate in front of eyes.

Serangiini. Monophyly confirmed (PP = 1.0). Diagnostic morphological characters include (Ślipiński and Burckhardt, 2006): (A) large and flattened 1-segmented antennal club; (A) triangular prosternum forming broad chin-piece anteriorly and broad, blunt prosternal process posteriorly.

Sukunahikonini. This group is most likely paraphyletic, and may need to be combined with Serangiini and included in an expanded Microweiseini. No well-defined morphological characters distinguish this tribe; a relatively narrow prosternal process and simple prosternum are used to separate this group from Microweiseini (Ślipiński and Tomaszewska, 2005).

Carinodulini. Monophyly confirmed (PP = 1.0). Diagnostic morphological characters include: (A) long maxillary palp with cultriform terminal segment; (A) pronotum with usually complete sublateral carina; (A) pronotum and prosternum with pits or foveae; (A) metaventral and abdominal postcoxal lines V-shaped. Larva unknown. Based on characters in adult morphology and DNA data from *Carinodulinka baja* Ślipiński and Tomaszewska (2005), this tribe is here classified in Microweiseinae. Ślipiński (2007) did not include Carinodulini in his original classification because adult characters alone were not conclusive. *Carinodula* Gordon et al. is unique in Microweiseinae in having an 11-segmented antenna, a mandible with subapical tooth very close to the apical one, and the molar region of the mandible narrow and prominent.

Subfamily Coccinellinae (sensu Ślipiński, 2007). Monophyly confirmed (PP = 1.0, Clade C). The only morphological synapomorphy of this large and diverse group is the complex tegmen of the male genitalia with a large penis guide, symmetrical, primarily articulated parameres, and articulated basal strut.

Monocorynini. Monogeneric. Diagnostic morphological characters include: (A) 8-segmented antenna with peculiar 1-segmented club; (A) pronotum with complete sublateral carina; (A) tegmen unique with almost completely reduced penis guide and a complex phallobase. Larva unknown.

The association of *Monocoryna* Arrow with *Mimolithophilus* Arrow (PP = 0.95) is surprising to us as there is no clear morphological evidence to support this placement – but is in agreement with Fürsch (1990). As reflected on the morphology tree, *Mimolithophilus* displays substantial morphological similarity to *Tetrabrachys* Kapur.

Coccinellini (including: Discotomini, Singhikalini, Halyzini, Tytthaspidini, Bulaeini). Monophyly confirmed (PP = 1.0). Diagnostic morphological characters: (A) unique glands associated with

female ovipositor; (L) highly mobile and aposematic with dorsal armature and without dorsal glands; (P) abdominal tergites with gin traps.

This is a large and ecologically and morphologically diverse group. Our data did not support any of the other tribes traditionally classified in Coccinellinae, congruent with the results of Giorgi et al. (2009) and a separate, robustly sampled molecular and morphological project focusing on the subfamily Coccinellinae (Li et al., unpublished data).

Epilachnini. (including: Eremochilini, Epivertini, Cynegetini). Monophyly confirmed (PP = 0.98). Diagnostic morphological characters: (A) labium with trapezoidal mentum; (A) mandible with multiple apices and no mola; (L) larvae with very complex dorsal armature but no dorsal gland openings.

Adult and larval Epilachnini are herbivorous and are characterized by modified mandibles, a distinctly hairy, medium-sized or large body, often with a contrasting, distinctive color pattern. There is no support for the tribes Cynegetini (=Madaini) and Epivertini. The position of *Eremochilus* Weise (Eremochilini) is here solely based on adult morphology and requires further research. The critical characters of this peculiar taxon are difficult to homologize with other Coccinellidae because of its strongly modified mouthparts (Gordon and Vandenburg, 1987).

Chilocorini (including: *Chilocorellus* Miyatake, *Hypoceras* Chappuis; excluding *Axion*). Monophyly confirmed (PP = 1.0). Diagnostic morphological characters (Giorgi, 2006): (A) short antenna inserted under expanded clypeus; (A) sperm duct in female genitalia composed of two sections of different diameters; (L) with branched processes and sclerotised gland openings.

Varying degrees of clypeal expansion have independently occurred in many unrelated groups, causing problems with the definition of Chilocorini. The surprising exclusion of *Axion* from Chilocorini is based solely on molecular evidence and requires further research. The placement of *Hypoceras* in this tribe is surprising but can be well justified in morphological terms.

Telsimini (including *Telsimia* and *Hypocyrema* Blackburn). Monophyly confirmed (PP = 1.0). Diagnostic morphological characters (Ślipiński et al., 2005): (A) short, 6–7-segmented, spindle-form antenna inserted in cavity under expanded clypeus; (A) heavily sclerotized prementum.

Aspidimerini Monophyly confirmed (PP = 1.0). Diagnostic morphological characters: (A) extremely short, geniculate antenna located in anteroventral fossa; (A) large mentum covering labial palpi from below; (L) disk-like habitus, with dorsal glands.

Platynaspini (excluding *Crypticolus* Strohecker). Monophyly confirmed (PP = 1.0). Diagnostic morphological characters: (A) antenna very short with large pedicel located under expanded clypeus; (A) mentum large covering insertion of labial palpi from below; (L) disk-like habitus, with dorsal glands. Despite apparent morphological similarities (Ślipiński and Tomaszewska, 2002), we found no support for association of the Malagasy genus *Crypticolus* with *Platynaspis* Redtenbacher.

Hyperaspini (including Brachiacanthini). Monophyly confirmed (PP = 1.0). Diagnostic morphological characters: (A) short antenna bearing spiniform club; (A) male abdomen with 9th tergite visible ventrally; (L) labial palpi 1-segmented. Our taxon sampling did not include dorsally pubescent *Blaisdelliana* Gordon and many South American genera of Hyperaspini.

4.2. Taxa that are not monophyletic or for which monophyly is uncertain

Ortalinae sensu Kovář (1996) is polyphyletic with taxa representing tribes Noviini and Ortaliini forming significantly supported clades (PP = 1.0) but very far removed from one another.

Sticholotidini (including: *Sticholotis* Crotch, *Synonychimorpha* Miyatake, *Jauravia* Motschulsky, *Nesolotis* Miyatake, *Nexophallus* Gordon, and *Habrolotis* Weise; excluding: *Lotis* Crotch, *Parinesa* Gordon, *Chaetolotis* Ślipiński). Molecular and combined datasets recovered a restricted version of this tribe, albeit without significant support (PP = 0.58), placing the remaining taxa among other groups. We have found no diagnostic morphological features to support this grouping while excluding other members of traditional "Sticholotidini".

Chnoodini (including Oryssomini) form two separate, weakly supported clades (PP = 0.91, 0.93) in the combined analysis, with one group subsuming *Tetrabrachys* (Tetrabrachini), traditionally recognized as a separate tribe or subfamily. This tribe is subsequently moved to unresolved Coccidulini.

Scymnilini has not been adequately sampled to test monophyly; however, we note that New World *Zilus* clusters with Telsimini while putative Australian members of the tribe *Chaetolotis* Ślipiński and *Bucolellus* Blackburn form a separate clade (PP = 1.0).

Coccidulini (including Stethorini, Scymnini, Selvadini, Cranophorini, Poriini) form an unresolved backbone of the tree and could not be conclusively divided into monophyletic groups.

Although deeper relationships among this clade were not resolved, our results include taxonomic implications for the following genera:

(a) The widespread, species-rich genus *Rhyzobius* appears to be polyphyletic: this suggests a need for revisionary work. All Australian species sampled here form a significantly supported clade including *Erithionyx* (PP = 1.0), whereas the two Old World *Rhyzobius* (specimens 219 and 222) appear together in the grade described in (b) below.

(b) The Australian genera *Apolinus* Pope & Lawrence and *Scymnodes* Blackburn do not form a monophyletic group as suggested by Pope and Lawrence (1990) and Poorani and Ślipiński (2009). The former clusters with South American *Poria* Mulsant (Poriini) (PP = 0.99); the latter is placed in the polytomy subtending (*Apolinus* + *Poria*), Aspidimerini, Hyperaspini, Platynaspini, Epilachnini, Old World *Rhyzobius,* and *Cranophorus*.

(c) Australian and Papuan *Cryptolaemus* Mulsant and *Rhynchortalia* Crotch form a significantly supported clade (PP = 1.0) placed as a sister taxon to 'Azyini'

(d) 'Scymnini' form a clade (PP = 0.9) except for the very far removed *Horniolus* Weise.

(e) Many small, wingless ladybeetles that morphologically resemble *Rhyzobius* form grades with other putative Sticholotidini ('Sticholotidini gen.") or Shirozuellini (e.g., *Microrhyzobius* Weise, *Poorani* Ślipiński, *Guillermo* Ślipiński etc.) these taxa cannot be placed here in any reasonable group, although we note that a sister-group relationship between *Poorani* and *Guillermo* is significantly supported (PP = 1.0).

4.3. Revised classification of Coccinellidae

Given the results of our analyses, we find no justification for the continued division of Coccinellidae into the six subfamilies currently in use. We hereby formally revise the subfamily classification of Coccinellidae to comprise two subfamilies: Microweisinae Leng 1920 and Coccinellinae Latreille, 1807 (*sensu* Ślipiński, 2007). The remaining groups for which monophyly was supported are designated as tribes within Coccinellinae. Tribes whose monophyly was not adequately tested are unchanged.

Family Coccinellidae Latreille, 1807

Subfamily Microweiseinae Leng, 1920

Tribe Microweiseini Leng, 1920
Tribe Serangiini Pope, 1962
Tribe Sukunahikonini Kamiya, 1960
Tribe Carinodulini Gordon et al., 1989
Subfamily Coccinellinae Latreille, 1807
Tribe Argentipilosini Gordon and Almeida, 1991
Tribe Aspidimerini Mulsant, 1850
Tribe Cephaloscymnini Gordon, 1985
Tribe Chilocorini Mulsant, 1846
Tribe Coccidulini Mulsant, 1846
= Azyini Mulsant, 1850
= Chnoodini Mulsant, 1850
= Exoplectrini Crotch, 1874
= Cranophorini Mulsant, 1850
= Oryssomini Gordon, 1974
= Poriini Mulsant, 1850
= Scymnini Mulsant, 1846
= Scymnillini Casey, 1899
= Stethorini Dobzhansky, 1924
= Tetrabrachini Kapur, 1948
Tribe Coccinellini Latreille, 1807
= Halyziini Musant, 1846
= Discotomini Mulsant, 1850
= Tytthaspidini Crotch, 1847
= Bulaeini Savoiskaja, 1969
= Singhikaliini Miyatake, 1972
Tribe Cryptognathini Mulsant, 1850
Tribe Diomini Gordon, 1999
Tribe Epilachnini Mulsant, 1846
= Cynegetini C. G. Thomson, 1866
= Epivertini Pang and Mao, 1979
= Eremochilini Gordon and Vanderberg, 1987
Tribe Hyperaspini Mulsant, 1846
= Brachiacanthini Mulsant, 1850
Tribe Limnichopharini Miyatake, 1994
Tribe Monocorynini Miyatake, 1988
Tribe Noviini Mulsant, 1846
Tribe Ortaliini Mulsant, 1850
Tribe Platynaspini Mulsant, 1846
Tribe Plotinini Miyatake, 1994
Tribe Shirozuellini Sasaji, 1967
Tribe Sticholotidini Weise, 1901
Tribe Telsimiini Casey, 1899
Tribe Selvadiini Gordon, 1985

4.4. Comparison to previously published phylogenies

The results of our analysis bear some resemblance to those of Giorgi et al. (2009), yet differ substantially from the results of Magro et al. (2010). The parsimony and Bayesian analyses conducted by Giorgi et al. (2009) produced similar results; in the interest of methodological consistency, we compare our results (Fig. 4) only with their Bayesian topology. Giorgi et al. used only two ribosomal genes (18S, 28S), but found support for a topology largely consistent with our results. The greatest difference between our results and those of Giorgi et al. is their finding of significant support for Coccinellini as the sister group to remaining tribes of Coccinellinae (instead of sister to Chilocorini, cf. Fig. 4, clade D). Chilocorini was found by Giorgi et al. to be the sister group to Telsimia, which in our results was far removed from the remainder of Chilocorini. This discrepancy is most likely due to the difference in taxon sampling between the two studies, particularly the fact that our use of morphological data enabled the inclusion of taxa unavailable for the purely molecular analysis of Giorgi et al.

The analysis conducted by Magro et al. (2010) was based on five, primarily ribosomal loci (COI, 12S, 16S, 18S, and 28S) but employed particularly asymmetrical taxon sampling: although Coccinellinae constitutes less than a fourth of the species in Coccinellidae, over half the sampled taxa were drawn from this subfamily. In addition, the 30 (mostly European) species of non-coccinellines sampled represented just 18 of the ~265 genera of non-coccinelline ladybirds. This disparity in taxon sampling is likely responsible for the following substantial differences in phylogenetic hypotheses between the current study and that of Magro et al.: The latter authors found Epilachninae (represented by two genera) to be the sister group to the remainder of Coccinellidae, with statistically significant support; a sister-group relationship between *Diomus* and *Hyperaspis* was strongly supported, and the epilachnine genus *Subcoccinella* was placed, remarkably, as the sister group to *Rodolia* with 100% bootstrap support. Although Magro et al. incorporated a substantial number of loci, their combined dataset failed to compensate for inappropriate taxon sampling and resulted in significant support for nonsensical relationships.

4.5. Utility of morphological data

The first simultaneous, combined Bayesian analysis of morphological and molecular data was conducted by Nylander et al. (2004), who used 3080 bp of sequence data and 166 morphological characters to infer relationships among gall wasps (Cynipidae). Nylander et al. found that: (1) statistical analysis of the morphology data yielded a topology similar to that of a parsimony analysis; (2) morphology contributed a notable amount of signal to the combined analysis; and (3) was not "swamped" by the signal from the much larger molecular dataset. Subsequent studies incorporating both datatypes found similar results: including morphology adds backbone support and helps to resolve the overall phylogeny (Wahlberg et al., 2005; Lee et al., 2009; Glenner et al., 2004; Bernhard et al., 2009; Lopardo et al., 2010)

In recent years, phylogenetic analyses have increasingly relied on molecular data to the exclusion of morphology. Although a purely molecular approach may be relatively rapid, less dependant on specialist knowledge and specimen preparation, and provides and enormous number of nucleotide characters, morphology has proven an extremely valuable data source in many studies, particularly those which incorporate fossil or extinct taxa, as well as specimens that may not have been preserved with DNA extraction. Moreover, the Mk1 model (Lewis, 2001) has also proven adequate for modeling the evolution of morphological data with no more spurious assumptions than in any parsimony analysis (Nylander et al., 2004). In addition, it is no longer necessary to destroy, macerate, or otherwise obscure the morphology of most arthropod specimens in order to collect molecular data: DNA has been successfully extracted and amplified from intact, dried, pinned specimens (Gilbert et al., 2007) as well as single legs of microscopic insects (e.g. Acs et al., 2010; Buffington et al., 2007)

The most valuable contribution of morphological data to mixed-datatype analyses may well be its ability to resolve deeper nodes within a phylogeny: in the current study, as well as in Lopardo et al. (2010), a combined morphology-DNA dataset provides deep resolution where neither datatype alone could. Even in molecular phylogenetic studies incorporating an extremely robust DNA dataset (e.g. Regier et al., 2009, with five protein-coding nuclear genes), "backbone" support remains elusive.

In light of the results of this study and those listed above, we strongly recommend the inclusion of morphological data in phylogenetic analyses whenever possible, as well as retention of morphological vouchers from individual extractions and permanent or semi-permanent slide mounting after sequence data has been obtained.

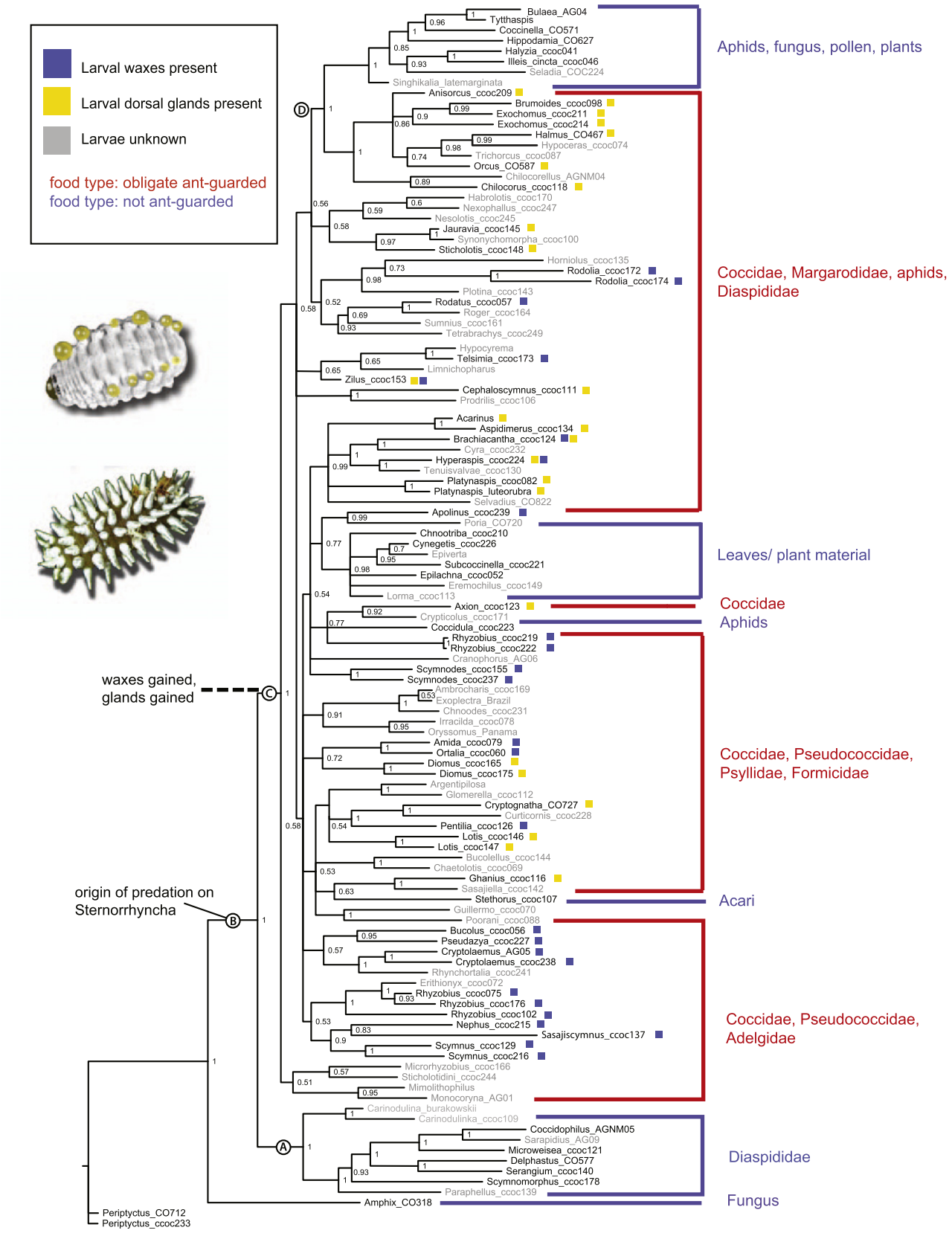

Fig. 5. Distribution and likely origins of larval defensive characters (dorsal glands; waxy exudates). Letters indicate key clades referred to in text.

4.6. Evidence for adaptive radiation in Coccinellidae

The diversity and evolutionary success of Coccinellidae is striking when compared to their closest phylogenetic relatives. Coccinellids comprise over 6000 species, more than the rest of the Cerylonid Series combined; they are also alone amongst Coleoptera in preying almost exclusively on sternorrhynchan insects. (Robertson et al., 2008; Leschen, 2000). Unlike the hyper-diverse beetle families Chrysomelidae and Curculionidae, the diversification of Coccinellidae cannot be understood as an escalating evolutionary "arms race" or adaptive radiation on plant hosts. Rather, coccinellid species richness is an example of dramatic diversification in a polyphagous group. The results of our analyses indicate four correlates of diversification, two ecological and two morphological (Fig. 5): an ancestral shift from fungivory to carnivory on armored scales, family Diaspididae (Fig. 5, clade B); a shift from feeding on armored scales to soft-bodied Sternorrhyncha (Fig. 5, clade C) and the origin of larval defensive glands and waxy exudates (Fig. 5, clade C).

Trophic shifts from fungivory to predation have occurred in a handful of species representing several lineages of fungus beetles, including at least four families of cucujoids; Leschen (2000) suggested that fungus feeders may be preadapted for this transition, proposing the following mechanism: (1) fungivory/ mycophagy is the ancestral condition for a beetle lineage; (2) beetles feed on sooty molds, which grow on the sugary "honeydew" of sternorrhynchan insects; (3) beetles switch to predation on sternorrhynchans (many of which are sedentary and soft-bodied, requiring little or no specialized predation behavior in beetles). Giorgi et al. (2009) inferred such a shift in the ancestral lineage of all coccinellids, from mycophagy (as in all other Cerylonid Series families) to obligate predation on Sternorrhyncha. Our results lend additional credence to this scenario, with a completely predatory Microweiseinae (clade A) sister to the remainder of the family.

Although this trophic shift is undoubtedly one of the most critical factors in the evolution of Coccinellidae, it alone cannot explain the family's diversity: many other groups in which similar shifts have occurred have not diversified so dramatically (Leschen, 2000). The second likely catalyst for coccinellid speciation is the origin of ant-specific larval defense mechanisms, including waxy exudates (Fig. 5, blue[3] squares) and dorsal defensive glands (Fig. 5, yellow squares), which most likely occurred in the common ancestor of Coccinellinae and have been secondarily lost in all non-sternorrhynchan-feeding groups, e.g. Coccinellini (aphids, fungus), Epilachnini (plant material) and *Stethorus* (mites).

These defensive characters may be necessary precursors to successful predation on scales and other soft-bodied sternorrhynchans. Whereas the armored scales (Diaspididae) are not attended by ants, the remaining families of sternorrhynchan prey – Coccidae, Margarodidae, Aleyrodidae, Pseudococcidae, Adelgidae, and Aphidae – are all visited or heavily guarded by ants in a well-documented mutualistic interaction (e.g. Way, 1963; Holldobler and Wilson, 1990; Gullan, 1997; Giorgi et al., 2009). Sedentary sternorrhynchans attended by ants are a lucrative food source: ant-guarded populations often persist longer, grow larger, and mature later (Holldobler and Wilson, 1990). Adult beetles may poach these "ant livestock" with the protection of explanate pronota, epipleura, and retractile appendages, but larval coccinellids have no such armor.

Dorsal glands in larvae are found throughout Coccinellinae, predominantly in taxa formerly placed in Chilocorinae and Sticholotidini. These glands clearly function in defense, but whether through repellent effects or through chemical camouflage depends on the taxon in question. Coccinellid larval dorsal gland secretions have been demonstrated to repel ants (e.g. Völkl, 1995; Finlayson et al., 2009; Happ and Eisner, 1961) and in some cases to function as a form of ant-specific chemical crypsis (e. g. *Diomus*; Vantaux et al., 2010).

Thick waxy larval exudates are common in a variety of coccinelline groups, particularly those formerly placed in Coccidulinae, Scymninae and Ortaliinae (Steinweder, 1929; Pope, 1979). These waxes often completely conceal the larvae and pupae; they may function in chemical or tactile mimicry of their prey (as in mealybug-feeding *Cryptolaemus*) or form a mechanical defense against ants. Harris (1921) noted that the wax shield of *Ortalia pallens* allows it to prey directly on ants in close proximity to their colony, without repercussion.

Interestingly, the two major lineages in which both defensive glands and waxes have been lost (Coccinellini and Epilachnini) are large-bodied, conspicuously aposematic beetles and exposed feeders; coccinellines feed on aphids, fungi, and plant material, while epilachnines feed exclusively on plant tissue. Reflex bleeding is used as a defense in both adults and larvae of these groups. Thus, reflex bleeding may be either effective enough against vertebrate predators or metabolically costly enough to merit warning colors in adults and larvae, while larval dorsal defensive glands are not.

5. Conclusion

The distribution of clade size across our phylogeny is consistent with a marked increase in species richness following the origin of feeding on soft-bodied, ant-tended Sternorrhyncha and the simultaneous origin of larval defenses against ants. The evolution of dorsal glands and waxes most likely allowed coccinellines to thrive and diversify on a diet of ant-guarded scale insects. Further study will be necessary to pinpoint the precise origin of soft scale feeding and its attendant larval defenses: specifically, ecological data is sorely needed for the *Monocoryna* group (sister to the remainder of Clade C) and the Carinodulini (sister to the rest of Clade A). No adult or larval trophic information is available for these genera: all specimens used in this and other studies have been collected in leaf litter siftate or pitfall traps, which in combination with their reduced eyes (e.g. Carinodulini, *Mimolithophilus*) suggests they are soil or leaf litter-dwellers.

Acknowledgments

We wish to thank the following persons and institutions for their generous and patient assistance, input, and sharing of data: Joe McHugh (University of Georgia), James Robertson (University of Arizona), and Matthew Brandley (University of Sydney). Specimens for this study were provided (and in many cases, collected) by Roger Booth, Mike Caterino, Vladimir Gusarov, Richard Leschen, Nathan Lord, Kelly Miller, Geoff Monteith, Gino Nearns, Hong Pang, J. Poorani, Alex Riedel, Nicolai Yunakov, Marek Wanat, David Kavanaugh, Stephanie Clutts and Michael Sharkey. We are particularly grateful to Stephen Cameron (CSIRO) and Michael Whiting (Brigham Young University) for support with laboratory resources and sequencing costs.

This study was supported by ABRS grant to A. Ślipiński, NSF AToL Grant EF-0531665 to M.F. Whiting, DEB-0531768 to B. Farrell, NSF PEET Grant DEB-0329115 to J.V. McHugh, M.F. Whiting, and K.B. Miller.

[3] For interpretation of color in Fig. 5, the reader is referred to the web version of this article.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2011.03.015.

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