

# Molecular phylogeny of Nitidulidae: assessment of subfamilial and tribal classification and formalization of the family Cybocephalidae (Coleoptera: Cucujoidea)

ANDREW R. CLINE<sup>1</sup>, TREVOR R. SMITH<sup>2</sup>, KELLY MILLER<sup>3</sup>,  
MATTHEW MOULTON<sup>4,5</sup>, MICHAEL WHITING<sup>5</sup> and PAOLO  
AUDISIO<sup>6</sup>

<sup>1</sup>California Department of Food & Agriculture, Plant Pest Diagnostics Center, Sacramento, CA, U.S.A., <sup>2</sup>Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, FL, U.S.A., <sup>3</sup>Department of Biology, University of New Mexico, Albuquerque, NM, U.S.A., <sup>4</sup>Department of Human Genetics, EIHG, University of Utah, Salt Lake City, UT, U.S.A., <sup>5</sup>Department of Biology and M.L. Bean Museum, Brigham Young University, Provo, UT, U.S.A. and <sup>6</sup>Department of Biology and Biotechnologies 'Charles Darwin', Sapienza Rome University, Rome, Italy

**Abstract.** We present a molecular phylogeny of Nitidulidae based on thirty ingroup taxa representing eight of the ten currently recognized subfamilies. Approximately 10 K base pairs from seven loci (*12S*, *16S*, *18S*, *28S*, *COI*, *COII* and *H3*) were used for the phylogenetic reconstruction. The phylogeny supports the following main conclusions: (i) Cybocephalidae are formally recognized as a distinct family not closely related to Nitidulidae and its constituent taxa are defined; (ii) Kateretidae are sister to Nitidulidae; (iii) Cryptarchinae are monophyletic and sister to the remaining nitidulid subfamilies; (iv) subfamily Prometopinae **stat. res.** is reinstated and defined, to accommodate taxa allied to *Axyra* Erichson, *Prometopia* Erichson and *Megauchenia* MacLeay; (v) Amphicrossinae, Carpophilinae and Epuraeinae are shown to be closely related taxa within a well-supported monophyletic clade; (vi) tribal affinities and respective monophyly within Nitidulinae are poorly resolved by our data and must be more rigorously tested as there was little or no support for prior morphologically based tribes or genus-level complexes; (vii) Nitidulinae are found to be paraphyletic with respect to Cillaeinae and Meligethinae, suggesting that they should either be subsumed as tribes, or Nitidulinae should be divided into several subfamilies to preserve the status of Cillaeinae and Meligethinae; (viii) *Teichostethus* Sharp **stat. res.** is not a synonym of *Hebascus* Erichson and the former is reinstated as a valid genus. These conclusions and emendations are discussed in detail and presented within a morphological framework.

## Introduction

Nitidulid beetles, also known as sap, pollen and picnic beetles, occur in almost every major biogeographical region and occupy a diverse array of niches. Nitidulidae biology is exceptionally varied and incorporates most major life history strategies known within Coleoptera, including, fungivory (including saprophagy and detritivory), phytophagy (including vegetative and reproductive structures), necrophagy, facultative predation,

pollination of numerous flowering plant families and inquilinism with social insects (reviewed by Audisio, 1993; Cline, 2005; Jelínek *et al.*, 2010). The most prevalent biological associations include phytophagy and fungivory, with some nitidulids commonly encountered as pests of crops and dried goods (e.g. several Carpophilinae, Epuraeinae and Nitidulinae). Recently, the small hive beetle (*Aethina tumida* Murray) has received much attention as a pest of honey bee colonies and its life history is now one of the best known within the family; other sap beetles are also becoming invasive in beehives and their respective life histories are being detailed as well (e.g., Marini *et al.*, 2013; Audisio *et al.*, 2014b).

Correspondence: Andrew R. Cline, Plant Pest Diagnostics Center, 3294 Meadowview Road, Sacramento, CA 95832, U.S.A. E-mail: andrew.cline@cdfa.ca.gov

The nitidulid lineage of Cucujoidea includes three families: Smicripidae, Kateretidae and Nitidulidae. Smicripidae was defined based on larval characters only (Böving & Craighead, 1931), which helped disentangle confusion that had persisted for several decades. Previous studies based on adult morphology (i.e. LeConte, 1878; Sharp, 1900; Casey, 1916; Leng, 1920; Hetschko, 1930), all placed Smicripidae within Monotomidae, whereas Horn (1879) placed them within Nitidulidae. Kateretidae (*sensu* Audisio, 1994, 1995; Cline and Audisio, 2010), unlike Smicripidae, have long been associated with Nitidulidae, generally considered a nitidulid subfamily until recently (Audisio, 1993). Like Smicripidae, Kateretidae was first elevated to family level based on larval characters (Verhoeff, 1923). More recently, Audisio (1984) suggested splitting Kateretidae from Nitidulidae based on adult features, which was further elaborated and formalized by Kirejtshuk (1986b). The nitidulid lineage of beetles was recovered as monophyletic in a morphological cladistic analysis of basal Cucujoidea (Leschen *et al.*, 2005).

Nitidulidae classification has a long and complex history. Characterization of the family is difficult and the literature is replete with examples of members from unrelated families being misplaced in Nitidulidae. Two main reasons for this confusion, and the resultant lack in overall understanding of the family, are the small body size of many groups and the need for dissections of males to ascertain species identity. Size-wise, taxa span more than one order of magnitude, from minute (~1) to ~15 mm in length. The overall morphology of the group is diverse with globose convex taxa and elongate parallel-sided brachypterous groups. Some are extremely setose whereas others are completely glabrous, and whereas some can be brilliantly coloured with metallic hues and contrasting black and orange or reds, others are shades of brown.

Nitidulids are a relatively old lineage of Cucujoidea with origins dating back to at least the Cretaceous (Ponomarenko, 1983; Kirejtshuk & Ponomarenko, 1990). Recent estimates based on molecular clock analyses (Hunt *et al.*, 2007) suggest a possible origin of Nitidulidae in the Early Cretaceous (c. 120–140 Ma). Nitidulidae are the second most diverse family of cucujoid beetles, comprising ~4000 species, and are divided into ten subfamilies (Jelínek *et al.*, 2010): Calonecrinae, Carpophilinae Erichson, Amphicrossinae Kirejtshuk, Meligethinae Thomson, Epu-raeinae Kirejtshuk, Nitidulinae Latreille, Cillaeinae Kirejtshuk and Audisio, Maynipeplinae Kirejtshuk, Cryptarchinae Thomson and Cybocephalinae Jacquelin duVal. Calonecrinae and Maynipeplinae are the only subfamilies with relatively restricted distributions; the former being found in Southeast Asia and the latter in central Africa.

Cybocephalids have been confused as nitidulids since their original description. When Erichson (1844) described *Cybocephalus* the tarsal formula was assumed to be 5-5-5, the fifth segment being minute and apparently difficult to discern at the time. The 5-5-5 tarsal formula, as well as some superficial morphological similarities, led Erichson to place the genus within Nitidulidae. Fifteen years later, with marginally better microscopy, Jacquelin du Val (1858) determined that *Cybocephalus* possessed only four tarsomeres and the genus was then placed in a separate subfamily. Murray (1864) agreed with

Jacqueline duVal and further supported the separation. Subsequently, Böving & Craighead (1931), after analysing Nitidulidae and *Cybocephalus* larvae, determined that cybocephalines were clearly separate from Nitidulidae. This was upheld by later studies, including two major revisionary works (Parsons, 1943; Audisio, 1993). Endrödy-Younga (1962, 1968) asserted Cybocephalidae was a distinct family, an opinion shared by Audisio (1993). However, some researchers retained Erichson's inclusion in Nitidulidae (Horn, 1879; Crowson, 1967); vacillated between family and subfamily status (Kirejtshuk, 1984, 1986b, 1998b, 2000, 2008; Kirejtshuk *et al.*, 1997); or kept them included based on historical convention (Vinson, 1959; Habeck, 2002). Smith & Cave (2006, 2007a,b) recently revised the Nearctic and West Indian Cybocephalidae and again recognized these beetles as a distinct family. Likewise, Hisamatsu (2013) did the same for the Japanese fauna.

Cybocephalidae fossils have been found in amber deposits dating from the early Miocene to the early Eocene (c. 53 Ma; Palmer *et al.*, 1957; Hieke & Pietrzeniuk, 1984; Kirejtshuk & Nel, 2008). The family is found in all major biogeographic regions with >150 total described species in eight extant genera (Yu & Tian, 1995; Tian, 2000). An additional genus, closely related to *Cybocephalus*, was recently described from Eocene French Amber (Kirejtshuk & Nel, 2008). The greatest species diversity occurs in the tropics, but the beetles are prevalent in temperate regions as well. *Cybocephalus* is Holarctic, with some members extending into subtropical and tropical areas. In the New World, *Pycnocephalus* Sharp can be found in Central and South America, whereas the remaining present-day cybocephalid genera – *Endrodiellus* Endrödy-Younga, *Hierro-nius* Endrödy-Younga, *Horadion* Endrödy-Younga, *Pastillodes* Endrödy-Younga and *Pastillus* Endrödy-Younga – are found in Africa and *Taxicephomerus* Kirejtshuk is restricted to Southeast Asia. The Chilean nitidulid genus *Nodola* Brethes may also belong to this family, but it could not be included in the present study. Cybocephalids are all predatory and, for taxa where the specific biology is known, they feed exclusively on hemipterous scale insects (see Silvestri, 1910; Smith & Cave, 2006; Jelínek *et al.*, 2010).

The status and monophyly of nitidulid subfamilies has never been tested until now. Herein, we present the first quantitative phylogenetic analysis, based on >10 000 base pairs of DNA sequence data, for eight of the ten purported nitidulid subfamilies. Cline (2005) predicted that Nitidulinae would be rendered paraphyletic and Cybocephalinae would be separated as a distinct family (the latter was also suggested by Bocak *et al.*, 2014); both of these hypotheses were tested as part of this study, as well as previously published hypotheses of tribal composition and affinities within Nitidulinae.

## Materials and methods

### DNA extraction, sequencing and alignment

Thoracic muscle tissue was extracted from each specimen, or whole specimens were used due to the small size

of some taxa. Genomic DNA was extracted using Qiagen DNEasy kits (Valencia, CA, USA) and the protocol for animal tissue. DNA-extracted specimens are preserved as primary DNA vouchers deposited in the Insect Genomics Collection at Brigham Young University, Provo, Utah, as well as the frozen tissue collection at the California State Collection of Arthropods, Sacramento, California, and all have BYU voucher numbers.

Seven genes were sequenced and utilized for analyses: 12S, 16S, 18S and 28S rRNA, cytochrome c oxidase I (*COI*), cytochrome c oxidase II (*COII*) and histone III (*H3*). Primers for PCR amplification and sequencing were obtained from several sources (see Table S3). DNA fragments were amplified via PCR using Taq Gold® (Applied Biosystems, Foster City, CA, U.S.A.) or Taq Platinum (Invitrogen, Carlsbad, CA, U.S.A.) on a DNA Engine DYAD® Peltier Thermal Cycler. PCR and sequencing reaction conditions are detailed in Tables S1, S2. Negative controls were used to monitor contamination. Each PCR product was examined and products were purified using MontagePCR<sub>96</sub> Cleanup Kit (Millipore, Billerica, MA, U.S.A.). Sequencing reactions were cycle sequenced using ABI Prism BigDye v3 (ABI, Fairfax, VA, U.S.A.) and products were purified using Sephadex G-50 Medium. The products were sequenced in both directions using an ABI 3730xl DNA analyser (DNA Sequencing Center, BYU). Sequence data were edited and initial sequence alignment was performed using Sequencher® v4.6 (Genecodes, 2007).

#### Taxon sampling

One species of Kateretidae (*Anthonaeus agavensis* (Crotch)) and 31 different species representing 25 genera of Nitidulidae were included as ingroup (see Table S4). As Kateretidae and Nitidulidae have had an intertwined taxonomic history (Jelínek & Cline, 2010; Jelínek *et al.*, 2010) with retention of the former as a subfamily of the latter or as a distinct family itself, *A. agavensis* was selected to determine whether Kateretidae should or should not be included within Nitidulidae.

The two genera *Glischrochilus* Reitter and *Paromia* Westwood were selected as exemplars of Cryptarchinae. *Glischrochilus* is a Holarctic taxon, whereas *Paromia* is found only in the Patagonian region of South America (Blackwelder, 1945). *Amphicrossus* Erichson, *Carpophilus* Stephens, *Epuraea* Erichson and *Trimenus* Murray were all selected to test the hypothesis of a purportedly monophyletic 'Carpophilin lineage'. *Fabogethes* Audisio & Cline was selected to determine whether the subfamily Meligethinae is sister to or contained within Nitidulinae, but not to ascertain the monophyly and intrageneric relationships of Meligethinae, which was recently addressed (Audisio *et al.*, 2009). The New World genera *Conotelus* Erichson and *Macrostola* Murray were selected to establish monophyly and placement of Cillaeinae with respect to other nitidulid subfamilies.

*Prometopia* Erichson, *Megauchenia* Macleay, *Nitidula* F., *Thalycra* Erichson, *Lobiopa* Erichson, *Triacanus* Erichson,

*Teichostethus* Sharp, *Stelidota* Erichson, *Aethina* Erichson, *Cratonura* Reitter, *Pallodes* Erichson, *Anthocorcina* Kirejtshuk, *Phenolia* Erichson, *Hebascus* Erichson, *Pocadius* Erichson, *Cychramus* Kugelann and *Niliodes* Murray were all selected to test previous hypotheses of tribal and generic complexes within Nitidulinae. Specifically, *Prometopia* and *Megauchenia* were chosen to assess the purported monophyly of the 'axyroid' group of genera and its phylogenetic placement within Nitidulidae. *Nitidula* was selected to assess its previously asserted basal position within Nitidulinae. *Thalycra*, *Teichostethus*, *Pocadius*, *Cychramus* and *Niliodes* were chosen to determine the boundaries and relatedness of Cychramini and the *Pocadius* and *Thalycra* genera complexes. *Triacanus* and *Pallodes* were selected to determine the status of the tribe Cyllochini and its relative placement in Nitidulinae. *Aethina* was selected as a representative of the *Aethina* complex of genera and *Anthocorcina* as a representative of the tribe Mystropini. *Lobiopa*, *Phenolia* and *Stelidota* have been tentatively placed in various places within Nitidulinae and they were included to determine their relative positions within the subfamily. *Cratonura* was selected due to its enigmatic position in Nitidulinae and as a representative of an odd assemblage of south temperate taxa from Chile.

Some genera of Nitidulidae had two species sampled. These multiple samples were analysed due to broad generic limits based on morphology and wide geographic ranges (i.e. *Carpophilus*, *Phenolia* and *Stelidota*), or to corroborate the purported monophyly and placement of the taxa (i.e. *Trimenus* see Jelínek, 1979, 1982). Members of the two monotypic and problematic Nitidulidae subfamilies (i.e. Calonecrinae and Maynipeplinae), were not available for molecular analyses, and therefore these subfamilies are therefore not treated here. However, their isolated position among the Nitidulidae, probably closer to the Carpophilin lineage, is well-defined based on adult morphology (Kirejtshuk, 1998b); a recent review of the family provides further details of their placement (Jelínek *et al.*, 2010). All Cucujoidea taxa are considered ingroups to assert Cybocephalidae's relationship with Nitidulidae. Outgroups were selected based on the historical and modern confusion surrounding Nitidulidae, availability and to provide a robust framework with which to analyse existing subfamilial and tribal classifications within Nitidulidae (i.e. Sphindidae, Endomychiidae, Coccinellidae, Silvanidae, Passandridae and Erotylidae) to assess the phylogeny of Nitidulidae and its relationship to both Cybocephalidae and Kateretidae.

Outgroup taxa included: two Tenebrionoidea taxa (1 Tenebrionidae and 1 Colydiidae) and three Cleroidea taxa (1 Trogossitidae, 1 Cleridae and 1 Melyridae).

#### Phylogenetic analysis, tree construction and statistical measures

Sequence alignment was carried out in the program Muscle (EMBL-EBI 2014) using default parameters.

Partitioned Bayesian analyses (combined data) were carried out in MrBayes v3.1 (Ronquist and Huelsenbeck, 2003) with mixed model settings. The model GTR+G+I

was determined to be the most justified using ModelTest (Posada & Crandall, 1998). Data were partitioned by gene (12S, 16S, 18S, 28S), as well as codon position in protein coding genes (combined *COI* and *COII* together) for a total of ten partitions. Branch lengths were estimated separately in the model for each of the partitions. Default prior and proposal settings were used. Two Markov Chain Monte Carlo runs, each with one cold and three incrementally heated chains, were performed with 5 million generations sampled every 1000th generation. The first 2.5 million generations were discarded in each run as burn-in.

#### Morphological examination

Standard protocols for dissection of specimens were followed, including soaking specimens in warm soapy water, utilization of minuten tools and fine forceps for genitalia and mouthpart extraction, and chemical cleaning with a dilute 10% aqueous KOH solution. Genitalia, mouthparts and internal sclerites were placed on temporary glycerin slide mounts for microscopic analysis. Some specimens were placed in a Branson® 1200 series sonicator to remove excess debris from the body surface. A Nikon® SMZ 1500 stereomicroscope and Leitz® Laborlux 12 compound microscope were used for observations. Measurements were made with a calibrated ocular micrometer. Type and nontype specimens analysed for the comparative morphology portions of this project were derived from institutions listed in the acknowledgments section.

All external images were made through a Diagnostic Instruments, Inc. digital camera (Model# 11.2 Colour Mosaic) attached to a Nikon® SMZ1500 dissecting microscope or using a Syncroscope AutoMontage® system attached to a Leica Z16 APO microscope. For the first system, multiple images were acquired and montages compiled using SPOT® Advanced software and CombineZP® freeware, and subsequently touched up using Adobe® Photoshop®. Scale bars were calibrated with an ocular micrometer using SPOT® Advanced software on images taken with the digital camera attached to the Nikon® SMZ1500. Scanning electron micrographs were prepared using a JEOL models JSM 6300 and JSM-5510LV SEMs.

## Results

#### Phylogenetic results

The resultant phylogram from the Bayesian analysis is presented in Fig. 4. Our results indicate novel findings with consequences for the higher-level phylogeny of Nitidulidae. These include first and foremost the need to formalize the family, Cybocephalidae, and support its exclusion from the nitiduloid lineage (i.e. Nitidulidae, Kateretidae and Smicripidae). Cybocephalidae is recovered in a clade with Sphindidae with weak statistical support, but clearly distant from Nitidulidae, thus corroborating recent findings by Bocak *et al.* (2014). Sphindidae was a previously recognized basal lineage of Cucujoidea (Beutel & Ślipiński, 2001). The morphological evidence (see

Table 1) and molecular data clearly indicate that Cybocephalidae should be considered a separate family and not contained within Nitidulidae. The pertinent taxonomic delimitation of Cybocephalidae is provided below.

Nitidulidae are recovered as monophyletic in all analyses. Kateretidae are likewise always placed as sister to the Nitidulidae ingroup taxa (Fig. 4). Although some subfamilies such as Cryptarchinae appear to be relatively stable, others will need more robust taxon sampling to fully establish their placement within Nitidulidae and their relationships to other subfamilies. The two exemplars of Cryptarchinae (*Glischrochilus* and *Paromia*) were always retrieved together and consistently placed as the sister group of the other subfamilies. The monobasic subfamily Amphicrossinae is associated with taxa from Epuraeinae and Carpophilinae, and placed in a clade containing Epuraeinae, Carpophilinae and Amphicrossinae. Carpophilinae, as represented here, are found to be sister to Epuraeinae.

A large and problematic, but well supported, clade containing members currently placed in Nitidulinae, Meligethinae and Cillaeinae is deeply subordinate within Nitidulidae. The two Cillaeinae genera, *Conotelus* and *Macrostola*, group together and they are placed with the nitiduline genera *Stelidota* and *Teichostethus*. Meligethinae are represented by a single species of the genus *Fabogethes*, and placed with *Thalycra* and *Lobiopa*. These results strongly suggest that Nitidulinae are paraphyletic, with respect to Meligethinae and Cillaeinae.

This 'Nitidulinae' clade comprises two well-supported subclades. The first contains *Nitidula*, *Thalycra*, *Lobiopa* and *Fabogethes*. The second clade contained the remainder of the taxa, with *Triacanus* being the sister to the remaining taxa. Within the second clade Cillaeinae are the sister of *Teichostethus* and *Stelidota*, whereas the sister group of this lineage comprises a clade consisting of the genera *Aethina*, *Cratonura*, *Pallodes*, *Anthocorcina*, *Phenolia*, *Hebascus*, *Pocadius*, *Cychramus* and *Niliodes*. These various clades within Nitidulinae suggest that current hypotheses of tribal composition and affinities are likely paraphyletic and incongruent with the phylogeny of the subfamily. Tribal classifications have never undergone rigorous quantitative analysis, except for Cyllodini (Leschen, 1999).

#### Taxonomic results

Family: Cybocephalidae Jacquelin DuVal

Cybocephalinae Jacquelin DuVal 1858 (pg. 151 lists 'Cybocephalites. Groupe 4.', which is contained within the family grouping 'Nitidulides')

Cybocephalidae; Böving and Craighead 1931 (larval characterization)

Cybocephalidae; Endrödy-Younga 1962a (adult and biological characterization)

Type Genus: *Cybocephalus* Erichson, 1844: 441

Included genera. *Cybocephalus*, *Endrodiellus*, *Hierronius*, *Horadion*, *Pastillodes*, *Pastillus*, *Pycnocephalus*, *Taxicephomeus* and †*Pastilloenicus* Kirejtshuk.

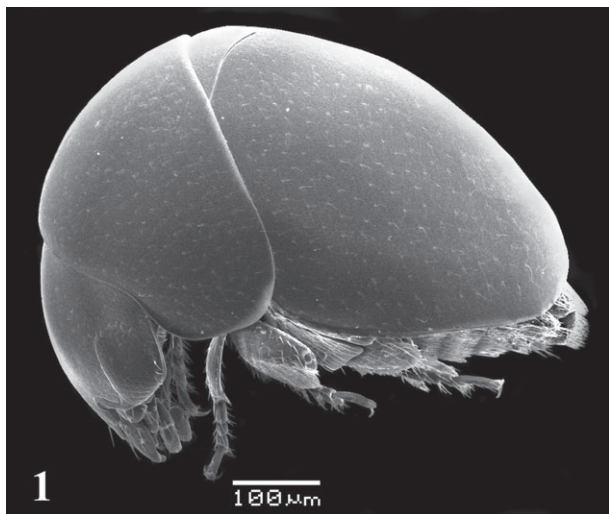
**Table 1.** Characters and character states differentiating Nitidulidae and Cybocephalidae.

#	Character	Cybocephalidae	Nitidulidae
<b>Adult</b>			
1	Predation on hemipterous insects	Ubiquitous	Rare, 1 genus
2	Tarsal formula	4-4-4	5-5-5
3	Number of abdominal ventral plates (excluding male anal plate)	5	6
4	Number of abdominal spiracles pairs	5	6
5	Body contractile, capable of conglobulation with mandibles resting on metasternum	Yes	No
6	Elytra abbreviated, exposing pygidium	Never	Often
7	Shape of prothoracic coxae	Circular/conical	Transverse
8	Prosternal process extending posteriorly beyond prothoracic coxal cavities	No	Yes
9	Antennal insertion separation	Narrow	Broad
10	Mesoventral procoxal rests	Absent	Present
11	Mesoventrite discrimen	Absent	Present
12	Metendosternite stalk	Absent	Present
13	Subocular antennal grooves on ventral head surface	Absent	Present
<b>Larvae</b>			
1	Predation on hemipterous insects	Yes	Unknown
2	Dorsal sutures on head capsule	Present	Absent
3	Pregomphi & urogomphi on terminus	Absent	Present
4	Hypostomal rods	Present	Absent
5	Long antennal chetae	Present	Absent
6	Orientation of hypostomal ridges	Divergent	Convergent
7	Hypopharynx with sclerome and bracons	No	Yes
8	Maxillary mola	Absent	Present
9	Annular spiracles with lateral air tubes	Absent	Present
10	Fringed mandibular prostecha	Absent	Present (except Meligethinae)
11	Distal sensory appendage on second antennal segment	Slender, shorter than third antennomere	Conical, longer than third antennomere
12	Spiracular air tube location	On outer sides of peritreme	Inside peritreme
13	Mandibular prostheca/retinaculum	Absent	Distinct
14	Abdominal dorsal setae	Capitate	Simple
15	Maxillary palpus	Two segmented	Three segmented
16	Paired lateral conical projections on eight and ninth abdominal segments	Absent	Present
17	Pronotal tergal plates	Absent	Present
18	Clypeolabral suture	Absent	Present
19	Frontal sutures	Contiguous	Separate

*Diagnosis.* Cybocephalidae differ from Nitidulidae and other Cucujoidea, in adult and larval morphology. Table 1 emphasizes the differences between Nitidulidae and Cybocephalidae, but these characters will differentiate Cybocephalidae from other Cucujoidea as well. Cybocephalidae adults can be differentially diagnosed by the following combination of characters: a 4-4-4 tarsal formula; five visible abdominal ventrites (excluding the male ‘anal plate’); and five pairs of abdominal spiracles; body contractile (capable of conglobulation) allowing the mandibles in repose to rest against the metasternum (Fig. 1). The larvae of Cybocephalidae (Fig. 2) can be differentially diagnosed by the following combination of characters: head without dorsal sutures; pregomphi and urogomphi absent on abdominal tergite XI; hypostomal rods present with divergent hypostomal ridges posteriorly; hypopharynx without a sclerome or bracons; mandibles without mola; mandibles without prostheca;

peculiarly long seta present on last antennomere (nearly as long as the whole antennae); and annular spiracles with two lateral air tubes. Cybocephalidae pupae differ from Nitidulidae and other Cucujoidea, in the shape and composition of the terminal abdominal segment (Fig. 3A, B).

*Comments.* Cybocephalids are obligate predators, feeding almost exclusively on scale insects. A few nitidulids have been reported to be predaceous; one on sternorrhynchous insects – *Cychramptodes murrayi* Reitter on *Cryptes baccatus* (Maskell); one facultatively predaceous on mosquito larvae – *Amphicrossus japonicus* Reitter (Kovac *et al.*, 2007); and a few species of the genus *Pityophagus* Shuckard are facultatively predaceous on weevil larvae (Audisio *et al.*, 2011). Some Neotropical nitidulids in the tribe ‘Cyllodini’ (*Eusphaerius* Sharp and related genera) have adults that are capable of



**Fig. 1.** *Cybocephalus iviei* T.R. Smith, lateral habitus of adult; scale bar = 100  $\mu$ m.

partial conglobulation; however, the mandibles do not appear to rest against the metasternum in repose. The larvae of Nitidulidae, with which cybocephalids have often been confused, have pregomphi and urogomphi, no hypostomal rods but with hypostomal ridges strongly convergent posteriorly, a hypopharynx with a sclerome and bracons, mandible with a raised mola, mandibles with a fringed protheca (except Meligethinae which share a simple or simply toothed inner mandibular edge), much shorter setae on last antennomere (usually shorter than the latter alone) and biforous spiracles (Hayashi, 1978; Audisio, 1993; Kirejtshuk *et al.*, 1997). Larval differences were illustrated by Böving & Craighead (1931) and Hayashi (1978). Many Cybocephalidae larvae also have peculiar trumpet-shaped setae (Fig. 2C), which are not known in Nitidulidae, nor many other Cucujoidea. Although not directly inferred from our data, a relationship of Cybocephalidae to the Cerylonid series of Cucujoidea may be tentatively proposed. Cybocephalidae exhibit a 4-4-4 tarsal formula, which is typical of Cerylonid series members. The presence of five abdominal plates also occurs in members of the Cerylonid series. Although absence of a character does not necessarily denote relatedness, numerous Cerylonid series taxa do not possess pregomphi or urogomphi as larvae, which is the condition in Cybocephalidae. Likewise, some Cerylonid series members are predaceous on hemipterous insects as both larvae and adults. Our data do not necessarily indicate a definitive relationship to Cerylonid-series families.

#### *Status of the subfamily Prometopinae*

One particular group of nitidulids was retrieved near Amphicrossinae, Epuraeinae and Carpophilinae, and in our analysis is composed of *Prometopia* and *Megauchenia*. These two genera have been regarded as Nitidulinae by previous authors, but were retrieved in our analysis as sister taxa to each other and residing outside of all other Nitidulinae. Our conclusion supports an

earlier classification by Böving and Craighead (1931) of subfamilial status for *Prometopia*, based solely on larval characters.

Subfamily: Prometopinae Böving and Craighead, 1931 **stat. res.**

Type Genus: *Prometopia* Erichson, 1843: 279

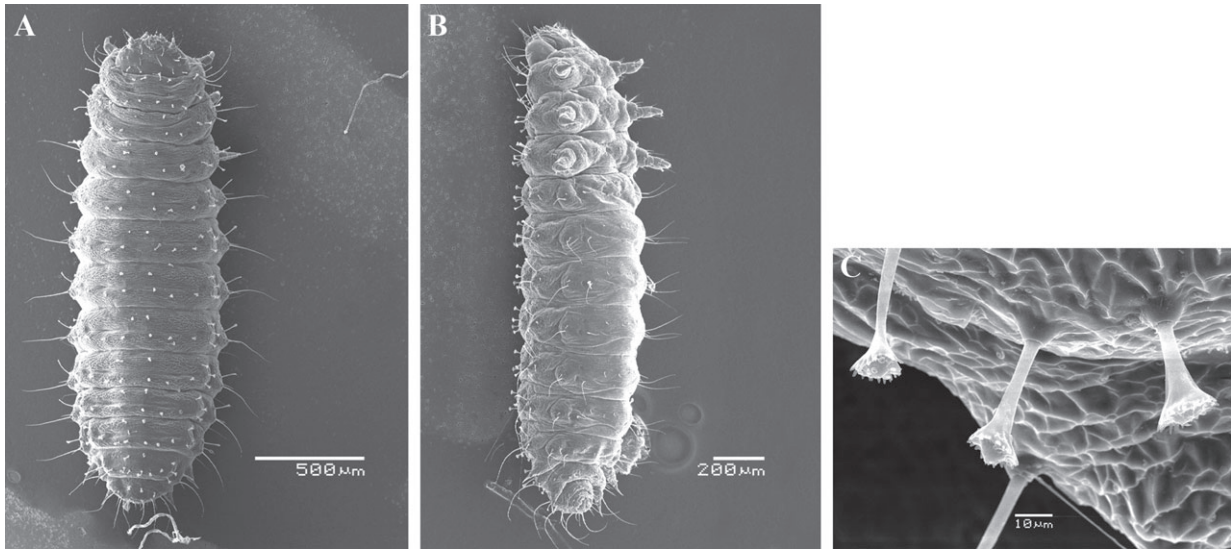
*Included genera.* *Axyra* Erichson (Fig. 7A), *Pseudoplatychora* Grouvelle, *Megauchenia* MacLeay, *Platychora* Erichson (Fig. 7B), *Prometopia* Erichson (Figs 5A, B, 6A–D, 7C), *Parametopia* Reitter, *Palaeometopia* Kirejtshuk (fossil from the Dominican amber; see Kirejtshuk & Poinar, 2007), *Taraphia* Audisio and Jelínek (Fig. 7D) and *Megaucheniodes* Audisio and Jelínek (see Audisio & Jelínek, 1993, Fig. 2).

*Diagnosis.* Prometopinae taxa can be differentiated by the following combination of characters: (i) presence of a shallow or deep sulcus (often delimited by a raised line) along lateral margin of head adjacent and typically posterior to each eye (faint in *Prometopia*); (ii) presence of deeply diverging metacoxal lines (Fig. 5A, B) on abdominal sternite I (reduced in *Taraphia*); (iii) meso- and metacoxae widely separated (Figs 5, 7C); (iv) tarsomeres simple, never bilobed or with densely setose ventral empodium; (v) antennomere 3  $\geq 2 \times$  length of antennomere 2; (vi) mycangium often present near prosternal suture or basal region of metacoxal axillary line (absent in *Prometopia*, *Parametopia* and *Platychora*). To date, only larvae of *Prometopia* have been described (Fig. 6A–D). However, *Prometopia* possesses urogomphi unlike any nitiduline taxon in that the urogomphi are oriented in a laterally flattened arrangement (Fig. 6D). Likewise, the presence of a multi-lobed mandible with deep central groove and bispinose mesal margin (Fig. 6B) are unlike other nitidulid larvae. These larvae also lack adhesive tarsungular seta. These were the characters that Böving and Craighead (1931) originally used to delimit the subfamily. Members of this subfamily are most likely to be confused with Nitidulinae taxa, specifically *Stelidota*, *Phenolia*, *Gaulodes* Erichson, *Temnoracta* Kirejtshuk and other subcortical nitidulines.

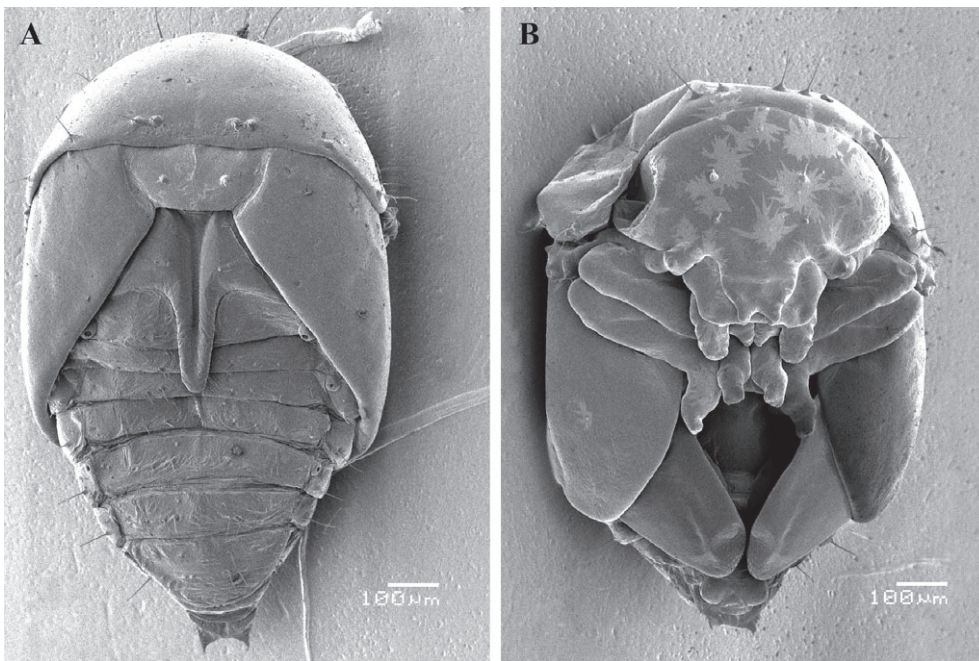
*Comments.* Jelínek (1982) provided an identification key to the adults of his *Axyra* group of genera. The key below is modified and expanded to include genera described since that publication. The recent synonymy of *Prometopia* and *Parametopia* (see Kirejtshuk, 2008) is not followed here based on the characters used in the couplet below; instead we continue to consider them as separate entities in the key in accordance with Jelínek (1982).

#### *Key to extant genera of Prometopinae*

1a. Meso- and metatibiae flattened, with a single lateral margin (Fig. 7B, C); mesosternum situated at nearly the same level as metasternum, appearing either slightly convex and somewhat transversely impressed or longitudinally roof-shaped; labrum with fine median incision with small lateral protuberances beside the incision ..... 2



**Fig. 2.** *Cybocephalus nipponicus* Endrödy-Younga larva: (A) dorsal view of mature larva, (B) ventral view of mature larva, (C) close-up of trumpet-shaped setae; scale bars: 500 µm (A), 200 µm (B) and 10 µm (C).



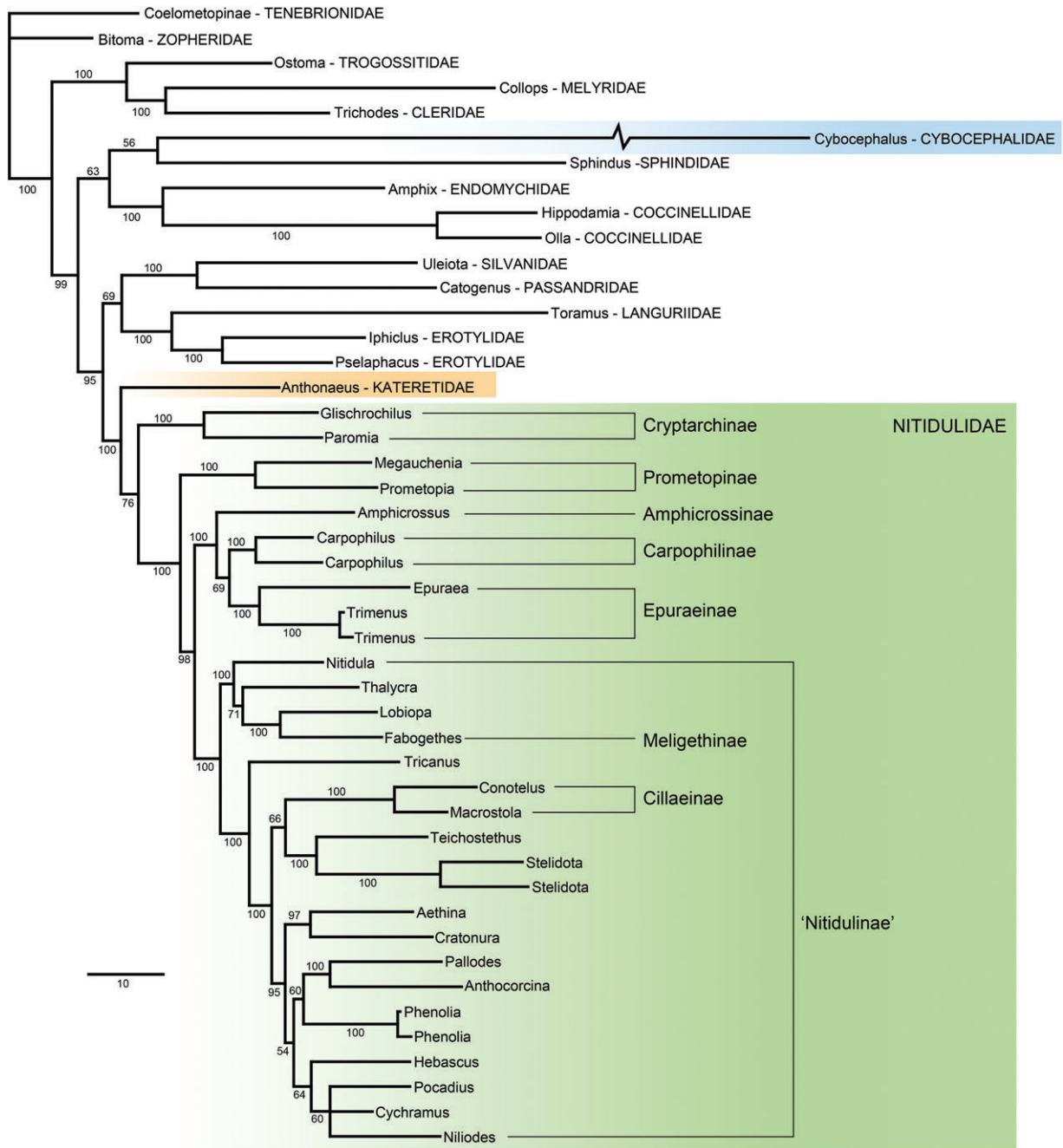
**Fig. 3.** *Cybocephalus nipponicus* Endrödy-Younga pupa: (A) dorsal view, (B) ventral view; scale bar = 100 µm.

1b. Meso- and metatibiae not flattened, with more than one lateral margin present (Fig. 7A, D); mesosternum situated more dorsad than metasternum, appearing sunken between the prosternum and metasternum; labrum deeply and broadly bilobed ..... 4

2a. Labrum and mentum transverse; elytral apices typically truncate and often exposing pygidium (Fig. 7B); pantropical ..  
..... *Platychora*

2b. Labrum and mentum appearing more hemispherical (somewhat transverse in *Parametopia*); elytral apices typically separately rounded and rarely exposing pygidium (Fig. 7C) ..... 3

3a. Antennal club symmetrical (Fig. 7C); body oblong oval to broadly oval; metasternal axillary space well developed and large; dorsal body surface variably setose, but pubescence distinct; basal margin of pronotum often bordered; pantropical and extending into the Nearctic and Palearctic ..... *Prometopia*



**Fig. 4.** Bayesian tree with posterior probabilities indicated below nodes. All families and Nitidulidae subfamilies are labelled accordingly.

3b. Antennal club asymmetrical; body broadly oval to round; metasternal axillary space not well developed, typically small and confined to lateral margins of sclerite; dorsal body surface typically indistinctly setose, appearing glabrous; basal margin of pronotum never bordered; Asia.....*Parametopia*

4a. Pronotum cordiform, lateral margin narrowly emarginated prior to posterior corners (Fig. 7D); Borneo and Sumatra.....*Taraphia*

4b. Pronotum variable, not cordiform; lateral margin not emarginated prior to posterior corners.....5

5a. Elytra serially punctuate; SE Asia.....*Megauchenia*

5b. Elytra confusedly punctuate, punctures not in well-defined series.....6

6a. Caudal marginal lines of mesocoxae separate between mesocoxae; metasternal axillary space closed (Fig. 7A); circum-tropical.....*Axyra*





**Fig. 5.** *Prometopia sexmaculata* (Say) adult: (A) SEM of ventral adult habitus, scale bar = 500  $\mu\text{m}$ ; (B) SEM of metacoxal line close-up.

- 6b. Caudal marginal lines of mesocoxae connected between mesocoxae; metasternal axillary space open ..... 7
- 7a. Body form sub-cylindrical, transversely convex and parallel sided; frons tuberculate, at least in males; Borneo .....  
..... *Megaucheniodes*
- 7b. Body form oval and convex; frons smooth, not tuberculate in males or females; SE Asia ..... *Pseudoplatychora*

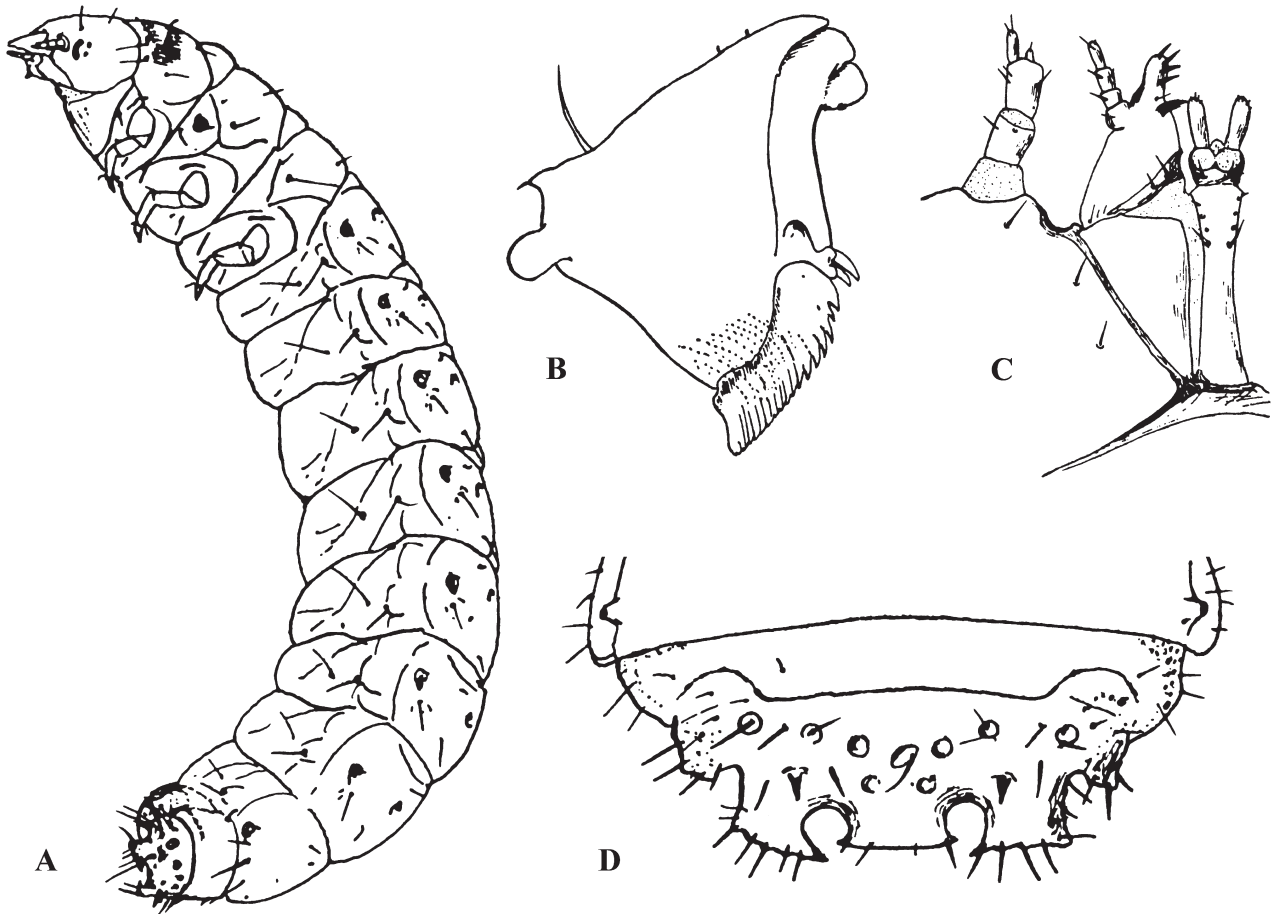
## Discussion

The transfer of *Cybocephalus* and related genera to a separate and distinct family not associated with Nitidulidae is not surprising, but had never been rigorously tested until now. Most cybocephalid taxonomists/systematists (Endrödy-Younga, 1962, 1968; Smith & Cave, 2006, 2007a,b) consistently applied the term Cybocephalidae to taxa related to *Cybocephalus*. However, others have misinterpreted characters that suggest a relationship to Nitidulidae and therefore included them in the latter. Several studies addressing broad-scale phylogenetics within Cucujoidea (Ślipiński & Pakaluk, 1992; Leschen *et al.*, 2005; Robertson *et al.*, 2008) did not include Cybocephalidae members as ingroup taxa. However, Leschen (1999) specifically addressed internal phylogenetic relationships within Nitidulidae and included representatives of Cybocephalidae. Leschen's

study placed *Cybocephalus* as sister to the Australian inquiline nitidulid *Cychramptodes*, which is the only known obligate predator within the family. This placement was supported by five characters, all of which appear to be convergent and associated with predation on hemipterous insects, in particular scale insects. These include glabrous convex bodies (difficult for tending ants to grab or hold onto with mandibles), hypognathous head (to allow feeding while being protected underneath a convex body) and modifications of the body as a result of convexity – shape of mesosternum and ratio of procoxal width to prosternum width.

Nitidulidae remains one of the most biologically diverse groups of beetles, with members exhibiting life history strategies including predation, fungivory, herbivory, frugivory and necrophagy. Our results indicate that the likely ancestral biology involved either subcortical fungivory or imbibing on sap flows (a type of fungivory via acquisition of yeasts or other fermenting microbials within sap flows). More robust in-group sampling of taxa is necessary to further develop hypotheses on the evolution of the multitude of life history strategies exhibited by nitidulids.

The two most recent catalogues dealing with Nitidulidae have included cybocephalids as a subfamily within Nitidulidae (Jelínek & Audisio, 2006; Kirejtshuk, 2008); however, inclusion of Cybocephalids in Nitidulidae by Jelínek & Audisio (2006) was a consequence of an editorial decision on the systematic

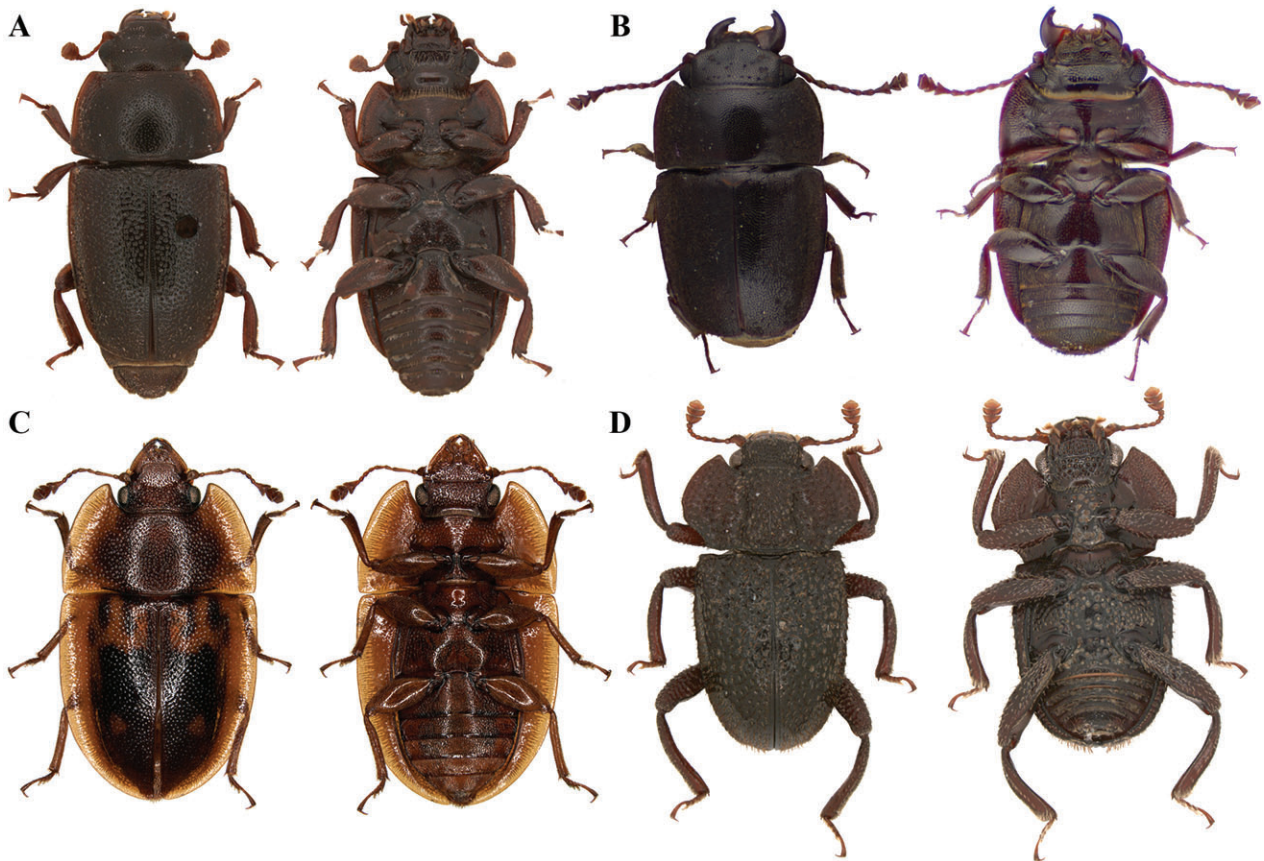


**Fig. 6.** *Prometopia sexmaculata* (Say) third instar larva: (A) habitus lateral view, (B) dorsal view of mandible, (C) ventral mouthparts, (D) dorsal view of urogomphi. (modified from Böving & Craighead, 1931).

family-level arrangement adopted in Loebli and Smetana's catalogue of Palaearctic beetles, consistent with classification proposed by Lawrence & Newton (1995). Yet Cybocephalidae is considered by the same authors to be a distinct family in the *Fauna Europaea* database (Audisio & Jelínek, 2005–2012). Likewise, relatively recent monographs of Nitidulidae have contained Cybocephalinae as a subfamily within Nitidulidae (Kirejtshuk, 1998a; Jelínek *et al.*, 2010). Herein, the morphological evidence from adults and larvae, as well as molecular data from seven different loci, fully establishes Cybocephalidae as a distinct and separate entity from Nitidulidae.

The monophyly of Nitidulidae and its sister-group relationship to Kateretidae is an expected conclusion based on morphological evidence (Cline, 2005; Leschen *et al.*, 2005), although it has been suggested fairly recently that Kateretidae is the sister taxon or closely related to Cryptophagidae (Kirejtshuk, 1986a), Monotomidae (Kirejtshuk, 1998b) or Boganiidae (Kirejtshuk, 1986b, 1998b) and Smicripidae (Kirejtshuk, 2000). The most recent molecular phylogeny of Cucujoidea (Robertson *et al.*, 2008), albeit focused on the Cerylonid Series of the superfamily, did not demonstrate a sister-group relationship between Nitidulidae and Kateretidae, but did not support a relationship with

Cryptophagidae either. However, in the more comprehensive and large-scale molecular study of Hunt *et al.* (2007), Kateretidae was placed more isolated in a rather heterogeneous assemblage including both related Cucujoidea (e.g. Silvanidae) and distantly related Chrysomeloidea (e.g. Cerambycidae) families. The outgroup sampling in that study was, however, too limited to allow for general conclusions with respect to the relationships of nitidulid lineages. Our results do not correspond well with the previous molecular Cucujoidea phylogeny with regard to the inclusion of Tenebrionoidea taxa within Cucujoidea as well as the sister-group relationship with Kateretidae. These disparate conclusions demonstrate the need for a robust phylogeny of all Cucujoidea families, as well as representatives of both Cleroidea and Tenebrionoidea to unequivocally address the monophyly of Cucujoidea with respect to these other two superfamilies. However, our molecular data and morphological evidence both support a sister-group relationship of Nitidulidae and Kateretidae. *Smicrips palmicola* LeConte was initially selected and processed to validate the placement of Smicripidae outside of, but sister to, Kateretidae and Nitidulidae, thereby testing monophyly of a 'nitidulid lineage' complex that includes Kateretidae, Smicripidae and Nitidulidae. However, we did not



**Fig. 7.** Dorsal and ventral habitus images of representative adult Prometopinae (A) *Axyra* sp., (B) *Platychora major* Grouvelle, (C) *Prometopia sexmaculata* (Say), (D) *Taraphia amplicollis* Audisio and Jelínek

succeed in amplifying sufficient sequences from *S. palmicola* to test this with molecular data, although it appears firmly supported by morphological datasets (Cline, 2005; Leschen *et al.*, 2005).

Preliminary views of subfamilial relationships were presented by Kirejtshuk (1982, 1986b, 1995) as hand-drawn diagrams. Kirejtshuk's (1982) dendrogram includes six subfamilies, one of which is Kateretinae (=Kateretidae), and depicts Meligethinae, Nitidulinae and Cryptarchinae as a derived polytomy. Based on his 1995 dendrogram, Kirejtshuk suggests two major nitidulid groups: the Carpophilin and Nitidulin lineages. His Carpophilin lineage corresponds to the subfamilies Epuraeinae, Carpophilinae, Amphicrossinae and Calonecrinae; with the Nitidulin lineage comprising Meligethinae, Nitidulinae, Cillaeinae, Cryptarchinae and Cybocephalinae. The split of Nitidulidae into two lineages was further elaborated in Kirejtshuk's (1998a) treatise on Himalayan Epuraeinae. Interestingly, Maynipeplinae was not included in this latest treatment, which was published the same year (Kirejtshuk, 1998b). In the 1995 dendrogram, Epuraeinae were depicted as ancestral within the Carpophilin lineage with a Carpophilinae, Amphicrossinae and Calonecrinae polytomy. Within the Nitidulin lineage, the Cybocephalinae were placed in the most ancestral position,

with Cillaeinae and Cryptarchinae forming a more derived grouping, and Meligethinae and Nitidulinae appear in a derived position with the Meligethinae taxon positioned within but not outside of Nitidulinae taxa. Discovery of Maynipeplinae later allowed Kirejtshuk (1998b) to place both Calonecrinae and Maynipeplinae as likely basal to his Carpophilin lineage. We believe that larval characters may help to disentangle the relationships among many of these groups. For instance, the monophyly of Amphicrossinae may be supported by characters of the ninth and tenth abdominal sclerites as well as the hypopharyngeal and epipharyngeal complexes.

Cryptarchinae are likely the most well-defined monophyletic assemblage within Nitidulidae (Jelínek, 1974). The entire subfamily is easily characterized by several adult morphological features that appear to be autapomorphic, but no quantitative phylogenetic analyses have tested the utility of these characters in a phylogenetic context. The diagnostic, presumably autapomorphic, characters include: fusion of the clypeus and labrum; mandibular prosthema partially free; presence of an occipital stridulatory file; and posterior portion of procoxal cavities externally open, with the proepimera not contiguous with the intercoxal region of the prosternal process. No comprehensive analyses of immature stages have been undertaken to establish

larval or pupal characters to define the subfamily; however, most larvae of the subfamily appear to have branched urogomphi and pupae have elongate paired tubercles on the vertex of the head (see Hayashi, 1978). The subfamily lacks any internal divisions into tribes based on phylogenetic analyses; however, a preliminary approach for assessing genus level classifications based on potential synapomorphies has been proposed (Jelínek, 1974).

The delimitation of the subfamily Prometopinae was interesting and unexpected. Jelínek (1982) proposed the concept of an 'Axyra-group' of genera forming a monophyletic lineage, corresponding to Prometopinae. Since Jelínek's original concept, the Axyra group has been expanded to include *Taraphia* and *Megaucheniodes* as well (Audisio & Jelínek, 1993). Jelínek (1982) suggested that the Axyra lineage comprises two sister groups: one group was proposed to contain *Platychora*, *Prometopia* and *Parametopia*, and the other Axyra, *Pseudoplatychora* and *Megauchenia*. This division of genera is likely well founded based on the structure and armature of the meso- and metatibiae, placement of the mesosternum in relation to the metasternum, and shape of the anterior margin of the labrum; the two groupings will probably need to be considered as two tribes based on these major morphological differences. Kirejtshuk (2003, 2008) erroneously attributed *Platychora* to an undefined 'Ipidia complex' and further suggested that this complex was related to his marginally defined 'Soronia complex'. Likewise, Kirejtshuk (2003) suggested that *Lobiopa*, a member of his 'Soronia complex' were related to *Prometopia*. Our data do not support Kirejtshuk's assertions about these taxa.

The position of members of Cillaeinae and its separation from members of Carpophilinae has been indicated by several authors (Murray, 1864; Watrous, 1980; Audisio, 1984), and the group was formally raised to subfamily by Kirejtshuk (1986a). Cillaeinae are a well-differentiated grouping of nitidulids. According to our data, Cillaeinae may need to be either subsumed as a tribe of Nitidulinae, or Nitidulinae will need to be divided into several subfamilies with Cillaeinae retaining subfamilial status. We have decided to make no classificatory changes with these taxa until more taxon sampling and inclusion of more datasets can be achieved. We agree with Leschen (1999) that monophyletic groups need to be clearly defined and based on phylogenetic analysis.

The inclusion of Meligethinae in Nitidulinae in our analysis was somewhat unexpected. A recent re-examination of Meligethinae completely rearranged the internal classification of the subfamily (Audisio *et al.*, 2009); however, no hypotheses were offered as to the placement of the subfamily within Nitidulidae. Meligethinae is certainly a monophyletic grouping supported by multiple adult and larval synapomorphies (Audisio, 1993; Jelínek *et al.*, 2010), but whether it should retain subfamily status or need to be subsumed within a broadly defined Nitidulinae as a well-established tribe remains to be resolved. Due to the lack of multiple meligethine taxa in our analysis, we have decided not to propose any classificatory emendations at this time. Our data indicate that this group needs to be further examined and its placement in the family should be established only after a large robust analysis can be performed with numerous meligethine taxa and multiple datasets (Audisio *et al.*,

2014a; molecular and morphological dataset on several African and Palearctic taxa).

Within Nitidulinae as historically defined, our results suggest several problems with traditional generic complexes and tribes. Specifically, Cyllodini and the *Pocadius* genus complex both appear to be polyphyletic. Cyllodini has been subjected to recent cladistic analyses and was found to be monophyletic (Leschen, 1999). Interestingly, Leschen's study utilized *Cybocephalus* as an outgroup taxon and determined that *Cybocephalus* was related to several nitiduline taxa, a result never retrieved in our study. *Pallodes* and *Triacanus* were included in our analysis to assess the monophyly of Cyllodini and the relationships of the two divisions within the tribe (i.e. the *Cyllodes* and *Oxycnemus* groups; see Leschen, 1999). We did not recover these two genera together in our analyses and they do not appear to be sister taxa. Thus, although the two divisions may be independently monophyletic, the tribe Cyllodini as a whole is not. We suggest that more robust character datasets, including adult and larval morphological characters as well as molecular markers, are necessary to more accurately define this nitiduline lineage. No classificatory emendations are therefore suggested until a more robust phylogenetic analysis can be undertaken. The synonymy of Amborotubini within Cyllodini (Kirejtshuk, 2006a) will need to be re-examined as its placement within Cyllodini will require further evidence than its position as a distinct tribe unrelated to Cyllodini as proposed by Leschen and Carlton (2004); especially with the evidence herein that Cyllodini is not monophyletic.

The *Pocadius* genus complex has received a great deal of taxonomic attention in recent years (Cline, 2005, 2008, 2009; Kirejtshuk, 2006b, 2009). Based on external morphology of adults and biology of the constituent members, previous studies have suggested affinities of the *Pocadius* complex to the *Thalycra* complex (Kirejtshuk & Leschen, 1998). Our results do not support a sister-group relationship between *Pocadius* complex taxa (*Hebascus*, *Pocadius* and *Niliodes* in our analysis) and *Thalycra*, contrary to that previously proposed by Kirejtshuk (2009). The *Pocadius* complex itself is also not monophyletic as currently defined. Our results indicate that *Teichostethus*, a long-attributed member of the *Pocadius* complex, is not placed near any of the other *Pocadius* complex taxa. *Teichostethus* and *Hebascus* were included in this study based on their systematic affinities, particularly in light of their recently proposed synonymy (Kirejtshuk, 2008). Our results indicate that these taxa are definitively not synonyms, and given the numerous morphological differences (Jelínek, 1975; Cline, 2009) and disparate position in our phylogenetic analyses, *Teichostethus* **stat. res.** is considered a valid genus. Also, *Cychramus*, which is typically considered unrelated to the *Pocadius* complex, was placed in association with other *Pocadius* complex genera.

Jelínek (1993) stated that the genus *Aethinopsis* Grouvelle (= *Cychramus*, syn. Kirejtshuk, 2008) along with *Cychramus* formed a monophyletic group of genera that was not associated with the *Aethina* complex of genera. Herein, we demonstrated that *Cychramus* formed a polytomy with *Pocadius* and *Niliodes*. These data therefore corroborate the position of *Cychramus* as not closely related to the *Aethina* complex of genera, as proposed by Jelínek (1993). Rather, they may be included in a

broadly defined tribe that also contains *Pocadius* complex taxa. The *Aethina* generic complex and some of its constituent members has received some recent taxonomic attention (Audisio & Kirejtshuk, 1983; Kirejtshuk & Lawrence, 1999; Cline & Carlton, 2004). Kirejtshuk & Lawrence (1999) suggested an affinity of *Aethina* complex taxa with the *Pocadius* complex and *Thalycra* complex. Our results do not support a close relationship between *Aethina* and either the *Thalycra* or *Pocadius* complexes. Instead, *Aethina* is consistently placed with *Cratonura*, an enigmatic genus from Chile. Kirejtshuk (2008) places *Cratonura* in a 'Perilopsis complex'; however, this lineage remains undefined from a morphological context and untested in a phylogenetic framework. Further analysis is necessary to determine the position of *Cratonura*, and another Chilean endemic genus *Epu-raeopsis*, within or sister to *Aethina* complex taxa.

## Conclusions

Overall, our results provide insights into one of the most enigmatic and taxonomically challenging groups of Cucujoidea. Nitidulidae is a diverse assemblage of variable forms and biologies. This diversity of forms and life styles is evolutionarily peculiar and likely will continue to intrigue Coleopterists. To add stability to the group, we have formalized Cybocephalidae as a distinct family, resurrected the nitidulid subfamily Prometopinae, and delimited problems with the large and unwieldy subfamily Nitidulinae. Our approach of sequence analysis and comparative morphology support the classificatory emendations above, but also suggest further study is necessary to resolve the currently proposed internal classification system for the family.

## Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/syen.12084

**Table S1.** Amplification conditions for PCR and sequencing reactions.

**Table S2.** Reagent types and volumes used for molecular analyses.

**Table S3.** Primers for PCR amplification and sequencing.

**Table S4.** Taxa with GenBank accession numbers.

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