

Further progress on the phylogeny of Noctuoidea (Insecta: Lepidoptera) using an expanded gene sample

JEROME C. REGIER¹, CHARLES MITTER¹, KIM MITTER¹,
MICHAEL P. CUMMINGS², ADAM L. BAZINET², WINIFRED
HALLWACHS³, DANIEL H. JANZEN³ and ANDREAS ZWICK⁴

¹Department of Entomology, University of Maryland, College Park, MD, U.S.A., ²Laboratory of Molecular Evolution, Center for Bioinformatics and Computational Biology, University of Maryland, College Park, MD, U.S.A., ³Department of Biology, University of Pennsylvania, Philadelphia, PA, U.S.A. and ⁴Australian National Insect Collection, CSIRO Ecosystem Science, Canberra, Australia

Abstract. Major progress has been made recently toward resolving the phylogeny of Noctuoidea, the largest superfamily of Lepidoptera. However, numerous questions and weakly supported nodes remain. In this paper we independently check and extend the main findings of multiple recent authors by performing maximum-likelihood analyses of 5–19 genes (6.7–18.6 kb) in 74 noctuoids representing all the families and a majority of the subfamilies. Our results strongly support the six family system of Zahiri *et al.*, with the former Lymantriidae and Arctiidae subsumed within the huge family Erebidae, and Noctuidae restricted largely to the subfamilies with so-called triline hindwing venation. Our data also strongly corroborate monophyly of the set of four families with quadrid forewing venation, to the exclusion of Notodontidae, and removal from the latter of Oenosandridae. Other among-family relationships, however, remain unsettled. Our evidence is equivocal on the position of Oenosandridae, which are sister group to either Notodontidae alone or to all other noctuoids. Like other recent nuclear gene studies, our results also provide no strong support for relationships among the four quadrid forewing families. In contrast, within families our analyses significantly expand the list of robustly resolved relationships, while introducing no strong conflicts with previous molecular studies. Within Notodontidae, for which we present the largest molecular taxon sample to date, we find strong evidence for polyphyly for some, or all, recent definitions of the subfamilies Thaumetopoeinae, Pygaerinae, Notodontinae and Heterocampinae. Deeper divergences are incompletely resolved but there is strong support for multiple ‘backbone’ nodes subtending most of the subfamilies studied. Within Erebidae, we find much agreement and no strong conflict with a recent previous study regarding relationships among subfamilies, and somewhat stronger support. Although many questions remain, the two studies together firmly resolve positions for over half the subfamilies. Within Noctuidae, we find no strong conflict with previous molecular studies regarding relationships among subfamilies, but much stronger resolution along the ‘backbone’ of the phylogeny. Combining information from multiple studies yields strongly resolved positions for most of the subfamilies. Finally, our results strongly suggest that the tribes Pseudeustrotiini and Prodeniini, currently assigned to the largest subfamily, Noctuinae, do not belong there. In sum, our results provide additional corroboration for the main outlines of family-level phylogeny in Noctuoidea, and contribute toward resolving relationships within families.

Correspondence: Charles Mitter, Department of Entomology, University of Maryland, College Park, MD 20742, U.S.A. E-mail: cmitter@umd.edu

Introduction

Noctuoidea (~42 400 species; van Nieuwerkerken *et al.*, 2011), the largest superfamily of Lepidoptera, have long presented difficult phylogenetic problems. Major progress toward solving these has been made in recent years, driven in part by the advent of molecular phylogenetics (Miller, 1991; Weller *et al.*, 1994; Mitchell *et al.*, 1997, 2000, 2006; Kitching & Rawlins, 1998; Fibiger & Lafontaine, 2005; Lafontaine & Fibiger, 2006; Zahiri *et al.*, 2011, 2012, 2013a, 2013b; Rota *et al.*, 2016). It is now firmly established that Noctuidae in the broad former sense (e.g. Kitching & Rawlins, 1998), excluding the former Lymantriidae and Arctiidae, are paraphyletic (Weller *et al.*, 1994; Mitchell *et al.*, 2000, 2006; Zahiri *et al.*, 2011). The six-family system of Zahiri *et al.* (2011), in which the former families Lymantriidae and Arctiidae are subsumed within the huge family Erebiidae, and Noctuidae are restricted largely to the families with triform hindwing venation, has been widely accepted. Monophyly of the set of four families with quadrid forewing venation, to the exclusion of Notodontidae, and removal from the latter of Oenosandriidae, have been strongly established (Miller, 1991; Mitchell *et al.*, 2006; Zahiri *et al.*, 2011). Major re-examinations and initial phylogenetic analyses have been carried out on all but Oenosandriidae and Euteliidae (Miller, 1991; Lafontaine, 1993; Poole, 1995; Mitchell *et al.*, 2006; Zahiri *et al.*, 2012, 2013a, 2013b). Numerous questions and weakly supported nodes remain, however, and much additional evidence and corroboration is needed.

The goal of this paper is to independently assess and, where possible, extend the main findings of recent authors (Miller, 1991; Mitchell *et al.*, 2006; Zahiri *et al.*, 2011, 2012, 2013a, 2013b), using a smaller taxon sample than in previous molecular studies but a larger gene sample. We analysed 5–19 genes (6.7–18.6 kb) in 74 noctuid species, representing all the families and more than half of the subfamilies. We find no strong conflicts with previous molecular results; we corroborate many nodes with previous strong support; and we strongly resolve a number of additional nodes that had little or no previous support, especially in Notodontidae and Noctuidae.

Materials and methods

Taxon sampling

Our 74 noctuid exemplars span all six noctuid families. They include two of eight species of Oenosandriidae; 22 species of Notodontidae representing seven of the nine subfamilies plus two unplaced genera recognized by Miller (1991); 22 species of Noctuidae s.s. representing 11 of the 19 subfamilies studied by Zahiri *et al.* (2013b); 22 species of Erebiidae representing 13 of the 18 subfamilies recognized by Zahiri *et al.* (2012); five species of Nolidae, representing five of eight subfamilies recognized by Zahiri *et al.* (2013a); and one species of Euteliidae. As outgroups we included 16 exemplars representing 11 families and all four other superfamilies of Macroheterocera, plus both families of Pyraloidea, the apparent sister group to Macroheterocera. All of the outgroups, and 32 of the noctuoids,

had previously been included in the 483-taxon analysis of Regier *et al.* (2013), although they did not discuss noctuid relationships.

Specimens for this study, obtained with the gracious assistance of collectors around the world (see Acknowledgements), are stored in 100% ethanol at -80°C as part of the ATOLep collection at the University of Maryland, USA. Nucleic acid extraction used only the head and thorax for species that have larger adults, leaving the rest of the body including the genitalia as a voucher. The entire specimen was used for species with smaller adults. Wing vouchers were retained for nearly all exemplars. DNA 'barcodes' were generated for all taxa, either by us using standard primer sequences with M13 tails (Regier & Shi, 2005) or, more frequently, by the All-Leps Barcode of Life project (<http://www.lepbarcoding.org>). *COI* DNA 'barcodes' were checked against the Barcode of Life Data system reference library (Ratnasingham & Hebert, 2007) to confirm specimen identifications and also to facilitate future identification of specimens whose identity is still pending (i.e. species listed as 'sp.' or 'unidentified' in the present report). In the case of undescribed species, the species interim epithet is not italicized.

Gene sampling

All species were sequenced for five protein-coding nuclear gene regions (6.6 kb) that have previously been shown to provide generally strong resolution within superfamilies (Regier *et al.*, 2009). To increase resolving power for deeper relationships, in 34 of the 74 noctuoids, spread across all families, and all 16 outgroup species, we sequenced an additional 14 genes for a total of up to 14.7 kb. The 14 additional gene regions are a subset of the 21 gene regions first tested across ditrysian Lepidoptera by Zwick *et al.* (2011) and Cho *et al.* (2011). Gene names, functions and full lengths of the individual gene regions are given in Table S1 of Cho *et al.* (2011). The number of gene regions attempted for each exemplar, the total amount of sequence obtained, and the GenBank accession numbers for these sequences, can all be found in Table S1.

Generation of DNA sequence data

A detailed protocol of all laboratory procedures is provided by Regier *et al.* (2008). Further descriptions, including gene amplification strategies, PCR primer sequences, and sequence assembly and alignment methods, can be found in Regier *et al.* (2008, 2009). To summarize, species-specific templates for mRNA amplification were prepared by first extracting total nucleic acids. Extracted nucleic acids were stored at -80°C in RNase-free deionized water (diethyl-pyrocyanate-treated). Specific regions of the cognate mRNAs were amplified by reverse transcription followed by PCR. Specific bands were gel isolated and re-amplified by PCR using hemi-nested primers, when available. Visible bands that were too faint to sequence were reamplified, using as primers the M13 sequences at the 5' ends of all gene-specific primers. PCR amplicons were

sequenced directly on a 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, U.S.A.). Sequences were edited and assembled using the *TREV*, *PREGAP4* and *GAP4* programs in the *STADEN* package (Staden, 1999). Individual sequences were concatenated, and alignments were made automatically using the 'Translation Align' software in the *GENEIOUS PRO v5.3.4* package [60]. In the alignment process, splitting of individual codons was not allowed. A data-exclusion mask of 1440 uncertainly aligned characters out of 20 373 total aligned characters (=7.1% of total) for all 90 species was applied.

Character partitions, taxon × gene dataset design and phylogenetic analyses

Three distinct datasets that include all sequences were constructed. The first consists of unaltered nucleotides from all three nucleotide positions (nt123). The second (nt123_partition) contains the same nucleotides, but with these partitioned into two nonoverlapping character sets that separate nonsynonymous-only from mostly synonymous change. These two complementary character sets are called noLRallInt2 and LRallInt3 [see table 1 in Regier & Zwick (2011) for complete definitions; also see <http://www.phylotools.com>]. We chose this bi-partition procedure over the more common tri-partition by codon position because the approach is simpler, having only two character sets, and yet generates a larger nonsynonymous-only set. Scripts to generate the two character sets are freely available (appendix 4 of Regier *et al.*, 2008; <http://www.phylotools.com>). The third dataset (nt123_degen1) is based on the degen1 approach [23], in which in-frame codons of the same amino acid are fully degenerated with respect to synonymous change (e.g. CAT → CAY). Leu codons (TTR + CTN) are degenerated to Leu + Phe (YTN) and Arg codons (AGR + CGN) are degenerated to Arg + Ser2 (MGN). Phe and Ser2 are degenerated to TTY and AGY, respectively. The basic idea of the degen1 approach is to capture the non-synonymous signal while excluding the synonymous signal and any compositional heterogeneity it produces. The degen1 script is freely available (Regier *et al.*, 2010; Zwick *et al.*, 2012; <http://www.phylotools.com>). The substitution model used in all analyses was a general time-reversible nucleotide model with a term for invariant sites and among site rate heterogeneity accounted for by a discrete gamma distribution (GTR + G + I). This model was applied separately to each character subset in the partitioned analysis. To test whether the missing data from taxa sequenced for only five genes had a marked effect on the results from the all-data matrix (5–19 genes), we carried out parallel analyses on a reduced gene sample including only the five gene regions that were sequenced in all taxa.

All phylogenetic analyses were based on the maximum-likelihood (ML) criterion as implemented in GARLI (Genetic Algorithm for Rapid Likelihood Inference; v2.0; Zwickl, 2006). We used the program default settings, including random step-wise addition starting trees, except that we halved the number of successive generations yielding no improvement in likelihood score that prompts termination (genthreshfortopterm = 10000),

as suggested for bootstrapping in the GARLI manual. Each search for the single best ML tree consisted of 990–1000 separate GARLI ML search replicates run to completion on each of the full datasets (nt123, nt123_partition, nt123_degen1). Bootstrap analyses consisted of 700–750 pseudo-replicates, each based on 15 heuristic search replicates run to completion. Optimal-tree searches and bootstrap analyses were parallelized using grid computing (Cummings & Huskamp, 2005) through The Lattice Project (Bazinet & Cummings, 2008). For consistency in the characterization of results, we will refer to bootstrap support of 70–79% as 'moderate,' 80–89% as 'strong' and ≥90% as 'very strong'. The all-data 5–19 gene data matrices and trees generated in our analyses will be archived in Dryad.

Results and Discussion

All five of our analyses yielded similar topologies and bootstrap values. These observations are summarized in Fig. 1, which shows the single best ML topology for the nt123 all-data unpartitioned analysis, with bootstrap values for all five analyses superimposed on the branches. (The outgroups are not shown.) Our discussion will proceed from the bottom to the top of the tree in Fig. 1.

Among-family relationships

Like other molecular studies, our results strongly support monophyly of a Noctuoidea that excludes Doidae. The latter now seem firmly established, on both molecular and morphological evidence, to belong to Drepanoidea (Regier *et al.*, 2009, 2013; Mutanen *et al.*, 2010; Bazinet *et al.*, 2013; Heikkilä *et al.*, 2015). The implied convergence in the tympanic organs of Noctuoidea and Doidae, the latter consisting of six species restricted to the southwestern United States and Central America, deserves further study.

In a landmark study, Miller (1991) erected a separate family for *Oenosandra* Newman and relatives, removing these from Notodontidae. Like other molecular studies, our results strongly support this decision. The phylogenetic position of Oenosandridae, however, is less clear. The basal split in Noctuoidea is hypothesized on the basis of morphology to separate Oenosandridae, comprising eight known species restricted to Australia, from all others (Miller, 1991). Molecular analyses, however, have been equivocal on this point. In the present study (Fig. 1) the oenosandrids are sister to Notodontidae in all 19-gene analyses, but with weak support, whereas they are sister to all other noctuids, with strong support from nt123, in all five-gene analyses. It thus appears that there is conflict among genes in our full dataset. The eight-gene study of Mutanen *et al.* (2010) found the single oenosandrid to be the earliest-diverging noctuid. In a more extensive molecular study of noctuid relationships, however, Zahiri *et al.* (2011) found that the position of Oenosandridae varied depending on the details of character inclusion/exclusion. Thus, we regard the question of the placement of Oenosandridae as incompletely settled.

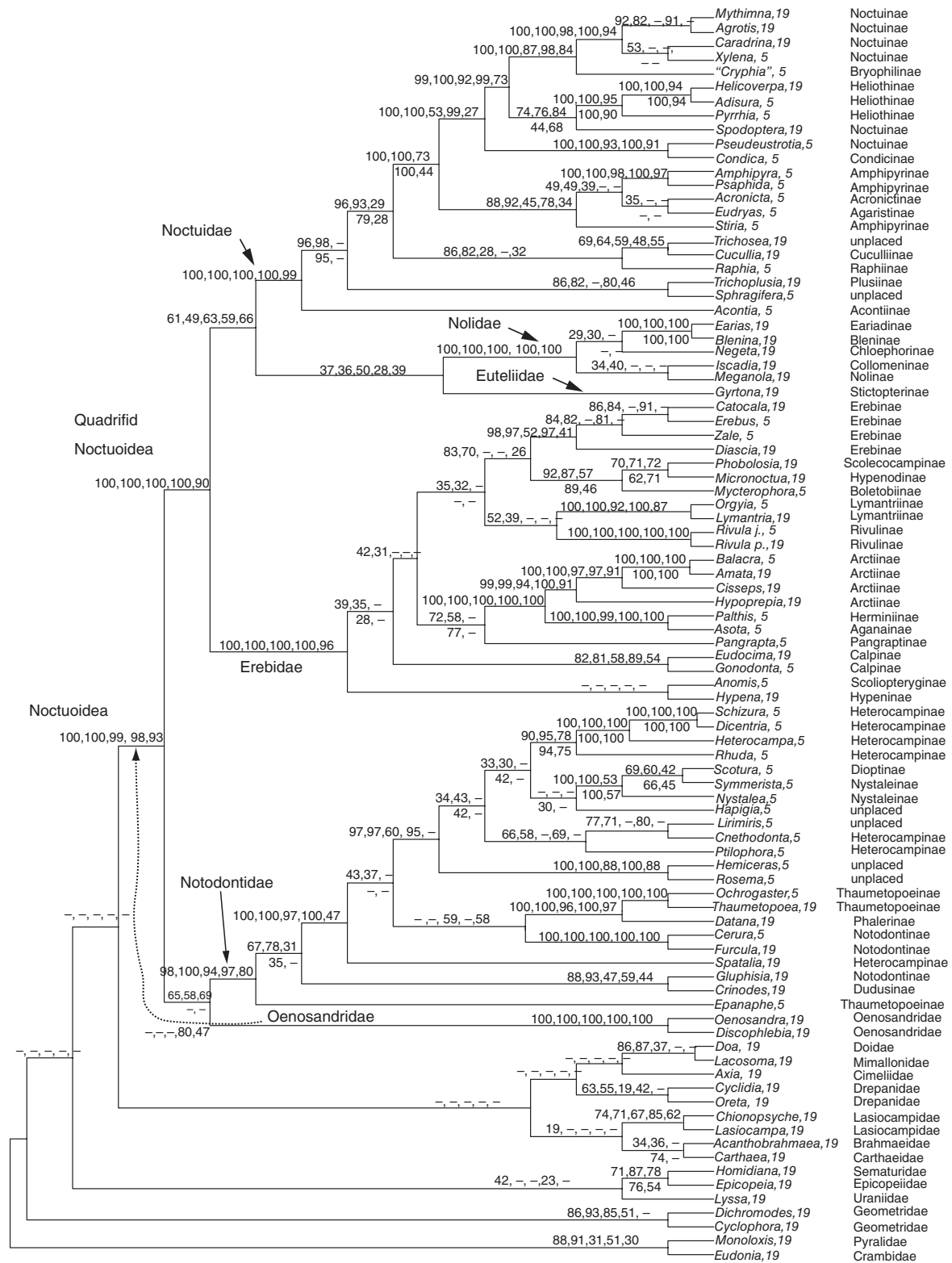


Fig. 1. Single best maximum-likelihood (ML) tree for nt123 (19 genes), for 74 noctuids (the 17 outgroups are not shown). Bootstrap support values (percentage) above and/or below branches: nt123 unpartitioned (19 genes), nt123 partitioned (19 genes), degen1 (19 genes), nt123 unpartitioned (5 genes), degen1 (5 genes). ‘-’, bootstrap value < 50. Classification follows Miller (1991) for Notodontidae, Lafontaine & Schmidt (2010, 2013) for all others.

Like previous nuclear gene studies, our results strongly support monophyly of the quadrid forewing noctuids and monophyly of each of the four constituent families, but are not decisive on relationships among the families. The strongest apparent support to date on the latter question comes from the mitogenomic study of Yang *et al.* (2015), who favour the hypothesis (Erebidae (Nolidae (Euteliidae + Noctuidae))).

In the sections below we review relationships within the three largest families.

Within-family relationships

Notodontidae. Notodontidae, consisting of about 3800 mostly tree-feeding species (Miller, 1992; van Nieukerken *et al.*, 2011), have been the subject of only one broad phylogenetic study (Miller, 1991). Our 22 exemplars are the largest sample of notodontids yet subjected to molecular-phylogenetic analysis. In Fig. 2 we compare our results on notodontids to those extracted from two earlier molecular studies (Mitchell *et al.*, 2006; Zahiri *et al.*, 2011). Greatly increased sampling is obviously needed, but the evidence so far already shows some strong groupings.

Notodontid subfamily concepts have been in flux. Based on his morphological-cladistic results, Miller (1991) recognized nine subfamilies. Schintlmeister (2008), in a monumental work on the Palaearctic fauna, presented a ten-subfamily system modified from Miller (1991), albeit without disclosing his reasoning, which treats *Thaumetopoeinae* as a separate, unrelated family. In a later catalogue of the world fauna (Schintlmeister, 2013), he recognized an additional eight subfamilies. Becker (2014) presented still another variant in conjunction with his checklist of the Neotropical fauna. Our results (Fig. 2) permit an initial test of some of these subfamily concepts, as well as Miller's (1991) hypothesis of among-subfamily relationships.

In Fig. 2A we show the subfamily placements under the classifications of Miller (1991), Schintlmeister (2008, 2013) and Becker (2014) for each genus sequenced. For six subfamilies, there were at least two representatives according to one or more classification systems, allowing partial tests of monophyly. Four of those subfamilies were polyphyletic in our tree, under one or more classification systems. First, although our results strongly confirm Miller's (1991) inclusion in Notodontidae of *Thaumetopoeinae* (the processionary moths), sometimes treated as a separate family, they also strongly suggest that this subfamily as currently defined is polyphyletic. The Afro-tropical endemic genus *Epanaphe* Aurivillius, used locally for silk production (Mbahin *et al.*, 2012), is separated by five nodes (Fig. 2A, nodes 2–6), two of which have bootstrap support of 100%, from the two other genera we sampled, the Australian *Ochrogaster* Herrich-Schäffer and the Palaearctic *Thaumetopoea* Hübner. The latter are united by 100% bootstrap support, as are *Thaumetopoea* and the Australian genus *Epicoma* Hübner in the tree of Zahiri *et al.* (2011; Fig. 2B). Thus, *Epanaphe* appears to be unrelated to a group consisting of all other *Thaumetopoeinae* for which sequence data exist. This very strong molecular result implies convergence in several morphological characters

(Miller, 1991) as well as the behavioural trait of subsociality (albeit of somewhat different forms).

Our results also imply polyphyly for some but not all concepts of Notodontinae. Miller (1991) proposed a substantial broadening of Notodontinae (also adopted by Lafontaine & Schmidt, 2010), to include not only *Notodonta* Ochsenheimer and close relatives but also three other family groups from previous authors, the latter all combined in his tribe Dicrurini. These are the Cerurinae of Forbes (1948) and predecessors; the Gluphisiini of Forbes (1948); and the Ptilophorinae of Matsumura (1929; = Ptilodontinae Packard 1864). Our tree strongly argues that these three groups are unrelated. *Gluphisia* Boisduval is separated from the common ancestor of *Cerura* Schrank + *Furcula* Lamarck and *Ptilophora* Stephens by three nodes (Fig. 2A, nodes 2, 3, 7), two of which have bootstrap support of 100%. *Cerura* + *Furcula* in turn are separated from *Ptilophora* by five nodes (nodes 4, 5, 8, 9, 10), one of which has bootstrap support of 97%. In the classifications of Schintlmeister (2008) and Becker (2014), by contrast, separate status is maintained for Cerurinae (*Furcula*, *Cerura*). Schintlmeister (2008) also separates the subfamily Ptilophorinae (*Ptilophora*). Becker (2014) follows Miller (1991) in retaining *Gluphisia* in Notodontinae, whereas Schintlmeister (2008) places *Gluphisia* in Pygaerinae. By Schintlmeister's narrow definition, the Notodontinae are not represented in our sample. Our results suggest that broader concepts (including that of Becker, 2014) are probably not monophyletic.

Pygaerinae sensu Schintlmeister (2008) are also polyphyletic on our tree, as *Gluphisia* and *Spatalia* Hübner are separated by three nodes (2, 3, 7) that include bootstrap supports of 88 and 100%. Our results confirm Schintlmeister's (2008) doubt that *Spatalia* actually belongs in Pygaerinae.

Finally, our results suggest that two of the definitions of Heterocampinae examined here are polyphyletic. Following suggestions by Forbes (1948), Miller included in this subfamily, in addition to *Heterocampa* Doubleday and close relatives (the Heterocampini of Forbes, 1948), the Old World groups Stauropinae, Spataliinae and Fentoninae of Matsumura (1929). Stauropinae and putative New World relatives were combined into Stauropini; most New World heterocampines were placed in Heterocampini; and *Spatalia* Hübner and *Fentonia* Butler were left unplaced as to tribe (Miller, 1991). Our results argue strongly against this expansion of Heterocampinae. In our tree, four New World genera including *Heterocampa* form a strongly supported 'core' group (node 11; BP=90), with strongly supported internal structure (nodes 12, 13; BP=100). The two sampled Old World heterocampines sensu Miller (1991), on the other hand, are separated by at least four nodes, from each other and from the New World heterocampines, with moderate to very strong support. *Spatalia*, an isolated, early-diverging lineage in our tree, is separated from *Heterocampa* and relatives by four nodes, one of which (node 8) has 97% bootstrap support. *Cnethodonta* Staudinger, an Old World member of Stauropini sensu Miller, is separated by four nodes, one (node 15) with 77% bootstrap support, from New World Heterocampinae, within which *Schizura* Doubleday, a member of Stauropini sensu Miller, is deeply nested. These results strongly suggest that

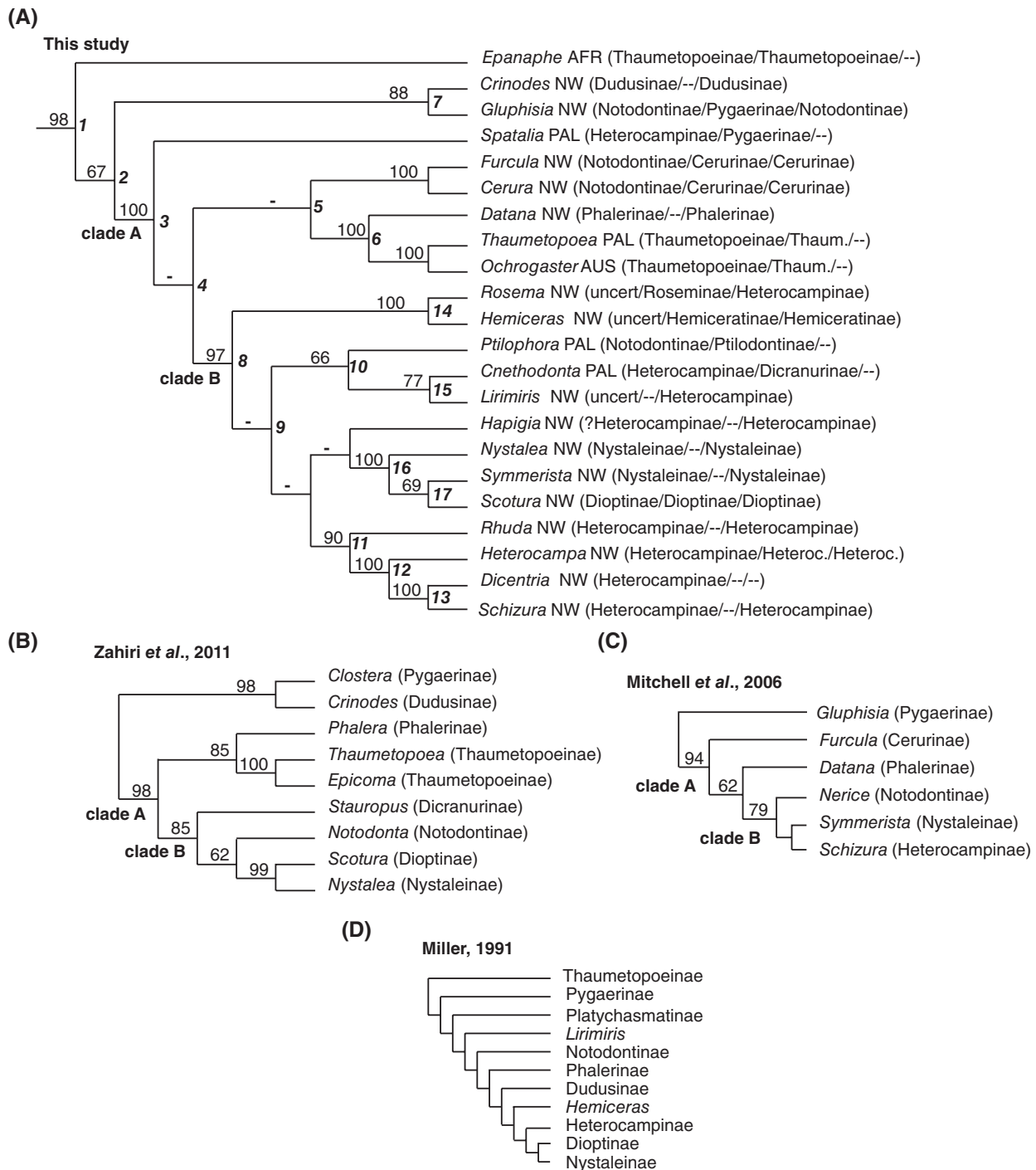


Fig. 2. Comparison of relationships among subfamilies of Notodontidae between the present study, Mitchell *et al.* (2006), Zahiri *et al.* (2011) and Miller (1991). (A) Relationships among subfamilies simplified from Fig. 1, with maximum-likelihood (ML) bootstraps for nt123 unpartitioned above branches. Geographic distribution shown after taxon names: AFR, Africa; AUS, Australia; NW, New World; PAL, Palaearctic. Subfamily names in parentheses according to Miller (1991)/Schintlmeister (2008, 2013)/Becker (2014). ‘--’, subfamily not specified by that author; ‘-’, bootstrap value <50; Thaum., Thaumetopoeinae; Heteroc., Heterocampinae. (B) Relationships among notodontid subfamilies extracted from larger noctuid ML phylogeny of Zahiri *et al.* (2011), with ML bootstraps above branches. Subfamily classification follows Lafontaine & Schmidt (2010, 2013). (C) Relationships among notodontid exemplars included in the two-gene, 141-taxon ML noctuid phylogeny of Mitchell *et al.* (2006), extracted from their Fig. 4. ML tree search used the GTR + G + I model, bootstraps based on Minimum Evolution search under GTR ML distance ignoring among-site rate variation. Subfamily classification follows Lafontaine & Schmidt (2010, 2013). (D) Relationships among subfamilies inferred from cladistic analysis of morphology by Miller (1991).

Stauropini in this sense are polyphyletic. The circumscription of Heterocampinae by Becker (2014) also disagrees with our tree, due to the strongly and moderately supported separation of *Hemiceras* Guenée and *Rosema* Walker, respectively, from the remaining heterocampines sensu Becker (2014). In our tree, the position of *Hapigia* Guenée, placed with doubt in Heterocampinae by Miller *et al.* (1997) and subsequently by Becker (2014), also contradicts monophyly of that subfamily, but *Hapigia* is separated from the largest cluster of Heterocampinae by only very weakly supported nodes. In sum, our evidence suggests that any monophyletic definition of Heterocampinae will probably restrict that subfamily to the New World. Whether the strongly supported 'core' group of genera around *Heterocampa* are related to other purported New World heterocampines remains unclear.

The relationships among notodontid subfamilies supported by our analyses include points of both agreement and disagreement with those of Miller (1991; Fig. 2D). The 'backbone' of our tree has two very strongly supported nested groupings (node 3, BP = 100 and node 8, BP = 97). The more inclusive grouping, which we term clade A, includes all taxa except *Epanaphe* (Thaumetopoeinae s.l.), *Crinodes* (Dudusinae) and *Gluphisia* (Pygaerinae sensu Schintlmeister, 2008). Among these three early-diverging taxa there is weak support for *Epanaphe* as the sister group to all other notodontids (node 2; BP = 67), and strong support for grouping *Gluphisia* with Dudusinae (node 7; BP = 88). The limited sampling of Zahiri *et al.* (2011; Fig. 2B) also shows a strongly supported grouping comparable to our Clade A, similar in excluding just Dudusinae and Pygaerinae (they did not sample *Epanaphe*), which are strongly grouped as in our study. A group analogous to Clade A is also strongly supported in the two-gene noctuid study of Mitchell *et al.* (2006; Fig. 2C), which sampled six notodontids. All three studies agree with Miller (1991; Fig. 2D) in identifying Pygaerinae as an early-diverging lineage. The initial divergences within Clade A are weakly supported, but there is very strong support for the grouping of *Datana* Walker (Phalerinae) with Thaumetopoeinae s.s. (node 6; BP = 100). This grouping of subfamilies was also strongly supported by Zahiri *et al.* (2011; Fig. 2B).

Within Clade A there is strong support for a group we term Clade B (node 8, BP = 97%) that includes all Clade A taxa except Thaumetopoeinae, Cerurinae sensu Schintlmeister (2008) and Phalerinae. An analogous clade, moderately to strongly supported, is found in the results of both Zahiri *et al.* (2013b; Fig. 2B) and Mitchell *et al.* (2006; Fig. 2C). Within Clade B, the basal divergences are very weakly supported, but there is very strong support for grouping of Nystaleinae with Diopinae (node 16; BP = 100), in agreement with Miller (1991) and Weller (1992). This result also corroborates Miller's assertion (Miller, 2009) that Diopinae, previously often treated as a separate family, are deeply nested within Notodontidae. Our tree suggests paraphyly of nystaleines with respect to diopines but bootstrap support for nonmonophyly is weak (node 17; BP = 69%). The grouping of Nystalaeinae + Diopinae is also reported by Zahiri *et al.* (2011; Fig. 2B), with strong support. The tree of Mitchell *et al.* (2006; Fig. 2C) suggests that

Notodontinae s.s. (Schintlmeister, 2008) are closely related to Nystalaeinae + Diopinae.

Clade B also contains the four genera stated or implied by Miller (1991) to be of uncertain position. Of these, *Hemiceras* Guenée and *Rosema* Walker are very strongly grouped together (node 14; BP = 100%), potentially providing the basis for expansion of either the subfamily Hemiceratinae recognized by Lafontaine & Fibiger (2006), or the subfamily Roseminae recognized by Schintlmeister (2013). Finally, the uncertainly placed Neotropical *Lirimiris* Walker is moderately strongly grouped with the Old World *Cnethodonta* (Dicranurinae sensu Schintlmeister, 2008; node 15; BP = 77%).

It might be argued that those aspects of our findings which appear to strongly conflict with morphological evidence are artifacts of sparse taxon sampling, which can include long branch attraction. We doubt that this is the case. In our experience with gene sets of this size in Lepidoptera, strongly supported, apparently artifactual groupings due to any effect of taxon sampling are rare. In a 123-taxon study across the families of Ditrysia, Cho *et al.* (2011) found no strongly supported unexpected groupings when the sampling was reduced to the 44 taxa having the most sequence. Within Noctuoidea, Mitchell *et al.* (1997, 2000, 2006) used only 7, 14 and 21 exemplars respectively to strongly circumscribe the huge clade now recognized as Erebidae, plus several groupings therein. These groupings, initially controversial because they contradicted monophyly for the traditional definition of Noctuidae, have invariably been corroborated in subsequent studies with much larger taxon samples. Moreover, the main notodontid clades identified in the current study are also well supported in the two other molecular studies that have included notodontids. We think they are likely to hold up. To summarize, the limited molecular information so far outlines multiple, strongly supported, nested major clades of notodontid subfamilies and provides strong evidence against some recent definitions of multiple subfamilies. Further study of notodontid relationships is one of the most important future tasks for noctuid systematics.

Erebidae. Erebidae, containing about 24 600 species (van Nieukerken *et al.*, 2011), are one of the largest families of Lepidoptera. In Fig. 3 we compare our results on relationships among subfamilies within Erebidae to those of Zahiri *et al.* (2012), using their classification. We sampled 13 of the 18 subfamilies. In both studies, support for groupings of subfamilies is often weak, especially at deeper levels. In the tree of Zahiri *et al.* (2012), 12 of the 16 nodes subtending multiple subfamilies have bootstrap support of 52% or less, whereas only four have support of $\geq 70\%$. Support in the present study is somewhat stronger, possibly due in part to the smaller taxon sample; six of 11 nodes subtending multiple subfamilies have BP $\geq 70\%$.

Although robust support is frequently lacking, its distribution across clades is very similar between studies. In both trees there is a strongly supported clade, here termed the 'Erebine lineage', consisting of Erebininae, Hypenodinae, Scolecocampinae, Boletoibiinae, Tinoliinae and Toxocampinae. (The last two were not sampled in the present study.) Considering just the subfamilies

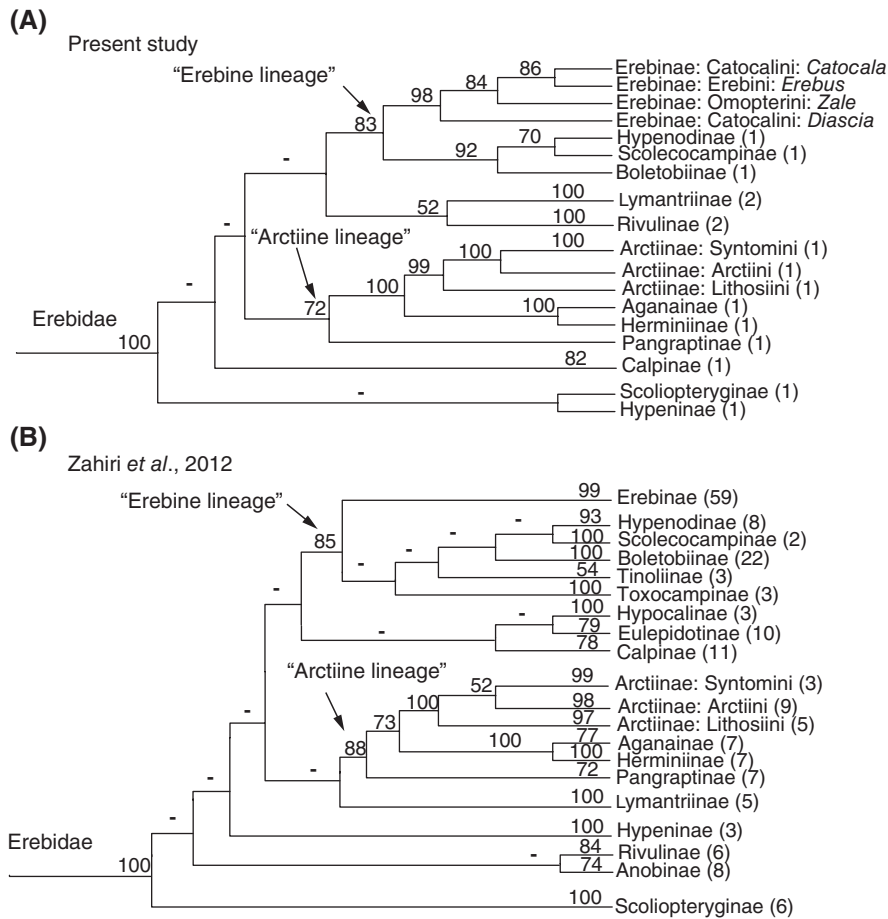


Fig. 3. Comparison of relationships among subfamilies of Erebidae between the present study and Zahiri *et al.* (2012). (A) Relationships among erebid subfamilies simplified from Fig. 1, with ML bootstraps for nt123 unpartitioned above branches. (B) Relationships among erebid subfamilies simplified from Zahiri *et al.* (2012), with ML bootstraps above branches. ‘-’, bootstrap value <50.

sampled in both, moreover, relationships within the ‘Erebine lineage’ are identical between studies; they are supported weakly in Zahiri *et al.* (2012) but moderately to strongly in the present study (Fig. 3). In addition, both studies moderately to strongly support a clade, here termed the ‘Arctiine lineage’, that consists of Pangraptinae, Herminiinae, Aganainae and Arctiinae. Relationships within this clade are the same (Pangraptinae, Arctiinae (Herminiinae, Aganainae)), and moderately to strongly supported, in both studies.

Outside of the two strongly supported lineages relationships are divergent, but even here some points of concordance can be discerned. Most notably, in both studies, Scolecocampinae and Hypeninae are among the first subfamilies to branch off. Overall, our study supports and extends the main conclusions of Zahiri *et al.* (2012). Although many questions remain, the two studies together firmly resolve positions for over half the subfamilies.

Noctuidae. Noctuidae s.s., containing about 11 700 species (van Nieukerken *et al.*, 2011) are the second-largest family of Noctuoidea. In Fig. 4 we compare our results on relationships within Noctuidae to those of Zahiri *et al.* (2013b; closely similar

to Rota *et al.* (2016)) and of Mitchell *et al.* (2006). Unlike previous studies (Fig. 4B, C), our current result (Fig. 4A) shows strong resolution throughout the ‘backbone’ of noctuid phylogeny, although this could in part reflect our smaller sample of subfamilies. Of the 14 bootstrap values for nodes subtending members of two or more subfamilies, there are 11 > 70%, 10 > 80% and 7 > 90%. Our strongly supported topology differs from those of both Mitchell *et al.* (2006) and Zahiri *et al.* (2013b), particularly among the earlier-branching lineages, but only in tree regions where support in those studies was weak. Of the subfamilies we studied, Acontiinae and Plusiinae + the unplaced *Sphragifera*, in that order, are strongly supported as the first two noctuid lineages to branch off.

There are multiple points of correspondence between the present study and that of Zahiri *et al.* (2013b). Although the taxon samples do not overlap entirely, we can recognize in both trees (Fig. 4A, C) a strongly supported node (BP = 100% in this study, 97% in Zahiri *et al.*, 2013b), here termed the ‘higher noctuid’ clade, consisting of Amphipyrynae, Acronictinae, Agaristinae, Bryophilinae, Condicinae, Heliiothinae and Noctuinae, and additionally (in the tree of Zahiri *et al.*, 2013b)

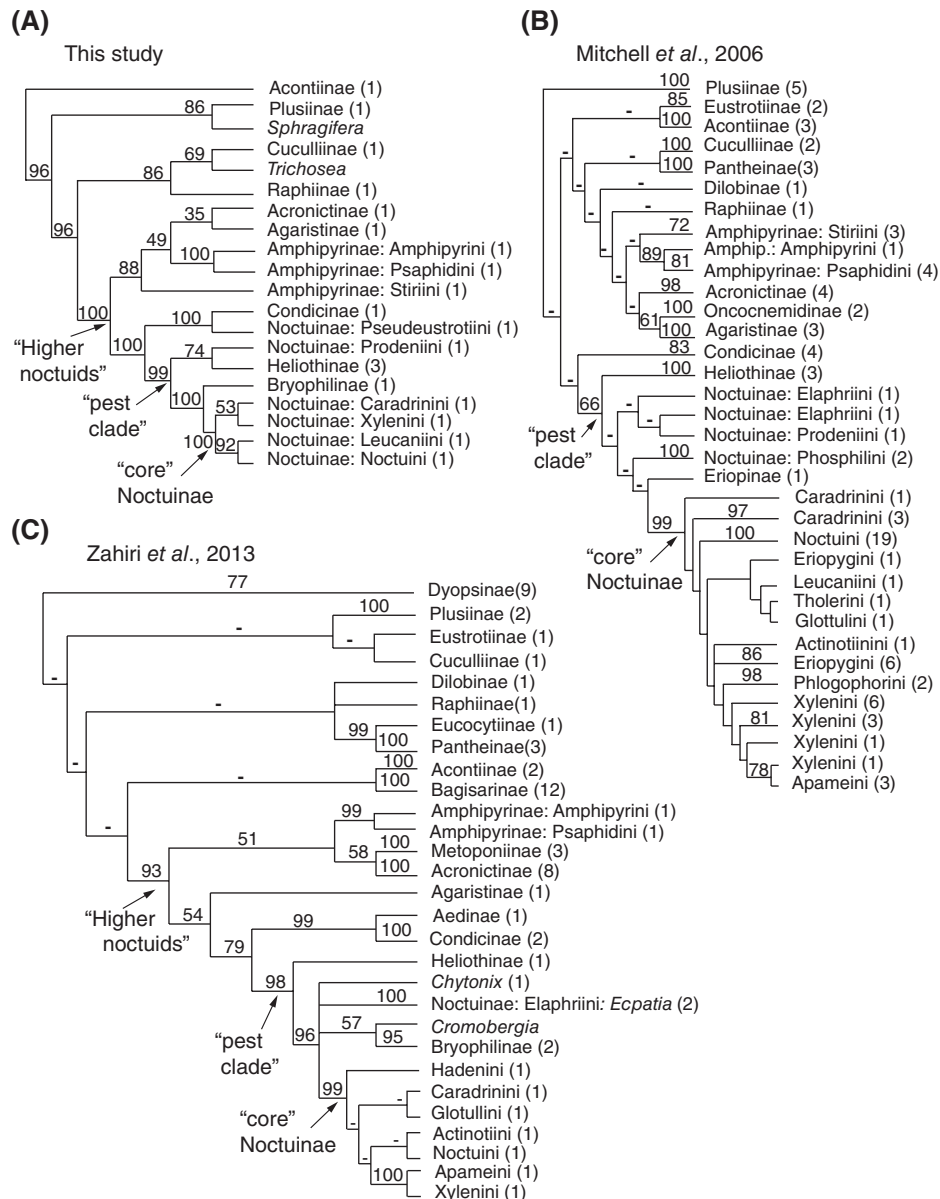


Fig. 4. Comparison of relationships among subfamilies of Noctuidae between the present study, Mitchell *et al.* (2006) and Zahiri *et al.* (2013b). (A) Relationships among noctuid subfamilies, and tribes of Noctuinae, simplified from Fig. 1, with bootstraps for nt123 unpartitioned above branches. Numbers in italics to right of branch points are node numbers referred to in text. (B) Relationships among noctuid subfamilies, and among tribes of Noctuinae, simplified from Mitchell *et al.* (2006; their Fig. 4), with ME bootstraps above branches. '-', or no marking, = bootstrap value <50. Numbers of genera sampled given in parentheses to the right of taxon names. (C) Relationships among noctuid subfamilies, and among tribes of Noctuinae, simplified from Zahiri *et al.* (2013b), with ML bootstraps above branches. '-', bootstrap value <50. Numbers of genera sampled given in parentheses to the right of taxon names.

Aedinae and Metoponiinae. Zahiri *et al.* (2013b) postulate two morphological synapomorphies for this clade, namely, basal abdominal brushes and pockets in the male (which, however, would have to have been repeatedly lost or reduced; Rota *et al.*, 2016), and presence of a raised, nodular tympanal sclerite, which appears to be unique.

Within the 'higher noctuid' clade, both studies strongly support a variant of the 'pest clade' of Mitchell *et al.* (2006),

consisting of Heliothinae, Noctuinae and related smaller subfamilies/tribes/unassociated genera. These total almost 7000 species, mostly herb feeders. (Noctuids outside this clade are a mixture of tree- and herb-feeding lineages; reviews in Mitchell *et al.*, 2006; Zahiri *et al.*, 2013b). In our tree, most of the 'higher noctuids' excluded from the 'pest clade' form a well-supported group containing Amphipyrinae, Acronictinae and Agaristinae (Fig. 4A, BP=88%). A comparable group is found, albeit

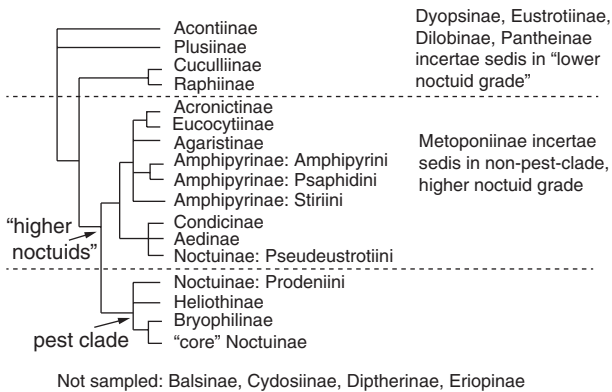


Fig. 5. Summary of current understanding of relationships within Noctuidae, combining groupings from the present study (Fig. 4A) and Zahiri *et al.* (2013b; Fig. 4C). Topology shown is a form of reduced semi-strict consensus of trees in Fig. 4A, C, produced by removing unassociated genera, collapsing all nodes in each tree with bootstrap support <80%, creating semi-strict consensus and removing terminals with highly ambiguous positions therein. Of the latter, Dilobinae, Dyopsinae, Eustrotiinae and Pantheinae are excluded from 'higher noctuids', whereas Metoponiinae are included in 'higher noctuids' but excluded from the 'pest clade'.

weakly supported, by Zahiri *et al.* (2013b; Fig. 4C; BP = 51%), except that Agaristinae are weakly grouped instead with the pest clade and near relatives. Metoponiinae, not sampled in this study, are grouped with Acronictinae and Amphipyrinae by Zahiri *et al.* (2013b), but only weakly (Fig. 4C).

In Fig. 5 we combine the information in Fig. 4A, C to depict what we can confidently say about relationships among the noctuid subfamilies. To construct the combined tree, we first removed the unassociated genera in both trees, then collapsed all the branches in each starting tree that had less than 80% bootstrap support. We then created a reduced consensus tree by including all unambiguous groupings of taxa that were supported (strongly) in at least one tree and not (strongly) contradicted by the other, and leaving out the four terminals lacking unambiguous placement in the consensus tree. Finally, we noted for the five deleted taxa what placements were consistent with the evidence (Fig. 5). The result is a relatively well-resolved set of strongly supported relationships among subfamilies, that also shows where the main remaining questions lie. Prominent among those questions is the position of the 'lower' noctuid subfamilies not sampled in this study.

A second major topic for future work is the circumscription and internal phylogeny of the huge subfamily Noctuidae s.l. (Lafontaine & Schmidt, 2010), which includes nearly 6000 species, over half of Noctuidae (Mitchell *et al.*, 2006). Morphology-based recognition of this clade (Beck, 1960, 1992; Lafontaine, 1993; Poole, 1995), which combines pieces of four large subfamilies in earlier classifications, was a major advance in noctuid systematics, as are recent detailed hypotheses such as that of Lafontaine & Schmidt (2010, 2013). Given the diversity of Noctuidae, it is to be expected that the process of sorting out exactly which lower-level groups do and do not belong will be protracted (Lafontaine & Schmidt, 2013). A consistent

result of molecular analyses, starting with the two-gene studies of Mitchell *et al.* (2000, 2006) and continuing through recent multi-gene analyses (Zahiri *et al.*, 2013b; Rota *et al.*, 2016; present study; see Fig. 4), has been very strong bootstrap support for a 'core' group of Noctuidae consisting of all tribes except Elaphriini, Prodeniini, Phosphilini and Pseudeustrotiini, but either no support for (Fig. 4C), or evidence against (Fig. 4A, B), monophyly of Noctuidae with one or more of the latter four tribes included. The evidence against monophyly, heretofore weak, is much stronger in the present study (Fig. 4A). The representative of Prodeniini is separated from 'core' Noctuidae by two nodes, one of which has 100% bootstrap support, whereas the representative of Pseudeustrotiini is separated from 'core' Noctuidae by three very strongly supported nodes. Although they need further testing, these results are unlikely to be an artifact of sparse taxon sampling as they are similar to, and not strongly contradicted by, those from the much larger taxon sample of Mitchell *et al.* (2006; Fig. 4B). Both larval and adult synapomorphies have been identified for Noctuidae s.l. (Lafontaine & Fibiger, 2006; Lafontaine & Schmidt, 2010), but no analyses have demonstrated that morphological characters as a whole (*sensu* Heikkilä *et al.*, 2015) support monophyly for this group, and morphological characters are not infallible (see Doidae, above). Thus, we consider the limits of a monophyletic Noctuidae to be an open question and predict that these may prove eventually to correspond to just the 'core' Noctuidae identified by molecular data.

Conclusions

We conducted an independent assessment, using the largest gene sample to date, of phylogenetic relationships within Noctuoidea inferred by recent authors. Our main findings are as follows:

- 1 Our data strongly corroborate the six-family system of Zahiri *et al.* (2011), including (i) removal of Oenosandridae from Notodontidae, (ii) exclusion of these two families from a clade containing the remaining families, and (iii) subordination of Arctiidae, Lymantriidae and most 'quadrifine' Noctuidae s.l. within the huge family Erebidae. However, our results, like those of previous molecular studies, are equivocal on the position of Oenosandridae and on relationships among the four 'quadrifid' forewing families.
- 2 Our evidence is much stronger on relationships within families. Within Notodontidae, we find strong evidence for polyphyly of one or more of the concepts of Thaumetopoeinae, Pygaerinae, Notodontinae and Heterocampinae expressed in recent classifications. Deeper divergences are only partially resolved, but our results, in combination with those of previous molecular studies, strongly support multiple nodes in an initial 'backbone' phylogeny estimate across the largest notodontid subfamilies.
- 3 Within Erebidae, relationships among subfamilies are likewise only partially resolved, but our results parallel those of Zahiri *et al.* (2012) in delimiting moderately to strongly supported 'arctiine' and 'erebine' lineages, with identical internal

relationships, that together encompass 8–10 of the 18 subfamilies.

- 4 Within Noctuidae, our results provide the strongest support to date for relationships among the subfamilies. There are no strong conflicts with the phylogeny of Zahiri *et al.* (2013b), and a semi-strict consensus of the robustly supported groupings in the two trees yields well-defined positions for most subfamilies.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/syen.12199

Table S1. A spreadsheet showing specimens sequenced, their classification, specimen accession numbers, number of genes attempted, total sequence length obtained and GenBank accession numbers.

Acknowledgements

We are greatly indebted to the following generous colleagues for providing specimens used in this study: James K. Adams, Joaquin Baixeras, Tom Burbidge, Robert F. Denno, Michael Fibiger, Nils Hyden, Akito Y. Kawahara, Ian J. Kitching, Ed C. Knudson, Robert LeClerc, Marcus J. Matthews, Andrew Mitchell, Judd Nelson, Kenji Nishida, Robert W. Poole and Ron Robertson. This publication was made possible by the Leptree project and the efforts of the entire Leptree team. We especially thank our co-P.I.s Donald Davis, Cynthia Parr and Susan Weller. We gratefully acknowledge the unflagging support of the team of Costa Rican ACG parataxonomists (Janzen & Hallwachs, 2011) who found and reared the specimens whose voucher numbers include ‘srmp’ (Table S1), and the team of biodiversity managers who protect and manage the ACG forests that host these caterpillars. We are grateful to the three anonymous reviewers, whose comments led to a much improved manuscript. Financial support was provided by: the U.S. National Science Foundation’s Assembling the Tree of Life program, award numbers 0531626 and 0531769; U.S. National Science Foundation awards DEB-1355028 and DBI-0755048; the Hatch funds of the Maryland Agricultural Experiment Station; U.S. National Science Foundation grants BSR 9024770 and DEB 9306296, 9400829, 9705072, 0072730, 0515699; the Wege Foundation; the International Conservation Fund of Canada; the Jessie B. Cox Charitable Trust; the Blue Moon Fund; the Dry Forest Conservation Fund, Area de Conservación Guanacaste; the Biodiversity Institute of Ontario; Permian Global; the Smithsonian Institution; and the University of Pennsylvania.

References

Bazinet, A.L. & Cummings, M.P. (2008) The Lattice Project: a grid research and production environment combining multiple grid computing models. *Distributed & Grid Computing – Science Made*

- Transparent for Everyone. Principles, Applications and Supporting Communities* (ed. by M.H.W. Weber), pp. 2–13. Tectum, Marburg.
- Bazinet, A.L., Cummings, M.P., Mitter, K.T. & Mitter, C. (2013) Can RNA-Seq resolve the rapid radiation of advanced moths and butterflies (Hexapoda: Lepidoptera: Apoditrysia)? An exploratory study. *PLoS One*, **8**, e82615.
- Beck, H. (1960) *Die larvalsystematik der eulen (Noctuidae)*. Akademie Verlag, Berlin.
- Beck, H. (1992) New view of the higher classification of the Noctuidae (Lepidoptera). *Nota Lepidopterologica*, **15**, 3–28.
- Becker, V.O. (2014) Checklist of New World Notodontidae (Lepidoptera: Noctuoidea). *Lepidoptera Novae*, **7**, 1–40.
- Cho, S., Zwick, A., Regier, J.C. *et al.* (2011) Can deliberately incomplete gene sample augmentation improve a phylogeny estimate for the advanced moths and butterflies (Hexapoda: Lepidoptera)? *Systematic Biology*, **60**, 782–796.
- Cummings, M.P. & Huskamp, J.C. (2005) Grid computing. *Educause Review*, **40**, 116–117.
- Fibiger, M. & Lafontaine, J.D. (2005) A review of the higher classification of the Noctuoidea (Lepidoptera) – with special reference to the Holarctic fauna. *Esperiana*, **11**, 7–92.
- Forbes, W.T.M. (1948) Lepidoptera of New York and neighboring states. Part 2. Notodontidae. *Cornell Agricultural Experiment Station Memoirs*, **274**, 203–237.
- Heikkilä, M., Mutanen, M., Wahlberg, N., Sihvonen, P. & Kaila, L.J. (2015) Elusive ditryasian phylogeny: an account of combining systematized morphology with molecular data (Lepidoptera). *BioMed Central Evolutionary Biology*, **15**, 260.
- Janzen, D.H. & Hallwachs, W. (2011) Joining inventory by parataxonomists with DNA barcoding of a large complex tropical conserved wildland in northwestern Costa Rica. *PLoS One*, **6**, e18123. DOI: 10.1371/journal.pone.0018123.
- Kitching, I.J. & Rawlins, J.E. (1998) Noctuoidea. *Handbook of Zoology. Lepidoptera, Vol. 1: Systematics and Evolution* (ed. by N.P. Kristensen), pp. 355–401. W. de Gruyter, Berlin.
- Lafontaine, J.D. (1993) Cutworm systematics: confusions and solutions. *Memoirs of the Entomological Society of Canada*, **165**, 189–196.
- Lafontaine, J.D. & Fibiger, M. (2006) Revised higher classification of the Noctuoidea (Lepidoptera). *Canadian Entomologist*, **138**, 610–635.
- Lafontaine, J.D. & Schmidt, C. (2010) Annotated check list of the Noctuoidea (Insecta, Lepidoptera) of North America north of Mexico. *ZooKeys*, **40**, 1–239.
- Lafontaine, J.D. & Schmidt, B.C. (2013) Comments on differences in classification of the superfamily Noctuoidea (Insecta: Lepidoptera) between Europe and North America. *ZooKeys*, **264**, 209–217.
- Matsumura, S. (1929) New species and genera of Notodontidae. *Insecta Matsumurana*, **4**, 36–48.
- Mbahin, N., Raina, S.K., Kioko, E.N. & Mueke, J.M. (2012) Biology of the wild silkmoth *Anaphe panda* (Boisduval) in the Kakamega Forest of Western Kenya. *International Journal of Forestry Research*, **2012**, Article ID 186549, 7 p. DOI: 10.1155/2012/186549.
- Miller, J.S. (1991) Cladistics and classification of the Notodontidae (Lepidoptera: Noctuoidea) based on larval and adult morphology. *Bulletin of the American Museum of Natural History*, **204**, 1–230.
- Miller, J.S. (1992) Host-plant associations among prominent moths. *BioScience*, **42**, 50–56.
- Miller, J.S. (2009) Generic revision of the Dioptinae (Lepidoptera: Noctuoidea: Notodontidae). Part 1: Dioptini. *Bulletin of the American Museum of Natural History*, **321**, 1–674.
- Miller, J.S., Janzen, D.H. & Franclemont, J.G. (1997) New species of *Euhapigiodes*, new genus, and *Hapigiodes* in Hapigiini, new tribe, from Costa Rica, with notes on their life history and immatures (Lepidoptera: Notodontidae). *Tropical Lepidoptera*, **8**, 81–99.

- Mitchell, A., Cho, S., Regier, J.C., Mitter, C., Poole, R.W. & Matthews, M. (1997) Phylogenetic utility of elongation factor-1a in Noctuoidea (Insecta: Lepidoptera): the limits of synonymous substitution. *Molecular Biology and Evolution*, **14**, 381–390.
- Mitchell, A., Mitter, C. & Regier, J.C. (2000) More taxa or more characters revisited: combining data from nuclear protein encoding genes for phylogenetic analyses of Noctuoidea (Insecta: Lepidoptera). *Systematic Biology*, **49**, 202–224.
- Mitchell, A., Mitter, C. & Regier, J.C. (2006) Systematics and evolution of the cutworm moths (Lepidoptera: Noctuidae): evidence from two protein-coding nuclear genes. *Systematic Entomology*, **31**, 21–46.
- Mutanen, M., Wahlberg, N. & Kaila, L. (2010) Comprehensive gene and taxon coverage elucidates radiation patterns in moths and butterflies. *Proceedings of the Royal Society B*, **277**, 2839–2848.
- Poole, R.W. (1995) Noctuoidea. Noctuidae (part). Cuculliinae, Stiiriinae, Psaphidinae (part). *Moths of America North of Mexico*, Fascicle 26.1. The Wedge Entomological Research Foundation, Washington, DC.
- Ratnasingham, S. & Hebert, P.D.N. (2007) BOLD: the barcode of life system (<http://www.barcodinglife.org>). *Molecular Ecology Notes*, **7**, 355–364.
- Regier, J.C. & Shi, D. (2005) Increased yield of PCR product from degenerate primers with nondegenerate, nonhomologous 5' tails. *BioTechniques*, **38**, 34–38.
- Regier, J.C. & Zwick, A. (2011) Sources of signal in 62 protein-coding nuclear genes for higher-level phylogenetics of arthropods. *PLoS ONE*, **6**, e23408.
- Regier, J.C., Shultz, J.W., Ganley, A.R.D. *et al.* (2008) Resolving arthropod phylogeny: exploring phylogenetic signal within 41 kb of protein-coding nuclear gene sequence. *Systematic Biology*, **57**, 920–938.
- Regier, J.C., Zwick, A., Cummings, M.P. *et al.* (2009) Toward reconstructing the evolution of advanced moths and butterflies (Lepidoptera: Ditrysia): an initial molecular study. *BioMed Central Evolutionary Biology*, **9**, 280–301.
- Regier, J.C., Shultz, J.W., Zwick, A. *et al.* (2010) Arthropod relationships revealed by phylogenomic analysis of nuclear protein-coding sequences. *Nature*, **463**, 1079–1083.
- Regier, J.C., Mitter, C., Zwick, A. *et al.* (2013) A large-scale, higher-level, molecular phylogenetic study of the insect order Lepidoptera (moths and butterflies). *PLoS One*, **8**, e58568.
- Rota, J., Zacharczenko, B.V., Wahlberg, N., Zahiri, R., Schmidt, B.C. & Wagner, D.L. (2016) Phylogenetic relationships of Acronictinae with discussion of the abdominal courtship brush in Noctuidae (Lepidoptera). *Systematic Entomology*, **41**, 416–429.
- Schintlmeister, A. (2008) *Notodontidae. Palaearctic Macrolepidoptera*, Vol. 1. Apollo Books, Stenstrup.
- Schintlmeister, A. (2013) *Notodontidae and Oenosandridae (Lepidoptera) (World Catalogue of Insects)*, Vol. 11. Brill, Leiden.
- Staden, R. (1999) *Staden Package*. MRC Laboratory of Molecular Biology, Cambridge. [WWW document]. URL <http://www.mrc-lmb.cam.ac.uk/pubseq/> [accessed on 19 September 2007].
- van Nieukerken, E.J., Kaila, L., Kitching, I.J. *et al.* (2011) Order Lepidoptera Linnaeus, 1758. Animal Biodiversity: An Outline of Higher-Level Classification and Survey of Taxonomic Richness (ed. by Z.-Q. Zhang). *Zootaxa*, **3148**, 212–221.
- Weller, S.J. (1992) Survey of adult morphology in Nystaleinae and related Neotropical subfamilies (Noctuoidea: Notodontidae). *Journal of Research on the Lepidoptera*, **31**, 233–277.
- Weller, S.J., Pashley, D.P., Martin, J.A. & Constable, J.L. (1994) Phylogeny of noctuid moths and the utility of combining independent nuclear and mitochondrial genes. *Systematic Biology*, **43**, 194–211.
- Yang, X., Cameron, S.L., Lees, D.C., Xue, D. & Han, H. (2015) A mitochondrial genome phylogeny of owlet moths (Lepidoptera: Noctuoidea), and examination of the utility of mitochondrial genomes for lepidopteran phylogenetics. *Molecular Phylogenetics and Evolution*, **85**, 230–237.
- Zahiri, R., Kitching, I.J., Lafontaine, J.D., Mutanen, M. & Kaila, L. (2011) A new molecular phylogeny offers hope for a stable family level classification of the Noctuoidea (Lepidoptera). *Zoologica Scripta*, **40**, 158–173.
- Zahiri, R., Holloway, J.D., Kitching, I.J., Lafontaine, D., Mutanen, M. & Wahlberg, N. (2012) Molecular phylogenetics of Erebiidae (Lepidoptera, Noctuoidea). *Systematic Entomology*, **37**, 102–124.
- Zahiri, R., Lafontaine, J.D., Holloway, J.D., Kitching, I.J., Schmidt, B.C., Kaila, L. & Wahlberg, N. (2013a) Major lineages of Nolidae (Lepidoptera, Noctuoidea) elucidated by molecular phylogenetics. *Cladistics*, **29**, 337–359.
- Zahiri, R., Lafontaine, J.D., Schmidt, B.C., Holloway, J.D., Kitching, I.J., Mutanen, M. & Wahlberg, N. (2013b) Relationships among the basal lineages of Noctuidae (Lepidoptera, Noctuoidea) based on eight gene regions. *Zoologica Scripta*, **42**, 488–507.
- Zwick, A., Regier, J.C. & Zwickl, D.J. (2012) Resolving discrepancy between nucleotides and amino acids in deep-level arthropod phylogenomics: differentiating serine codons in 21-amino-acid models. *PLoS One*, **7**, e47450. DOI: 10.1371/journal.pone.0047450.
- Zwick, A., Regier, J.C., Mitter, C. & Cummings, M.P. (2011) Increased gene sampling yields robust support for higher-level clades within Bombycoidea (Lepidoptera). *Systematic Entomology*, **36**, 31–43.
- Zwickl, D.J. (2006) *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. PhD Thesis, The University of Texas at Austin.

Accepted 22 June 2016