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# Disarticulating and Reassembling Amphipyrinae (Lepidoptera, Noctuoidea, Noctuidae)

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**ABSTRACT**

Just as every home has a junk drawer, most diverse higher taxa have one or more subordinate groups for their putative members that are unassignable to neighboring subordinate groups or are otherwise poorly understood. Amphipyrinae have long been a catchall taxon for Noctuidae, with most members lacking discernible synapomorphies that would allow their assignment to one of the many readily diagnosable noctuid subfamilies. Here we use data from seven gene regions (>5,500 base pairs) for more than 120 noctuid genera to infer a phylogeny for Amphipyrinae and related subfamilies. Sequence data for fifty-seven amphipyrine genera—most represented by the type species of the genus—are examined (most for the first time). We present the first large-scale molecular phylogenetic study of Amphipyrinae *sensu lato*; propose several nomenclatural changes for strongly supported results; and identify areas of noctuid phylogeny where greater taxon sampling and/or genomic-scale data are needed. We also discuss morphological (both adult and larval) and life history data for several of the higher taxonomic groupings most relevant to our results. Amphipyrinae are significantly redefined; many former amphipyrines, excluded as a result of our analyses, are reassigned to one of eight other noctuid subfamily-level taxa. For some amphipyrine genera for which phylogenetic placement to subfamily remains uncertain, we assign them to *incertae sedis* positions in Noctuidae.

Key Words: *Amphipyra*, *Cropia,* Stiriinae, Grotellinae, Chamaecleini Keegan & Wagner, phylogenetics, larval morphology

## INTRODUCTION

Amphipyrine noctuids have been described from all biogeographic regions (minus Oceania and Antarctica); they are believed to be least diverse in tropical regions and most diverse in the Northern Hemisphere – particularly in the southwestern Nearctic. Lafontaine & Schmidt (2010, 2015), recognize approximately 220 species in 73 genera from North America north of Mexico, with much of the subfamily's generic and species diversity occurring in the deserts and badlands.

Amphipyrinae have long been a taxon of uncertain identity, with its constituent tribes and subtribes defined largely by the absence of features characteristic of phylogenetically proximate noctuid subfamilies. In the case of some its tribes and subtribes, placement within the subfamily has been simply a matter of nomenclatural convenience with no characters (or lack thereof) supporting their inclusion (Poole, 1995). In essence, amphipyrines have been have-nots, lacking defining characters. As a consequence, taxonomic concepts of what is and is not an amphipyrine have varied greatly through time, both across continents and among workers. For discussions of characters (or the absence thereof) used to circumscribe the subfamily and its constituents see treatments in Poole (1995), Kitching & Rawlins (1998), and Fibiger & Lafontaine (2005).

Most North American noctuid collections, Internet resources, and taxonomic literature are still organized according to Franclemont & Todd's (1983) checklist of Nearctic moths found north of Mexico. Their concept of the subfamily included more than five dozen genera presently classified as Noctuinae; many genera now assigned to Balsinae, Bryophilinae, Condicinae, Eriopinae, Metoponiinae; more than two dozen "unassociated genera," most of which were reclassified by Lafontaine & Schmidt (2010) into other subfamilies; as well as a few erebids and a nolid.

In Africa, Australia, and parts of Asia the subfamily's limits have been nebulous, sometimes overlapping completely with Acronictinae and other seemingly distinct subfamilies (Hampson 1898; Nielsen *et al*., 1996). Kitching & Rawlins (1998) were so vexed by what is and what is not an amphipyrine that they embraced the opposite extreme, restricting membership to the nominate genus and a few closely allied genera, but offered no morphological characters that defined their concept of the taxon. The opposite problem, i.e., exclusion of true amphipyrines, has also plagued previous classification schemes. For example, psaphidines, which we regard to be closely allied to the nominate genus *Amphipyra*, have been pulled out as a separate subfamily in many taxonomic treatments (e.g., Poole, 1995; Kitching & Rawlins, 1998; Fibiger & Hacker, 2004; Fibiger & Lafontaine, 2005).

The currently accepted concept for the Amphipyrinae in North America, and the guide for our taxon sampling, is that of Lafontaine & Schmidt (2010, 2015). They transferred more than 60 former amphipyrine genera into the Noctuinae and Bryophilinae; recognized that some putative Nearctic amphipyrines were, in fact, New World Metoponiinae; and subsumed the Psaphidinae and Stiriinae as tribes within Amphipyrinae. Our results indicate that even with these recent taxonomic changes, the Amphipyrinae still contain species and genera that belong in no less than nine other subfamilial taxa!

Our study uses 5,508 base pairs from seven genes to explore phylogenetic relationships among predominantly Nearctic amphipyrines. Taxon sampling emphasized type species of genera. Although we have not yet had a chance to sequence members of several New World amphipyrine

genera, our study represents the most comprehensive assessment of the subfamily and the Noctuidae to date with more than 120 generic-level noctuids sampled, representing 25 subfamilies. In this effort, we limit our nomenclatural recommendations to well-supported results (clades), and identify areas of noctuid phylogeny, proximate to the Amphipyrinae, where greater taxon sampling is needed. Much of the discussion is given to providing adult and larval characters that we associate with the major clades affected by the results of this study, and use these characters to guide our taxonomic recommendations.

## METHODS

### *Taxon sampling*

Sixty-three species representing 61 genera, few of which have previously been included in a molecular phylogenetic study are included in our study. Fifty-seven of the 76 genera in Amphipyrinae, as circumscribed by Lafontaine & Schmidt (2010, 2015), are included among the 61 genera; represented are all three amphipyrine tribes, all eight subtribes, and all seven of their *incertae sedis* genera (see Table S1 in supplementary materials). Most amphipyrine genera (47 of 57) in the study are represented by their type species. For amphipyrine genera for which the type species was not sampled, morphologically similar and/or COI-proximate congeners were selected. The majority of specimens were collected as adults, pinned, and air-dried. Some adult specimens were collected directly into 95% ethanol; larval specimens were stored in 95% ethanol. Some larval specimens were briefly boiled in water prior to deposition into ethanol to prevent decay within the digestive tract. Single specimens of each species were used; voucher specimens, newly collected for this study, were deposited according to Table S1. In order to aid

readability, all taxon author names were moved from the text to a table in the supplementary materials (Table S5).

*Gene Sampling*

Seven genes were sampled that have been used in previous studies capable of resolving phylogenetic relationships of Lepidoptera at differing evolutionary depths: CO1, EF-1α, GAPDH, IDH, MDH, RpS5, and wingless (Cho *et al*., 1995; Fang *et al*., 1997; Mitchell *et al*., 2006; Wahlberg & Wheat, 2008; Zahiri *et al*., 2011, 2013; Rota *et al*., 2016; Regier *et al*., 2017). Both CO1 and EF-1α were sequenced in two parts making for a total of nine loci for this study. Another gene, CAD, that has been used to infer lepidopteran phylogenies (Zahiri *et al*., 2011, 2013; Rota *et al*., 2016), was abandoned due to its low amplification success during PCR.

*DNA Extraction, PCR, and Sequencing*

All DNA extractions were done using the NucleoSpin Tissue 250 kit manufactured by Macherey Nagel using 1-2 legs from each specimen. Once extracted, DNA was stored in a refrigerator at ~4º C until needed for PCR. The PCR profiles and primers outlined in Wahlberg & Wheat (2008) were used. PCR products were sent to Macrogen Europe Inc. (Amsterdam, the Netherlands) or Macrogen USA Inc. (Rockville, Maryland) for sequencing. For the majority of loci, single forward reads were used, although some nuclear loci with fragmented PCR products required reverse reads. Sequence chromatograms were visually inspected for base call errors and heterozygous loci in Geneious® 8.1.9 (http://www.geneious.com, Kearse *et al*., 2012). Consensus sequences for dual-read loci were also generated in Geneious. To ensure sequences were attributed to the correct species, a local BLAST (Altschul *et al*., 1990) search was conducted in Geneious to compare the manually named sequence files with the unnamed sequences from

Macrogen. Sequences were then checked against sequences available in GenBank (NCBI Resource Coordinators 2017) and BOLD (Ratnasingham & Hebert, 2007) to detect misdeterminations and contamination. Sequences were then exported to FASTA files, visually aligned to reference lepidopteran sequences for each locus using AliView version 1.18 (Larsson, 2014), and then concatenated using AMAS version 0.95 (Borowiec, 2016). Phylogenies were built for each locus to detect possible contamination. Our alignment file and its TreeBASE identification number can be found in the supplementary materials [Note: these to be added upon acceptance]. GenBank accession numbers for newly generated sequences can be found in Table S3 [Note: these to be added upon acceptance]. GenBank accession numbers for sequences generated for previous studies can be found in Table S4.

*Phylogenetic Inference and Tree Visualization*

Our 567 newly generated sequences were analyzed in conjunction with 810 published noctuoid sequences from Zahiri *et al*. (2011, 2013) and Rota *et al*. (2016). Phylogenies were inferred with RAxML using the RAxML BlackBox web-server (Stamatakis *et al*., 2008) and MrBayes v. 3.2.6 using the CIPRES web server (Miller *et al*., 2010). Stationarity of MCMC parameters used in MrBayes was assessed with Tracer v 1.6.0 (Rambaut *et al*., 2014). See supplementary online materials for alignment, tree, and log files from these analyses. Tree files and alignments have also been deposited in TreeBASE [Note: these to be added upon acceptance]. We used the R (R Core Team, 2017) package ggtree (Yu *et al*., 2017) in R Studio v 1.0.136 (R Studio Team, 2015) to visualize and annotate the trees. Further annotation was done using GIMP and Adobe Photoshop image editing software.

*Morphological and Life History Assessment*

Clade membership and topological positions of all amphipyrine genera were evaluated in terms of their male genital characters by JDL. At least one dissection was examined or newly prepared for most genera, and in all instances where a genus fell outside of the Amphipyrinae, Metoponiinae, and Stiriinae as depicted in Fig. 1. Likewise, the phylogenetic positions in Fig. 1 were evaluated in terms of larval biology and morphology by DLW. We report findings that reinforce or refute the relationships inferred in Fig. 1 in the Discussion.

## RESULTS

Our dataset consists of concatenated sequences of 154 noctuoid species with a maximum of 5,508 sites for the combined seven gene regions (and nine loci)—2,009 (36.5%) of the sites were parsimony informative. On average, each taxon's sequence data consisted of 25.1% missing or ambiguous sites. See Table S2 (supplementary materials) for sequence coverage by gene and taxon. We found no major signs of sequence contamination, no major conflicts between the maximum likelihood and Bayesian phylogenetic analyses, and no convergence problems in the Bayesian analysis. Although there was modest support for many of the deeper nodes, branch lengths were often too short to resolve intersubfamilial relationships (an indication that noctuids radiated rapidly or that the genes used were inadequate for resolving many deeper divergences).

Amphipyrinae proved to be surprisingly polyphyletic, with its genera being strongly supported as members of no less than ten subfamily-level noctuid lineages (Fig. 1). Our analysis suggests a much restricted Amphipyrinae (Amphipyrinae s.s.) consisting largely of the tribes Amphipyrini

and Psaphidini with (nearly all of) Stiriini excluded—the most species-rich tribe of the Amphipyrinae as circumscribed by Lafontaine & Schmidt (2010, 2015).

The current tribal and subtribal-level taxonomy within the Amphipyrinae s.s. (as depicted in Figs 1 A,B) was also found to contain paraphyletic and polyphyletic taxa as delimited in existing taxonomic treatments. For instance, *Emarginea*, is currently classified in the subtribe Nocloina, yet is strongly supported (BS=72) as a member of subtribe Feraliina. *Miracavira*, which is currently classified in Feraliina, is not strongly supported as a feraliine and instead clusters with weak support with nocloines. A detailed (species-level) study of phylogenetic relationships within North American Amphipyrinae and Stiriinae is underway by KLK.

The Stiriini is shown with strong support to be polyphyletic, with Lafontaine & Schmidt's (2010) subtribe Stiriina, forming the core of a subfamily-level taxon (BS=73) sister to the clade containing Metoponiinae + Grotellinae **Revised Status**. The clade, Stiriinae **Revised Status**, shown in olive (Fig. 1), includes *Narthecophora* (formerly a member of subtribe Azeniina), and subtribe Annaphilina which we elevate to Annaphilini **Revised Status**, and two genera listed as Stiriini *incertae sedis* by Lafontaine & Schmidt (2010): *Argentostiria*, and *Bistica.* Four stiriine genera presently placed in Stiriini *incertae sedis* by Lafontaine & Schmidt (2010) are strongly supported (BS=97) as sister to Acontiinae: *Chamaeclea, Hemioslaria, Heminocloa, Thurberiphaga* (all of which we transfer to Chamaecleini Keegan & Wagner **New Tribe**) (see below).

Stiriini is shown with moderate support (BS=65) as sister to the clade containing Cydosiinae, Metoponiinae, and Grotellinae. The former stiriine genus *Azenia* was shown with moderate

support (BS=64) to group within the clade containing Metoponiinae and Cydosiinae. Also clustering here were two amphipyrine genera (*Sexserrata* and *Tristyla*) that were shown with strong support (BS=100) as sister to one another, and the amphipyrine genus *Metaponpneumata*, which was shown with strong support (BS=93) as sister to Cydosiinae. This grouping of Metoponiinae and Cydosiinae in turn clustered to Grotellinae with strong support (BS=88).

Three former amphipyrine genera were shown with strong support to cluster with more remote subfamilies. *Nacopa* was strongly supported (BS=100) as sister to other Noctuinae included in this analysis. *Acopa* was strongly supported (BS=99) as nesting within the Bryophilinae. *Aleptina* was strongly supported (BS=100) as a member of Condicinae. Male genitalic characters support these results—see below.

A surprising discovery was that the genus *Oxycnemis* contains both amphipyrines and oncocnemidines; the type species of *Oxycnemis, O. advena,* clustered within triocnemidine Amphipyrinae s.s,, whereas *O. gracillinea* and *Leucocnemis perfundis* clustered together within Oncocnemidinae, with strong support (BS=100) (see discussion below).

Although many deeper-level relationships remain unresolved in our study, most of the subfamilial groupings reported by Zahiri *et al*., (2013) and Regier *et al*., (2017) are strongly supported. A summary of the taxonomic changes we recommend is shown in Table 1.

## DISCUSSION

Our results confirm the suspicions and misgivings of generations of workers that the Amphipyrinae are an unnatural grouping, and staggeringly so—the 61 amphipyrine genera that we surveyed fall into ten different subfamily-level taxa. Many taxonomic changes are needed in order to render the Amphipyrinae and other family group taxa monophyletic. Here, we recommend formal taxonomic changes only for those results that we believe (using our seven-gene data set and collective knowledge of larval morphology, adult morphology, and ecology) to be robust and unlikely to be affected by additional taxon sampling. For several of the nodes and taxonomic groupings in Figs 1A-C, more taxa (or genetic data) will be needed before formal taxonomic and nomenclatural changes can be convincingly justified, e.g., the parsing of amphipyrines and metoponiines into tribes and subtribes). Our discussion is broken down by taxon, first outlining what we regard to be true Amphipyrinae, and then reviewing the fate of genera that fell outside of Amphipyrinae. For many of the tribes or subfamilies affected by our findings, we offer a brief characterization of morphological and life history data supporting recommended taxonomic decisions.

Amphipyrinae sensu stricto

The Amphipyrinae s.s. are represented by the green branches in Figs 1A,B. In addition to the nominate tribe, the 26 amphipyrine s.s. genera in our analysis include members of the Psaphidini and its four subtribes: i.e., Psaphidina, Feraliina, Nocloina, and Triocnemidina, and two species of *Nacna*, (an Asian amphipyrine). Excluded from the Amphipyrinae are all four subtribes of Stiriini: Stiriina, Grotellina (except *Hemigrotella*, which is shown to group with Triocnemidina), Azeniina, and Annaphilina. The placement of these four subtribes and their constituent genera is discussed below.

In large measure, the amphipyrine and psaphidine genera from Lafontaine & Schmidt's (2010, 2015) checklist are confirmed as amphipyrines, a laudable feat given that the subfamily was widely regarded to be an unnatural group that lacked defining synapomorphies. We, however, found little support for the monophyly of either Amphipyrini or Psaphidini, with the 24 Psaphidini genera in our analysis clustered into roughly six groups (Figs 1A,B), although only two of these are well supported: informally recognized here as the amphipyrine, brachionychine, feraliine, nocloine, psaphidine, and triocnemidine groups. The feraliine group includes the East Asian genus *Nacna*, connected by a deep split to four North American genera. Given the shortness of several (deeper) internal branches and weak nodal support, it is our recommendation that it would be premature to formally delimit amphipyrine tribes and subtribes before more sampling is done across amphipyrine genera (especially beyond the Nearctic Region).

Some previous workers have recognized Psaphidini as a subfamily, a position we find problematic. Firstly, because we have little evidence that psaphidines as presently circumscribed are monophyletic, and secondly because recognition of a demonstrably monophyletic "Psaphidinae" might require recognition of >1-4 additional subfamily-level taxa (e.g., for *Nacna* + Feraliina) within a group of moths that are arguably rather uniform morphologically, ecologically, and not especially species rich. Thirdly, splits between the various subordinate groups (e.g. between the amphipyrine and feraliine group) are short in our analysis (all poorly supported), and not enough of the world fauna has been sampled to understand both membership and taxonomic consequences of recognizing psaphidines at the subfamily rank. Further, larval characters and known life histories argue to subsume the two subfamilies into a single entity as discussed by Wagner *et al*. (2008).

Oncocnemidinae and Agaristinae

Support for a sister relationship between Oncocnemidinae and Agaristinae is shown to be weak, although the latter is represented by just a single Australian exemplar (*Periscepta*). Such a relationship was hinted at with weak support in Mitchell *et al*. (2006), but not until this study have members from both subfamilies been included again in the same phylogenetic analysis. We currently are sampling across both subfamilies to test this association. We also note that the two subfamilies were placed next to each other in Lafontaine & Schmidt's (2010) checklist, based on Troubridge's (2008) finding of a shared feature of their thoracic-abdominal connection.

Acronictinae

Topological relationships within the subfamily as recovered by Rota *et al*. (2016) were unaffected in our analysis with *Lophonycta* coming out as sister to all other dagger moths. As noted above, deeper nodes among noctuid subfamilies were often without robust support and branches emanating from these nodes often short, as is the case with the node and branch connecting Acronictinae to the clade containing Stiriinae, Metoponiinae, and Grotellinae.

Stiriinae

As suggested by their larvae and life histories (Crumb, 1956; Wagner *et al*., 2011), adult morphology (Poole, 1995), and a recent molecular study of the Noctuidae (Regier *et al*., 2017), the Stiriini of authors represent a distinct subfamily, Stiriinae, which is represented in Figs 1A,C by the 14 genera rendered in olive. As defined here, its contents are trimmed relative to previous concepts (Franclemont & Todd, 1983; Poole, 1995; Lafontaine & Schmidt, 2010, 2015). Our

findings suggest that Stiriinae should be restricted to just what Lafontaine & Schmidt regarded as (the subtribe) Stiriina, and subtribe Annaphilina, with the addition of *Narthecophora*. And even from this pruned concept of Stiriinae, four genera—all believed to be associated with Malvaceae host plants—*Chamaeclea*, *Heminocloa*, *Hemioslaria*, and *Thurberiphaga* should be excluded (see below).

Annaphilina are well supported as the sister group to what were formerly held as the core Stiriinae. It would be helpful to include *Axenus arvalis* in future phylogenetic assessments given that it is the only genus in Annaphilina other than *Annaphila.*

Stiriinae are distributed mainly in western North America, and reach greatest diversity in deserts and adjacent aridlands. We suspect their species richness in Mexico will greatly exceed that found north of the border. Within core Stiriinae, all but one (*Argentostiria*) are thought to be specialists on Asteraceae; *Argentostiria* feeds on *Psorothamnus* (Fabaceae). Most included taxa are reliant on reproductive tissues, either flowers or callow seeds, as larvae. *Annaphila* are leaf and flower specialists on Boraginaceae, Montiaceae, and Phrymaceae. The subfamily is currently the focus of a species-level phylogenetic and biogeographic study by KLK.

<u>Acontiinae</u>

The genera *Chamaeclea*, *Hemioslaria*, *Heminocloa*, and *Thurberiphaga* form a monophyletic group sister to the two acontiines included in the analysis: *Acontia lucida* and *Emmelia trabaelis* (Figs 1A,C). Although the depth of the split between these former stiriine genera and Acontiinae in our analysis is on par with other subfamily-level depths in our analysis, we include them in the

Acontiinae on the basis of morphological characters, and group them in Chamaecleini Keegan & Wagner **New Tribe**.

In *Chamaeclea*, *Hemioslaria*, *Heminocloa*, and *Thurberiphaga* the alula is enlarged and replaces the tympanal hood of most noctuids as the apparent protection for the tympanum, and there are scattered setae on the scaphium in the male genitalia, both characters diagnostic for Acontiinae. *Chamaeclea*, *Heminocloa*, and *Thurberiphaga* all feed on Malvaceae as do most Acontiinae (Crumb, 1956; Wagner *et al*., 2011; DLW unpublished data). The larvae of all three genera are seed predators that bore into ripening fruits, with plump, smooth, grub-like caterpillars, which is far less common than leaf feeding among Nearctic acontiines (Crumb, 1956; DLW unpublished data).

<u>Metoponiinae + Cydosiinae</u>

This grouping of taxa (rendered in blue in Figs 1A,C) is the most unorthodox and perplexing in our study. We do not know if the group is comprised mostly of long-branch misfits or if it is a natural, but phenotypically divergent, assemblage. Perhaps the cluster of taxa is so unsettling due to the sparseness of sampling in and around this cluster of what could prove to represent multiple subfamily-level taxa. More than any other part of the tree, there is a need to expand the taxon sampling across this curious collection of genera to better understand the phylogenetic relationships of this assemblage.

Of the seven included genera, the phenotypic outlier is *Cydosia*, a small genus of moths with exquisitely colored adults and magnificent larvae (Figs 2C,D). *Cydosia* has had a dynamic taxonomic history having been classified in numerous noctuid subfamilies. Early American

workers commonly held the genus to be in Acontiinae (McDunnough, 1938; Franclemont & Todd, 1983). Lafontaine & Schmidt (2010) transferred *Cydosia* into its own subfamily. The analyses of Zahiri *et al*. (2013) and Rota *et al*. (2016) grouped their shared exemplar of *Cydosia* within Metoponiinae with weak support, rendering Metoponiinae paraphyletic in treatments that accord subfamilial rank to Cydosiinae. Our analysis reaffirms their findings and suggests four additional genera may be metoponiines: *Metaponpneumata, Azenia, Sexserrata,* and *Tristyla. Metaponpneumata* is shown with strong support to be sister to *Cydosia.*

That *Cydosia* would share a recent common ancestor with *Metaponpneumata*, a small, gray, nondescript denizen of North American deserts would not have been predicted (Figs 2A-D). Regier e*t al.* (2017) were similarly perplexed as to the position of Metoponiinae and relationships in this part of the noctuid phylogeny. When we remove *Metaponpneumata* from our analysis we recover the same topology with respect to *Flammona, Panemeria*, and *Cydosia* (results not shown) as Zahiri *et al*. (2013) and Rota *et al*., (2016). Interestingly, both *Cydosia* and *Metaponpneumata* are dietary generalists; so far as known the other Metoponiinae and Grotellinae are known or believed to be hostplant specialists (DLW unpublished data). Given the surprising relationships in this part of the tree, but sparse taxon sampling, we refrain from delineating subfamilies for now, and place these former amphipyrine genera into *incertae sedis* (See Table 1).

<u>Grotellinae</u>

The clade including *Grotella*, *Neogrotella*, and *Grotellaforma* (Figs 1A,C), is non-problematic; it is well supported by molecular, adult, larval, and life history data. Given its sister-group relationship to the clade containing Metoponiinae + Cydosiinae and relative age (branch depth)

we recognize this group as a subfamily, the Grotellinae. The Grotellinae are endemic to North American deserts and contain approximately 23 described species (Poole, 1989). So far as known, all species are dietary specialists of Nyctaginaceae; while several feed on leaves, especially in early instars, most are flower and seed predators, with phenologies closely tied to that of a single local host.

Noctuinae and Bryophilinae

*Acopa* and *Nacopa* are transferred to Bryophilinae and Noctuinae, respectively. Male genitalic characters support the new assignments of both genera. The male genitalia of *Acopa* would not immediately be recognized as belonging to the Bryophilinae because the valve is short, 2× as long as the sacculus, and heavily sclerotized, whereas in most Bryophilinae the valve is long, usually 3× as long as the valves and is weakly sclerotized. Two features of the valve are similar to those found in the Bryophilinae: the uncus is flattened and slightly spatulate apically and the clasper appears to arise from the costal margin of the valve, a feature common to many *Bryophila* in Eurasia. We have not sequenced any specimens of "*Cryphia"* from North America, so the relationship of *Acopa* to the other New World representatives of the subfamily remains unclear, but some species (e.g., "*Cryphia" olivacea*) have a minute rounded clasper at the same position on the costa and same orientation as *Acopa*. As for *Nacopa*, it is placed sister to the rest of the Noctuinae included in our analysis, and as such the genus may provide evolutionary insight into early aspects of the radiation of Noctuinae—one that produced one of the most ecologically successful and economically important clades of Lepidoptera (Zhang 1994). *Nacopa* has unusual valves for the subfamily Noctuinae in that the sacculus is massive, occupying about three quarters of the volume of the valve, but like other Noctuinae the clasper is

high on the valve but still connected to the lower margin by the thin sclerotized band discussed by Lafontaine & Poole (1991: 21). Larvae are unknown for either genus.

Condicinae

The type species *Aleptina inca*, is strongly supported as sister to *Hemicephalis alesa* (Figs 1 A,B). Early North American workers placed *Aleptina* in the Acontiinae (McDunnough, 1938; Franclemont & Todd, 1983; Todd *et al*., 1984). The genus was transferred without explanation to Triocnemidina (Amphipyrinae) by Lafontaine & Schmidt (2010). Three years later, the genus was associated with the Condicinae by Zahiri *et al*., (2013). The larvae, recently revealed to be specialists on various species of *Tiquilia* (Boraginaceae) (DLW unpublished data), are consistent with those of other condicines, but have only two SV setae on abdominal segment one, like most higher Noctuidae, and unlike other genera of Condicinae. *Aleptina* larvae resemble miniature versions of the condicine *Diastema*: the head is partially retracted into the prothorax, the prolegs on both A3 and A4 are modestly reduced, A8 is humped, and the spiracular stripe (when present) runs from the spiracle on A8 down the anal proleg. Interestingly, McDunnough (1938) had placed *Aleptina* and *Diastema* proximate in his checklist – a position unchanged in Franclemont & Todd (1983) and now supported by our findings. Before the nuclear and mitochondrial DNA phylogeny of Zahiri *et al*. (2012) few would have thought *Hemicephalis* (previously held to be an erebid) would in fact belong in the Condicinae, let alone the Noctuidae.

Oncocnemidinae

Lafontaine & Schmidt (2010) placed *Leucocnemis* in the subtribe Triocnemidina. Its type, *Leucocnemis perfundis*, groups with both oncocnemidines in our study: *Sympistis atricollaris*

and *Catabenoides vitrina* (Figs 1A,C). Consistent with this placement, the larva has the first two pairs of prolegs greatly reduced; the setae are relatively long and borne from minute white warts; and the D2 setae on A8 arise from warts on a sharply angled, transverse ridge. The caterpillar's fitful, prolonged alarm response is typical for oncocnemidines, but unknown from amphipyrines. Because *L. perfundis* is the type species, we transfer *Leucocnemis* into the Oncocnemidinae; but leave open the possibility that some *Leucocnemis* may in fact be triocnemidine amphipyrines.

The polyphyly we found in *Oxycnemis* based on our molecular data is supported by life history data and larval characters. Both *Oxycnemis advena* and its California cousin, *O. fusimacula,* are *Krameria* (Krameriaceae) feeders. *Oxycnemis gracillinea,* which groups with Oncocnemidines, feeds on *Menodora* (Oleaceae) as do other Oncocnemidinae (Wagner *et al*., 2011; DLW unpublished data). The caterpillar of O. *gracillinea* differs from those *O. advena* in having no obvious rump over A8, smaller head spots over each lobe, and less conspicuous dorsal pinacula; its dorsal pinstripping and reduced prolegs on A3 and A4 are common to oncocnemidines.

<u>*Cropia* Walker, 1858</u>

*Cropia*, a moderately diverse Neotropical genus, has long been recognized as an anomalous member of the Amphipyrinae. We were only able to include *C. connecta* in our analysis, a species with genitalia substantially different from those of the type species, *Cropia hadenoides*, and we are warned by Dan Janzen (pers. comm.) that the larva of *C. hadenoides* differs markedly from other species of the genus. Interestingly, *C. connecta* grouped outside Amphipyrinae and neighboring subfamilies. The male genitalia of *Cropia connecta* are somewhat reflective of our molecular findings in that they are odd for Noctuidae: they are relatively large, weakly

sclerotized, and set with a curious abundance of soft, piliform setae. However, the male genitalia of *C. hadenoides* differ markedly from those of *C. connecta*. Given the possibility that *Cropia* represent two distinct lineages, we are collecting additional sequence data of its type species (*C. hadenoides*) and other Old World genera, to which it may be related.

As noted above, the seven genes we chose provided considerable data for resolving relationships within virtually every subfamily-level taxon in the study, but frustratingly only modest or ambiguous character support for the phylogenetic relationships among the various subfamilies, and thus support the suggestions of others that the early radiation of the Noctuidae was a rapid one (Wahlberg *et al.,* 2013, Zahiri *et al.,* 2013).

## CONCLUSION AND FUTURE DIRECTIONS

It would seem that no two major taxonomic works have agreed on the bounds of the Amphipyrinae. Its realm has waxed and waned for more than a century. More expansive concepts have spanned the subfamilies that were the focus of this effort (e.g., Nielsen, 1997) and waned to efforts that restricted its content to little more than the nominate genus (e.g., Kitching & Rawlins, 1998). In most checklists and faunal works Amphipyrinae served as a repository for noctuids that lacked the synapomorphies of acontiines, acronictines, bagisarines, cuculliines, oncocnemidines, plusiines, and others. Our contribution is a step forward and provides phylogenetic scaffolding around which future taxonomic efforts can be anchored. Our effort is deficient or at least decidedly biased in its geographic coverage, i.e., in its emphasis of generic coverage of Nearctic taxa, where Amphipyrinae are known to be especially diverse. Future

efforts will need to add genera from other biogeographic regions, especially in the southern hemisphere and East Asia.

Much remains to be done with the fauna of North America. Central and northern Mexico could prove to be the cradle for much the New World diversity of Amphipyrinae, Grotellinae, Metoponiinae, and Stiriinae. We have yet to sample the type species for more than a dozen genera included in the Amphipyrinae by Lafontaine & Schmidt (2010), and it is not improbable that other amphipyrines, unrecognized as such, still reside within other subfamilies. In addition to amphipyrines, our study revealed that the monophyly of proximate subfamilies are also in need of closer scrutiny: e.g., Metoponiinae, Oncocnemidinae, and Stiriinae.

So much as possible, in this assessment, we emphasized type species because it was clear at the outset that several amphipyrine s.l. genera were polyphyletic, such as *Oxycnemis* and *Leucocnemis*. Other genera that appear to our eyes to be unnatural assemblages include *Aleptina*, *Azenia*, *Nocloa, Paratrach*ea*,* and *Plagiomimicus*.

It is our hope that the relationships hypothesized in this work will facilitate efforts to identify morphological and life history data that can be used to corroborate or refute the relationships revealed in this study. Given the weak branch support for some clusters, larval, anatomical, behavioral, and life history details could do much to test our findings. Even in those cases where branch support is strong, such information is needed to add biological meaning to the inferred clades and their taxonomic concepts.

**ACKNOWLEDGEMENTS**

Many of the taxa in this study are desert dwellers, tied to the stochasticity of desert rains, and as such are immensely difficult for one person to collect in a single year or even lifetime. The authors owe an immense debt to those who provided “amphipyrine” taxa. Three were especially generous with their specimens, time, and unpublished data: John Palting, Evan Rand, and David Wikle. The latter made a special effort to fill taxonomic gaps in our preliminary analyses. Additional specimens used here were supplied by Robert Behrstock, John DeBenedictis, Cliff Ferris, Ann Hendrickson, Sam Jaffe, Ed Knudson, and Eric Metzler – without their help this effort would have been greatly diminished. Our sequence data were interwoven into a pre-existing latticework built by Reza Zahiri and co-workers. While not used directly in this effort, many provisional species identifications were molecularly confirmed using sequences and software in BOLD v4 (http://www.boldsystems.org/). Marek Borowiec assisted with initial sequence alignment and an initial phylogenetic analysis. KLK is supported by grants from the Society of Systematic Biologists (Graduate Student Research Award), the Department of Ecology and Evolutionary Biology at the University of Connecticut (Zoology Award), as well as many generous donors that contributed to an Instrumentl [sic] crowd-funding grant. Egbert Friedrich provided superb photographs for some of the European exemplar species in Figs 1A-C, and Kennedy Marshall assisted with figure design. Jim Dice, Gina Moran, and the California Department of Food and Agriculture assisted with collecting permits for Anza-Borrego Desert State Park in 2016 and 2017. The Steele-Burnand Desert Research Institute hosted stays in both years. We also thank Jonena Hearst and Raymond Simpson for assisting with permitting and logistics for Big Bend and Guadalupe Mountains National Parks. NW acknowledges support from the Swedish Research Council. DLW is supported by USFS Co-op Agreement 14-CA-

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**Table 1.** Recommended taxonomic changes for taxa formerly regard to be Amphipyrines according to Lafontaine & Schmidt (2010, 2015).

| Taxon | Current Membership | Recommended Change |
|---|---|---|
| *Acopa* Harvey, 1875 | Amphipyrinae | Bryophilinae |
| *Aleptina* Dyar, 1902 | Amphipyrinae | Condicinae |
| *Cropia* Walker, 1858 | Amphipyrinae | Noctuidae incertae sedis |
| *Hemigrotella* Barnes & McDunnough, 1918 | Amphipyrinae (Stiriini) | Amphipyrinae (Psaphidini) |
| *Leucocnemis* Hampson, 1908 | Amphipyrinae | Oncocnemidinae |
| *Metaponpneumata* Möschler, 1890 | Amphipyrinae | Noctuidae *incertae sedis* |
| *Nacopa* Barnes & McDunnough, 1924 | Amphipyrinae | Noctuinae |
| *Sexserrata* Barnes & Benjamin, 1922 | Amphipyrinae | Noctuidae *incertae sedis* |
| *Tristyla* Smith, 1893 | Amphipyrinae | Noctuidae *incertae sedis* |
| *Azenia,* Grote, 1882 | Amphipyrinae | Noctuidae *incertae sedis* |
| *Chamaeclea* Grote, 1883 | Amphipyrinae | Acontiinae (Chamaecleini) |
| *Heminocloa* Barnes & Benjamin, 1924 | Amphipyrinae | Acontiinae (Chamaecleini) |
| *Hemioslaria* Barnes & Benjamin, 1924 | Amphipyrinae | Acontiinae (Chamaecleini) |
| *Thurberiphaga* Dyar, 1920 | Amphipyrinae | Acontiinae (Chamaecleini) |
| Stiriina Grote, 1882 | Amphipyrinae (Stiriini) | Stiriinae |
| Grotellina Poole, 1995 | Amphipyrinae (Stiriini) | Grotellinae |

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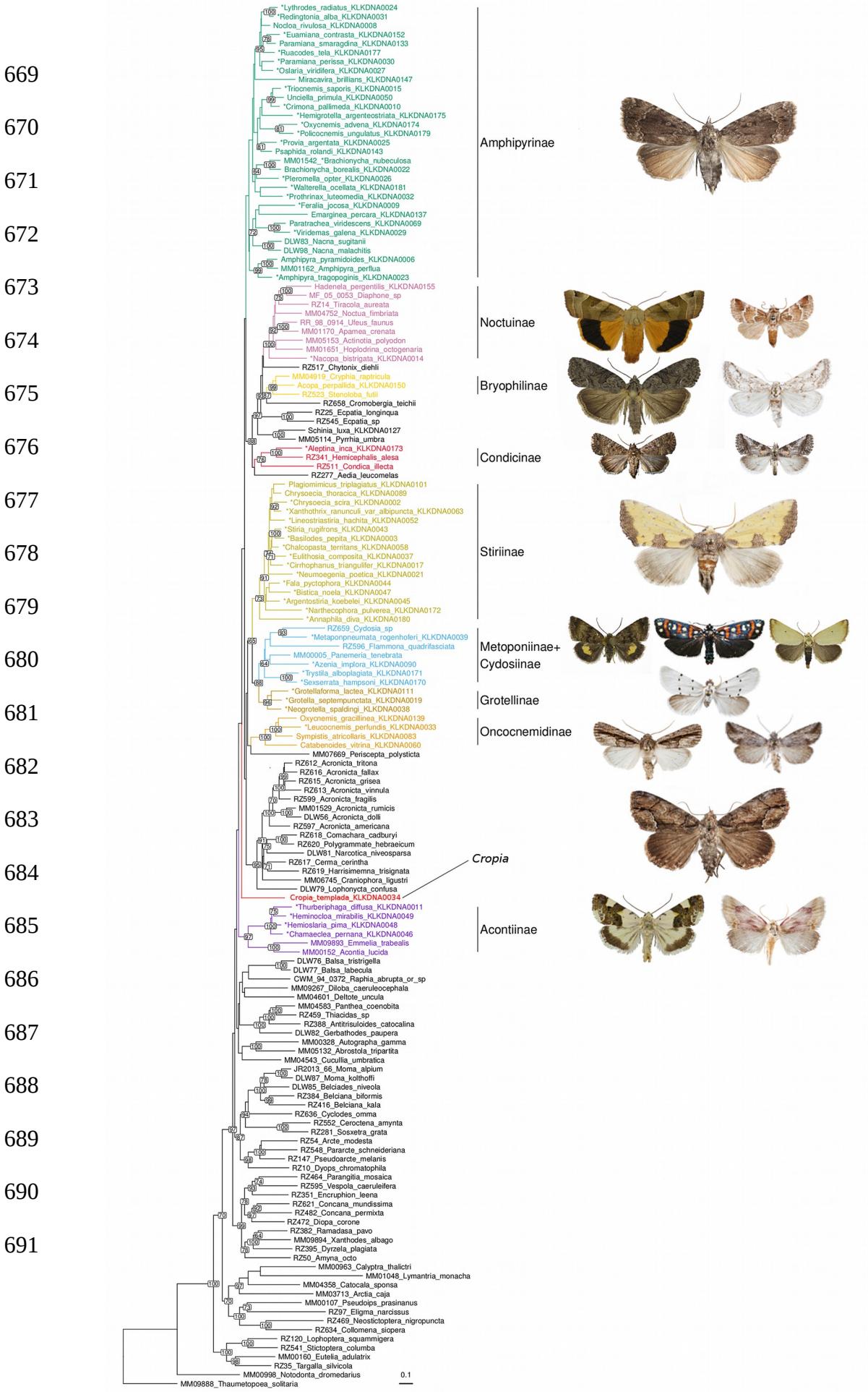
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Noctua_rivulosa_KLKDNA0008
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Paramiana_smaragdina_KLKDNA0133
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*Paramiana_perissa_KLKDNA0030
*Oslaria_viridifera_KLKDNA0027
Miracavira_brillians_KLKDNA0147
*Triocnemis_saporis_KLKDNA0015
Unciella_primula_KLKDNA0050
*Crimona_pallimeda_KLKDNA0010
*Hemigrotella_argenteostriata_KLKDNA0175
*Oxycnemis_advena_KLKDNA0174
*Policocnemis_ungulatus_KLKDNA0179
*Provia_argentata_KLKDNA0025
Psaphida_rolandi_KLKDNA0143
MM01542_*Brachionycha_nubeculosa
Brachionycha_borealis_KLKDNA0022
*Pleromella_opter_KLKDNA0026
*Walterella_ocellata_KLKDNA0181
*Prothrinax_luteomedia_KLKDNA0032
*Feralia_jocosa_KLKDNA0009
Emarginea_percara_KLKDNA0137
Paratrachea_viridescens_KLKDNA0069
*Viridemas_galena_KLKDNA0029
DLW83_Nacna_sugitanii
DLW98_Nacna_malachitis
Amphipyra_pyramidoides_KLKDNA0006
MM01162_Amphipyra_perflua
*Amphipyra_tragopoginis_KLKDNA0023
Amphipyrinae
Hadenela_pergentilis_KLKDNA0155
MF_05_0053_Diaphone_sp
RZ14_Tiracola_aureata
MM04752_Noctua_fimbriata
RR_98_0914_Ufeus_faunus
MM01170_Apamea_crenata
MM05153_Actinotia_polyodon
MM01651_Hoplodrina_octogenaria
*Nacopa_bistrigata_KLKDNA0014
Noctuinae
RZ517_Chytonix_diehli
MM04919_Cryphia_raptricula
Acopa_perpallida_KLKDNA0150
RZ523_Stenoloba_futili
RZ658_Cromobergia_teichii
RZ25_Ecpatia_longinqua
RZ545_Ecpatia_sp
Schinia_luxa_KLKDNA0127
MM05114_Pyrrhia_umbra
Bryophilinae
*Aleptina_inca_KLKDNA0173
RZ341_Hemicephalis_alesa
RZ511_Condica_illecta
RZ277_Aedia_leucomelas
Condicinae
Plagiomimicus_triplagiatus_KLKDNA0101
Chrysoecia_thoracica_KLKDNA0089
*Chrysoecia_scira_KLKDNA0002
*Xanthothrix_ranunculi_var_albipuncta_KLKDNA0063
*Lineostriastiria_hachita_KLKDNA0052
*Stiria_rugifrons_KLKDNA0043
*Basilodes_pepita_KLKDNA0003
*Chalcopasta_territans_KLKDNA0058
*Eulithosia_composita_KLKDNA0037
*Cirrhophanus_triangulifer_KLKDNA0017
*Neumoegenia_poetica_KLKDNA0021
*Fala_pyctophora_KLKDNA0044
*Bistica_noela_KLKDNA0047
*Argentostiria_koebelei_KLKDNA0045
*Narthecophora_pulverea_KLKDNA0172
*Annaphila_diva_KLKDNA0180
Stiriinae
RZ659_Cydosia_sp
*Metaponpneumata_rogenhoferi_KLKDNA0039
RZ596_Flammona_quadrifasciata
MM00005_Panemeria_tenebrata
*Azenia_implora_KLKDNA0090
*Trystila_alboplagiata_KLKDNA0171
*Sexserrata_hampsoni_KLKDNA0170
Metoponiinae+
Cydosiinae
*Grotellaforma_lactea_KLKDNA0111
*Grotella_septempunctata_KLKDNA0019
*Neogrotella_spaldingi_KLKDNA0038
Grotellinae
Oxycnemis_gracillinea_KLKDNA0139
*Leucocnemis_perfundis_KLKDNA0033
Sympistis_atricollaris_KLKDNA0083
Catabenoides_vitrina_KLKDNA0060
MM07669_Periscepta_polysticta
Oncocnemidinae
RZ612_Acronicta_tritona
RZ616_Acronicta_fallax
RZ615_Acronicta_grisea
RZ613_Acronicta_vinnula
RZ599_Acronicta_fragilis
MM01529_Acronicta_rumicis
DLW56_Acronicta_dolli
RZ597_Acronicta_americana
RZ618_Comachara_cadburyi
RZ620_Polygrammate_hebraeicum
DLW81_Narcotica_niveosparsa
RZ617_Cerma_cerintha
RZ619_Harrisimemna_trisignata
MM06745_Craniophora_ligustri
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Cropia
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*Hemioslaria_pima_KLKDNA0048
*Chamaeclea_pernana_KLKDNA0046
MM09893_Emmelia_trabealis
MM00152_Acontia_lucida
Acontiinae
DLW76_Balsa_tristrigella
DLW77_Balsa_labecula
CWM_94_0372_Raphia_abrupta_or_sp
MM09267_Diloba_caeruleocephala
MM04601_Deltote_uncula
MM04583_Panthea_coenobita
RZ459_Thiacidas_sp
RZ388_Antitrisuloides_catocalina
DLW82_Gerbathodes_paupera
MM00328_Autographa_gamma
MM05132_Abrostola_tripartita
MM04543_Cucullia_umbratica
JR2013_66_Moma_alpium
DLW87_Moma_kolthoffi
DLW85_Belciades_niveola
RZ384_Belciana_biformis
RZ416_Belciana_kala
RZ636_Cyclodes_omma
RZ552_Ceroctena_amynta
RZ281_Sosxetra_grata
RZ54_Arcte_modesta
RZ548_Pararcte_schneideriana
RZ147_Pseudoarcte_melanis
RZ10_Dyops_chromatophila
RZ464_Parangitia_mosaica
RZ595_Vespola_caeruleifera
RZ351_Encruphion_leena
RZ621_Concana_mundissima
RZ482_Concana_permixta
RZ472_Diopa_corone
RZ382_Ramadasa_pavo
MM09894_Xanthodes_albago
RZ395_Dyrzela_plagiata
RZ50_Amyna_octo
MM00963_Calyptra_thalictri
MM01048_Lymantria_monacha
MM04358_Catocala_sponsa
MM03713_Arctia_caja
MM00107_Pseudoips_prasinanus
RZ97_Eligma_narcissus
RZ469_Neosticloptera_nigropuncta
RZ634_Collomena_siopera
RZ120_Lophoptera_squammigera
RZ541_Sticloptera_columba
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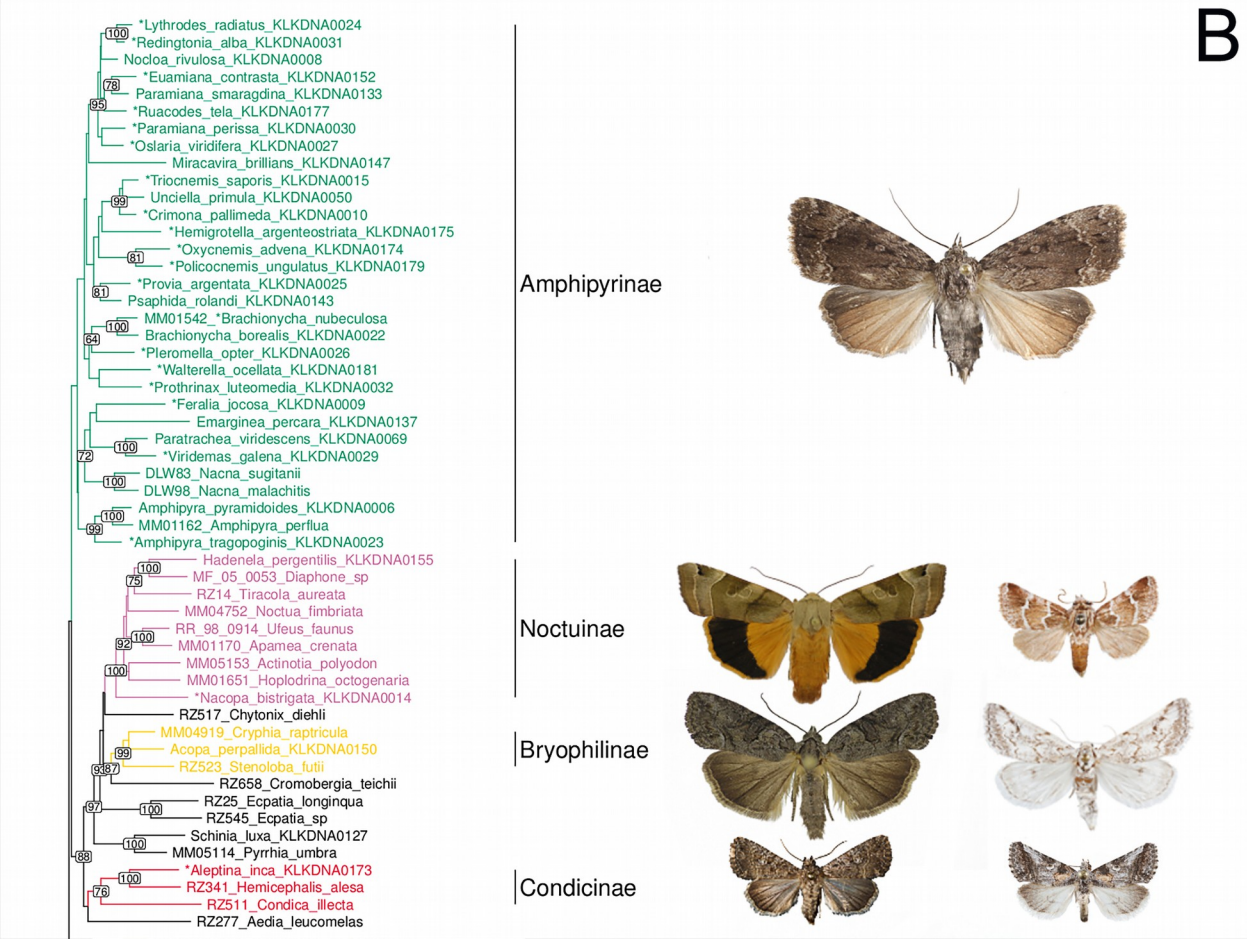
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Condicinae

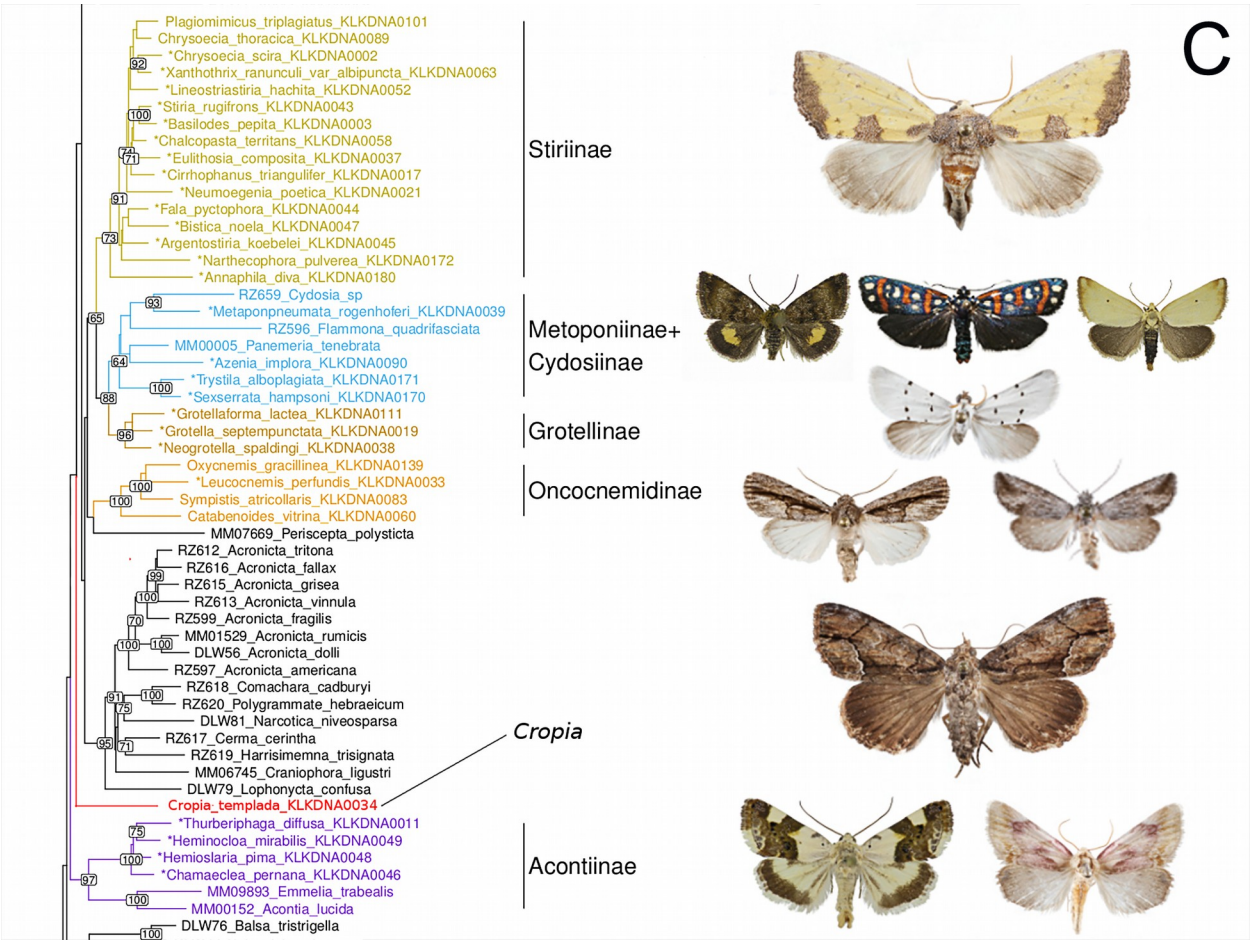


**Fig 1.** Results of seven-gene RAxML phylogenetic inference. (A) Full tree. (B) Amphipyrinae-Condicinae focus. (C) Stiriinae-Acontiinae focus. Clades containing at least one of the 57 amphipyrine genera included in this analysis are shown in color and labeled. Amphipyrinae taxa used in this analysis that are the type of their genus are denoted with an asterisk. An exemplar species (image) from each clade used in the analysis is shown. See Results and Discussion for information on clade names. Nodal support values are bootstraps, with values of > 63% shown, indicating a moderately to strongly supported clade. Exemplar species pictured from top to bottom (and left to right): *Amphipyra tragopoginis*, *Noctua fimbriata*, *Nacopa bistrigata*, *Cryphia*

*raptricula*, *Acopa perpallida*, *Condica concisa, Aleptina inca*, *Stiria rugifrons*, *Panemeria tenebrata*, *Cydosia curvinella*, *Aegle kaekeritziana*, *Grotella septempunctata*, *Sympistis atricollaris*, *Oxycnemis gracillinea*, *Cropia connecta*, *Acontia lucida*, *Chamaeclea pernana*. Scale bar shows substitutions per site.

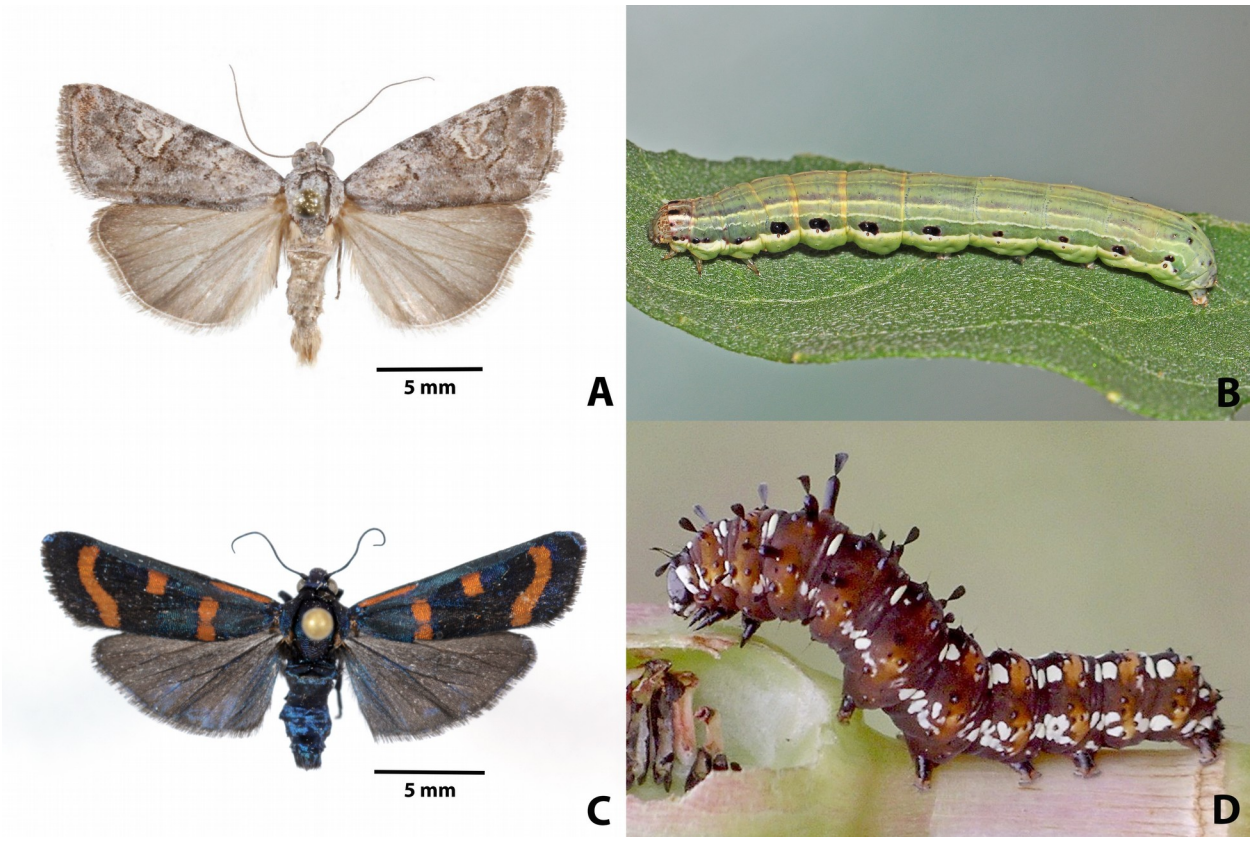


**Fig 2.** Comparison of adults and larvae of *Cydosia* and *Metaponpneumata*. (A) *Metaponpneumata rogenhoferi* adult. (B) *M. rogenhoferi* last instar. (C) *Cydosia aurivitta* adult. (D) *C. aurivitta* last instar. (*Cydosia* images courtesy of Berry Nall.)