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Molecular phylogeny of the Lichen Tiger Moths (Lepidoptera: Erebidae: Arctiinae: Lithosiini) of the Western Hemisphere

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Abstract

Few molecular-based studies have tested the monophyly of the Lithosiini subtribes proposed by Bendib and Minet (2000), and those have been limited by meager taxon sampling. Several studies have suggested some subtribes are not monophyletic as they are currently defined. We conducted a molecular phylogenetic study of representatives of the North American lithosiine fauna which are currently classified within the subtribes Acsalina, Lithosiina, Cisthenina and Eudesmiina, the latter having never been included in a molecular-based analysis before. Based on analyses of cytochrome oxidase subunit I (COI), ribosomal protein S5 (RPS5) and the large subunit 28S ribosomal DNA (28S), we assign some of the North American genera to subtribe for the first time and re-assign others contrary to previous placements. Then, we discuss the morphological characters that Bendib and Minet (2000) proposed to define subtribes and re-consider them in the context of our inferred phylogeny.

We report high support for a monophyletic Lithosiina+ *Agylla* + *Inopsis* + *Gnamptonychia*, three genera being unplaced or of uncertain placement (*Agylla*) by Bendib and Minet (2000). We remove *Gardinia* from the subtribe Lithosiina and place it in the Cisthenina, along with *Eudesmia*, formerly placed in its own subtribe, the Eudesmiina. Two other genera, *Bruceia* and *Ptychoglène*, not previously assigned to a subtribe are found to be members of the Cisthenina. We remove *Clemensia* from the Cisthenina and report it, along with the neotropical *Pronola*, as part of undefined clade. After these changes, our phylogeny shows strong support for the monophyly of Cisthenina + *Gardinia* + *Eudesmia* + *Bruceia* + *Ptychoglène*. We find *Acsala anomala* occurs on a long branch by itself, confirming the uniqueness of this species and its placement in a monotypic subtribe.

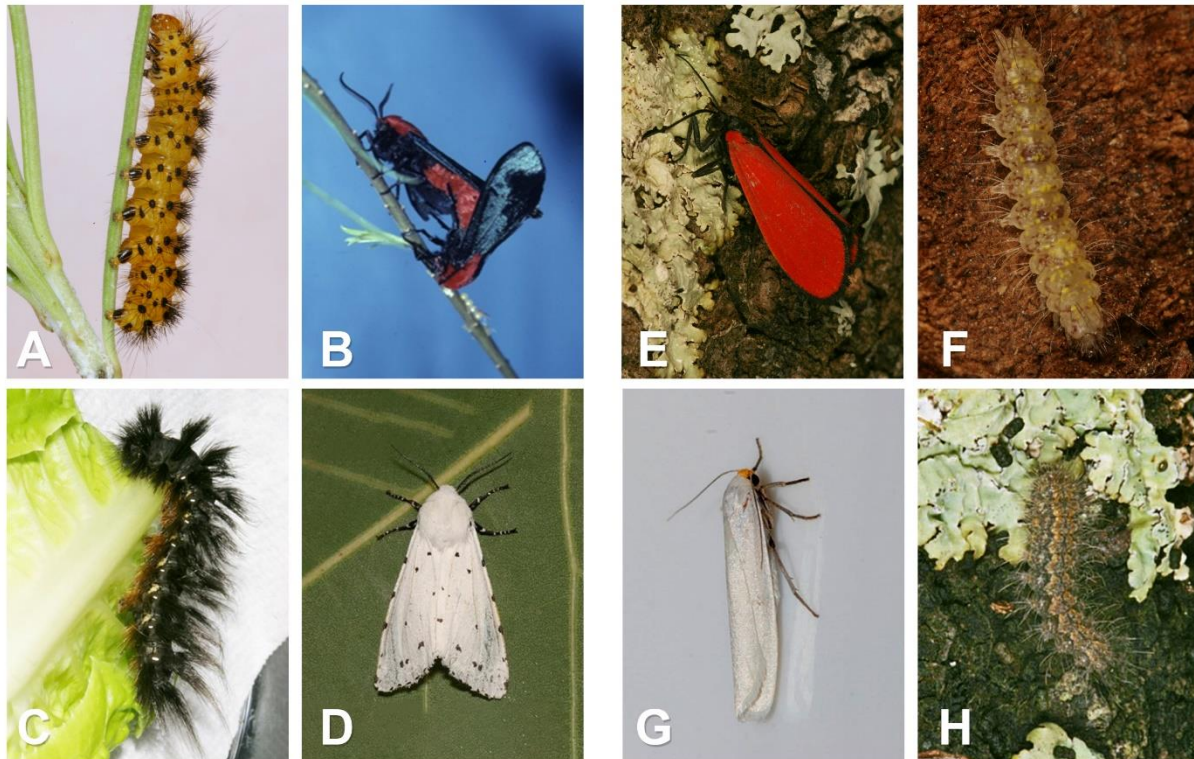
Finally, we confirm that *Afrida exegens*, sometimes considered a member of the Lithosiini, is not even an erebid, but rather it is a member of the Nolidae, as proposed by Holloway (1998) and Kitching and Rawlins (1998).

Keywords: Arctiinae, Lithosiini, Lichen Tiger Moths, Cisthenina, Lithosiina, Eudesmiina, Acsalina, Nolidae, Afridinae

Introduction:

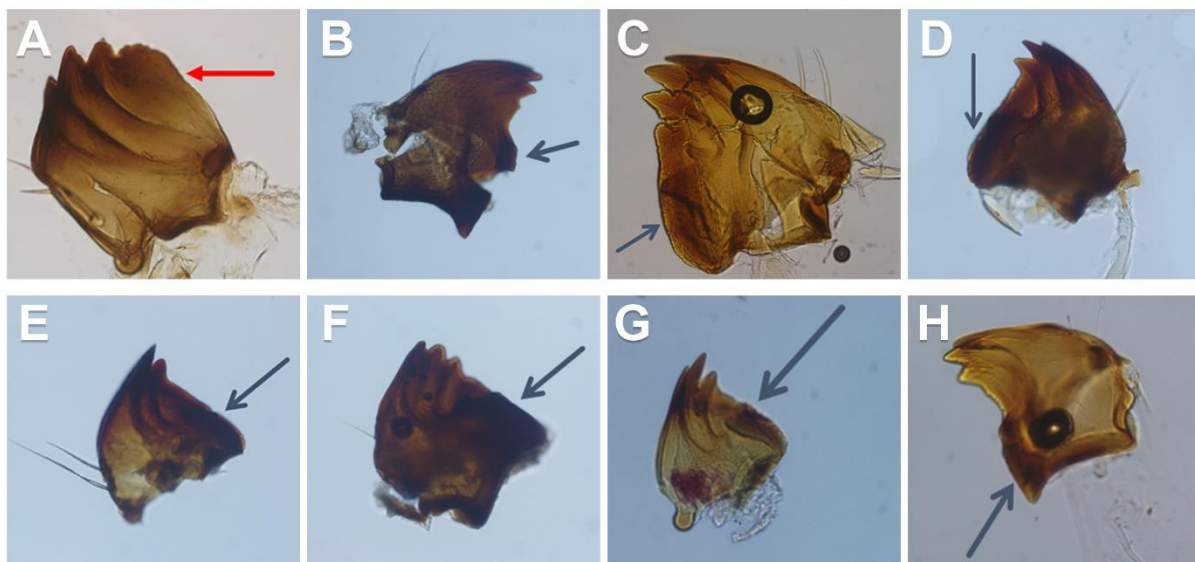
While a vast majority of caterpillars are phytophagous, the larvae of the tribe Lithosiini, commonly known as the Lichen Tiger Moths, feed on lichens, a tough and often toxic mutualism of algae, fungus and cyanobacterium. The ability to utilize these peculiar, but widely available organisms as food is likely behind the successful radiation of lithosiines across the globe. The tribe Lithosiini currently consists of some 3600 described species of small to medium, often brightly colored moths of nearly worldwide distribution. Not only do lichens offer a food source generally free from competition, they possess a chemical arsenal that can be exploited. It has been demonstrated by many researchers that the larvae of lithosiines possess metabolic pathways to deal with lichen toxins and actually sequester modifications of these for their own protection in all life stages (Hesbacher et al. 1995; Wagner et al. 2008; Conner 2009; Scott et al. 2014; Anderson et al. 2017; Scott-Chailvo et al. 2018). Adults of most lithosiines exhibit aposematic coloration, however the larvae are dull-colored, secretive nocturnal feeders (Wagner 2005, Conner 2009). Hiding by day and feeding at night likely is an adaptation to avoid bird predation, as lichens offer little in the way of protective cover from visual predators. Because they are nocturnally active, lithosiine larvae are seldom encountered and poorly known, in contrast to the often conspicuous feeding of their well-known woolly bear relatives, the Arctiini (**Fig. 1**).

Figure 1



While no adult apomorphy uniting lithosiines has been identified to date, the larval stages provide two: a presence of a mandibular molar and the chaetotaxy of the labrum, specifically the unique arrangement of the labral setae M1 and M2 (Bendib and Minet 2000). We confirm the presence of both these features across the larvae reared as part of this study, many of which were previously unknown. The modified mandibular molars of some of these are shown in **Fig. 2**. This peculiar modification of the mandible, presumably for grinding the tough lichen thallus for digestion, was noted rather early on. The apical ends of the forth and sometimes the third scissorial teeth of the mandible have been modified from the blade-like structure seen in arctiines and noctuids (Gilligan and Passoa 1985) to a flattened grinding structure in lithosiines (Gardner, 1943; Issiki et al., 1965; Garcia-Barros, 1985; McCabe, 1981; Lafontaine et. al., 1982; Rawlins, 1984; Habeck, 1987, Bendib and Minet 2000).

Figure 2



Despite being one of the larger radiations among the ditrysian Lepidoptera, few attempts had been made to define relationships among the numerous genera of lithosiines until Bendib and Minet (2000) proposed seven lineages and placed many of the genera into them. Originally considered part of the family Arctiidae, changes in taxon ranks and names were required when this family was subordinated under the Erebidae (Zahiri et al. 2012). The original Bendib and Minet classification was published prior to this change, thus their proposed tribes are now subtribes of the tribe Lithosiini. Of these proposed seven subtribes, only four that contain New World species are considered in the present study; the Lithosiina, Cisthenina, Eudesmiina and Acsalina. The other subtribes are the Phryganopterygiina (Madagascar), Nudariina (Old World) and Eudrosiina (Palearctic).

While these putative clades are not well defined by morphological apomorphies, Bendib and Minet (2000) note that there is “obvious morphological diversity in the imagines,” including size, color and resting posture that help support their subtribal classifications. The structure of the second abdominal sternite in the adults (Fig. 2) is described in detail and proposed as one possible apomorphy, the Cistheniina and Eudesmiina possessing the long, forked apodemes, while the Lithosiina (and arctiines) have shortened apodemes.

Among the North American fauna, adults of the Lithosiina are often on the larger side with elongate wings and a distinct adult resting posture, with the forewing covering most of the distal half of the other forewing. Most assume an almost flat profile when resting, while some genera like *Crambidia* Packard, 1864, rest in a cigar-like fashion, with the wings curved around the body. Adults of the Lithosiina are usually more somberly colored than many of the other tribes, with many members that are mostly white, grey or black. Bendib and Minet intended to publish a second paper focusing just on the Lithosiina, stating the subtribe is “extremely comprehensive and deserves, in our opinion, to be split into several precisely defined (sub)tribes.” Larvae of the Lithosiina have prominent verrucae, with an unusual arrangement of meso and metathoracical verrucae D and SD2. Genera assigned to this subtribe that occur in the US are *Crambidia*, with eleven species and *Eilema* Hubner, 1819, with one species. *Agylla* Walker, 1854 was initially assigned to this subtribe by Bendib and Minet (1998), but later they reconsidered and gave it uncertain status (Bendib and Minet 2000).

The Cisthenina are generally small moths and are often black banded with contrasting colors such as red or orange. The adults tend to hold their wings “roof-like” over their back without the wings overlapping. A suggested apomorphy is the lack of verrucae in the larvae (Bendib and Minet 2000), with the setae generally being short and sparse. US genera included by Bendib and Minet (2000) are the type genus *Cisthene* Walker, 1854 (with 20 U.S. sp.), *Hypoprepia* Hubner, 1831 (5 U.S. sp.), *Lycomorpha* Harris, 1839 (5 U.S. sp.), *Lycomorphodes* Hampson, 1900 (1 U.S. sp.) and *Rhabdatomis* Dyar, 1907 (1 U.S. sp.). The distinctive genus *Clemensia* Packard, 1844 (3 U.S. sp.) was also tentatively placed under the Cistheniina, although the authors found them different enough from other cisthenines that they suggested they be placed in a separate subtribe, the Clemensiiti. They listed three apomorphies for this designation: presence of a pair of metascutal membranous areas, sternum A2 with curved, moveable anterolateral processes and the abdomen of the female having corethrogynae.

The subtribe Eudesmiina are brightly colored like the Cisthenina, often with alternating black and orange bands, but they are larger and their wing surface area a bit broader. Like Cisthenina, the wings are held over their back like a tent, without overlapping. The larval stages, known at least for the genus *Eudesmia* Hubner, 1823, are rather shortened like larvae of Megalopygidae, with verrucae bearing tufts of long, soft setae. Two species in this subtribe, both of the genus *Eudesmia*, occur in the U.S.

The aptly named *Acsala anomala* Benjamin, 1935 is the only known member of the subtribe Acsalina. The life history of this enigmatic arctic lithosiine has been described by Lafontaine et al. (1982). Originally considered a lymantriid (tussock moth), it was later recognized as a lithosiine from the larvae, which feed on various lichens, taking several years to mature with the cold temperatures and short daylength of the high arctic. Bendib and Minet (2000) placed this species in its own subtribe based on many peculiar autapomorphies, including the hindwing venation, the translucent scales on the wings and flightless females.

Only a few molecular-based phylogenetic studies have tested the monophyly of the subtribes proposed by Bendib and Minet (2000) and those few have been limited by meager taxon sampling (Scott et al. 2014, Scott-Chailvo et al. 2018, Zenker et al. 2016). Even so, results of these studies suggest that some subtribes do not represent monophyletic groups. Thus, the present subtribal classification within the Lithosiini is tentative and will likely continue to be refined as more research is performed. Here we add to our knowledge of lithosiine evolution by conducting a molecular phylogenetic study which include representatives of the North American lithosiine fauna, which are classified within the subtribes

Acsalina, Lithosiina, Clsthenina and Eudesmiina (representatives of the latter have never been included in a molecular-based analysis before).

Materials and Methods:

Taxon sampling

A total of 181 lithosiine specimens were collected from across North America (or obtained from Europe as comparators) from 2010-2018, with 121 yielding useful DNA (66%). All taxa used in this study are listed in Supplementary Table 1. Sequences for additional taxa were acquired from GenBank and the Barcode of Life Database. GenBank accession numbers for all sequences used in this study are listed in Supplementary Table 1. DNA voucher specimens used for this study have all been deposited in the University of Arizona Insect Collection (accession numbers UAIC 3250-3299, 3500-3599, 3900-3914 and 4162-4182).

DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from right middle leg or the abdomen of each specimen the Qiagen® DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA), according to manufacturer suggested protocol. Total genomic DNA was stored in buffer at -80°C. All DNA extractions used in this study are listed in Supplementary Table 1.

Three genes that have been found to be phylogenetically informative in Lepidoptera were amplified: the barcoding region of the mitochondrial gene cytochrome oxidase subunit 1 (COI) using primers LCO1490 and HCO2198 (Hebert et al. 2003), nuclear marker RPS5 (Ribosomal Protein S5) and a ribosome marker 28S (nuclear large subunit rRNA 28S D2 loop), using primers and PCR protocols per Scott et al. (2014)

PCR products were cleaned, quantified, normalized and sequenced in both directions at the University of Arizona's Genomic and Technology Core Facility using a 3730 or 3730XL Applied Biosystems automatic sequencer. Chromatograms were assembled and initial base calls were made for each gene with Phred (Green and Ewing 2002) and Phrap (Green 1999) as orchestrated by Mesquite Ver. 3.6 (Maddison and Maddison 2018) and Chromaseq vers. 1.5 (Maddison and Maddison 2018). Final base calls were made in Mesquite and ambiguous bases were designated by a standard ambiguity code. Resulting sequences were deposited in GenBank (Table 1).

Alignment and phylogenetic analyses

Single gene matrices were aligned using default settings in MAFFT v. 7 as implemented in Mesquite. Aligned matrices were concatenated in Mesquite.

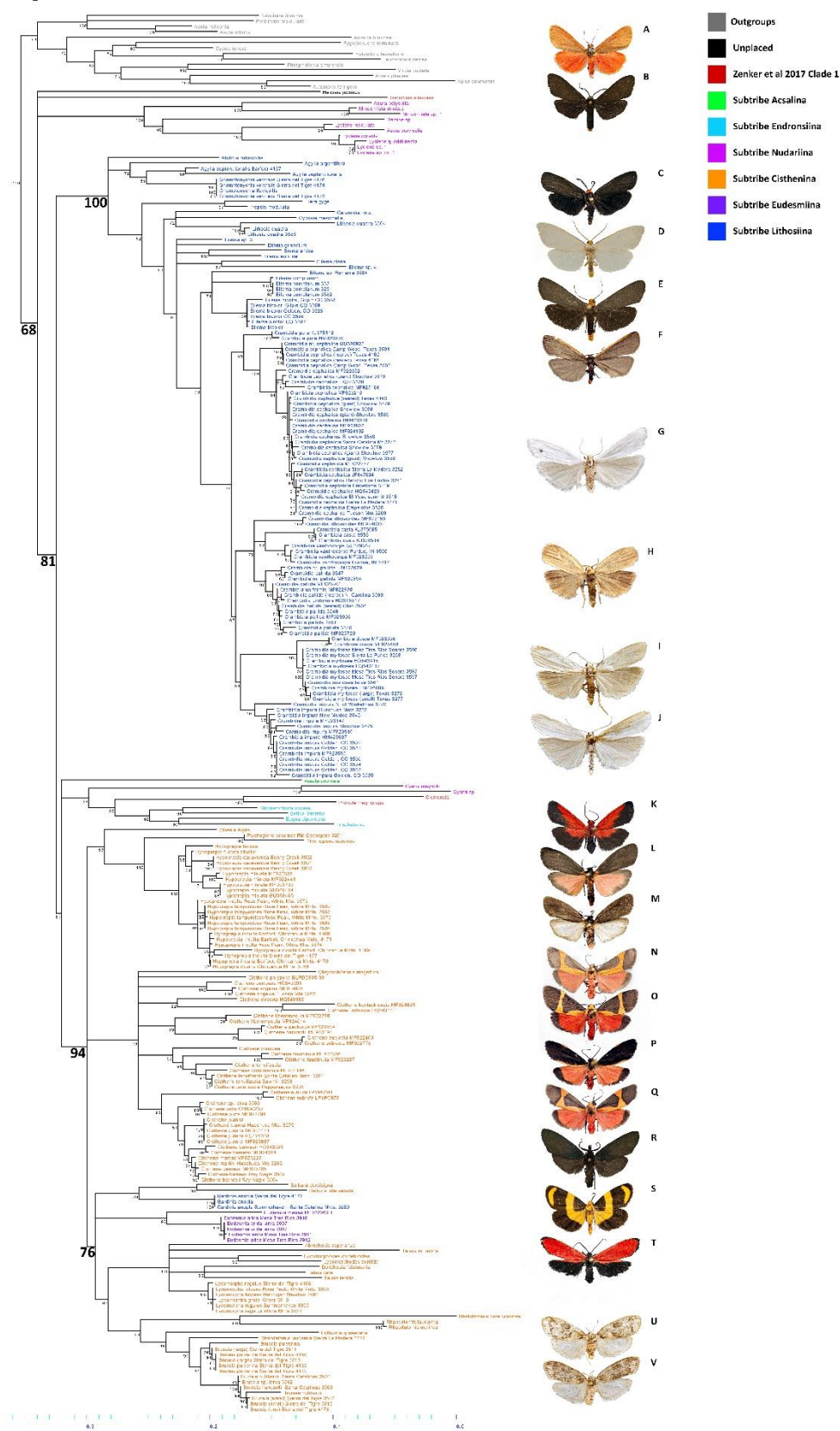
Maximum likelihood analyses were conducted on each gene individually using IQ-TREE version 1.6.10 (Nguyen et al. 2015), as orchestrated by the CIPRES Science Gateway (Miller et al 2010). The ModelFinder feature within IQ-TREE (Kalyaanamoorthy et al. 2017) was used to find the optimal character evolution models. The MFP model option was used for 28S, and the TESTMERGE option for the protein-coding genes. The TESTMERGE option sought the optimal partition of sites, beginning with the codon positions in different parts. In addition, analyses with the concatenated data were

conducted, with the TESTMERGE option also being used, beginning with each codon position for each gene as a separate part (thus, the analysis began allowing for up to 7 parts (three for each of the two protein-coding fragments, and one for 28S). One hundred searches were conducted for the maximum-likelihood tree for each matrix; for bootstrap analyses, 500 replicates were used.

Results:

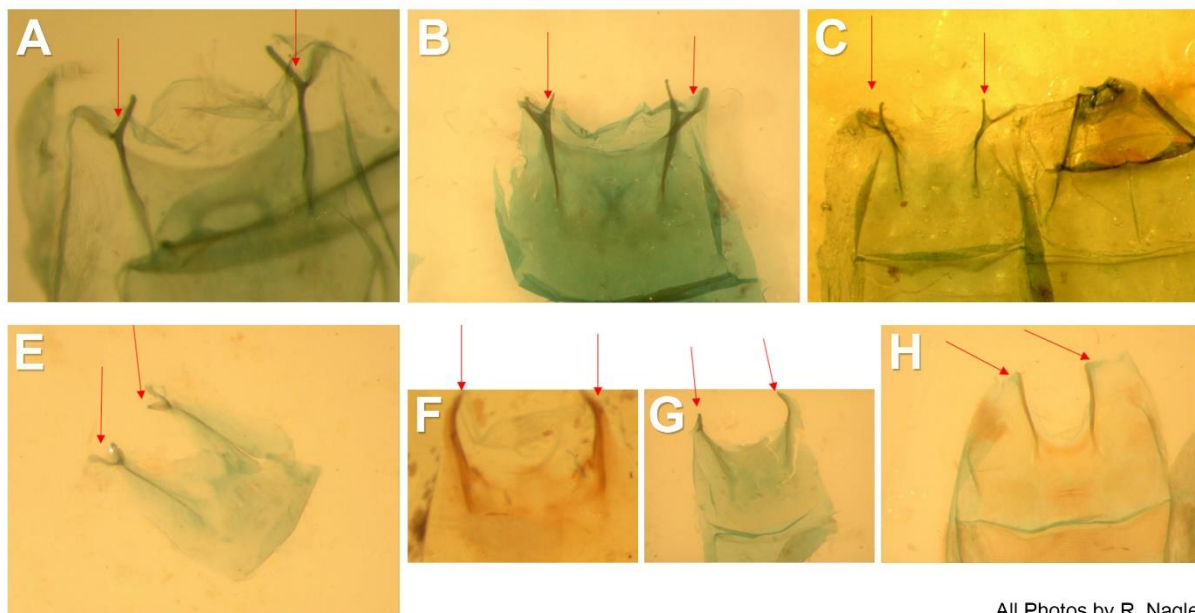
The maximum likelihood tree and bootstrap support values resulting from analysis of the concatenated dataset is in **Fig. 3**. Results of single gene analyses are presented as Supplement Fig. 1-3.

Figure 3



One of the surprises is that our study challenges the separation of the Eudesmiina, represented here by *Eudesmia arida* Skinner, 1906 and *Eudesmia menea* Drury, 1782 as separate subtribe of the lithosiines. Despite obvious differences in the larval morphology from other Cisthenina, we find strong support that *Eudesmia* is a clade of the Cisthenina and not a separate subtribe of lithosiines. This creates a problem in the definition of the Cisthenina, the proposed apomorphy being that the larval stages lack verrucae (Bendib and Minet 2000). During the course of our research we reared *Eudesmia arida* and found the larvae indeed possess verrucae (**Fig. 5B**). While many larvae of Cisthenina (*Cisthene*, *Hypoprepia*, for example) lack verrucae, the placement of *Eudesmia* in the Cisthenina would require a new larval synapomorphic definition. In addition to the presence of verrucae, the larval stages of *E. arida* possess exceedingly long, soft setae, unlike the short, stiff setae of *Cisthene*, *Hypoprepia* etc. As observed by Bendib and Minet (2000), the second abdominal sternite of the imago has long apodemes (**Fig. 4C**), a feature shared with Cisthenina (and the Old World Nudariina). The sternite of *Cisthene barnesii* Dyar, 1904 is shown for comparison (**Fig. 4A**).

Figure 4



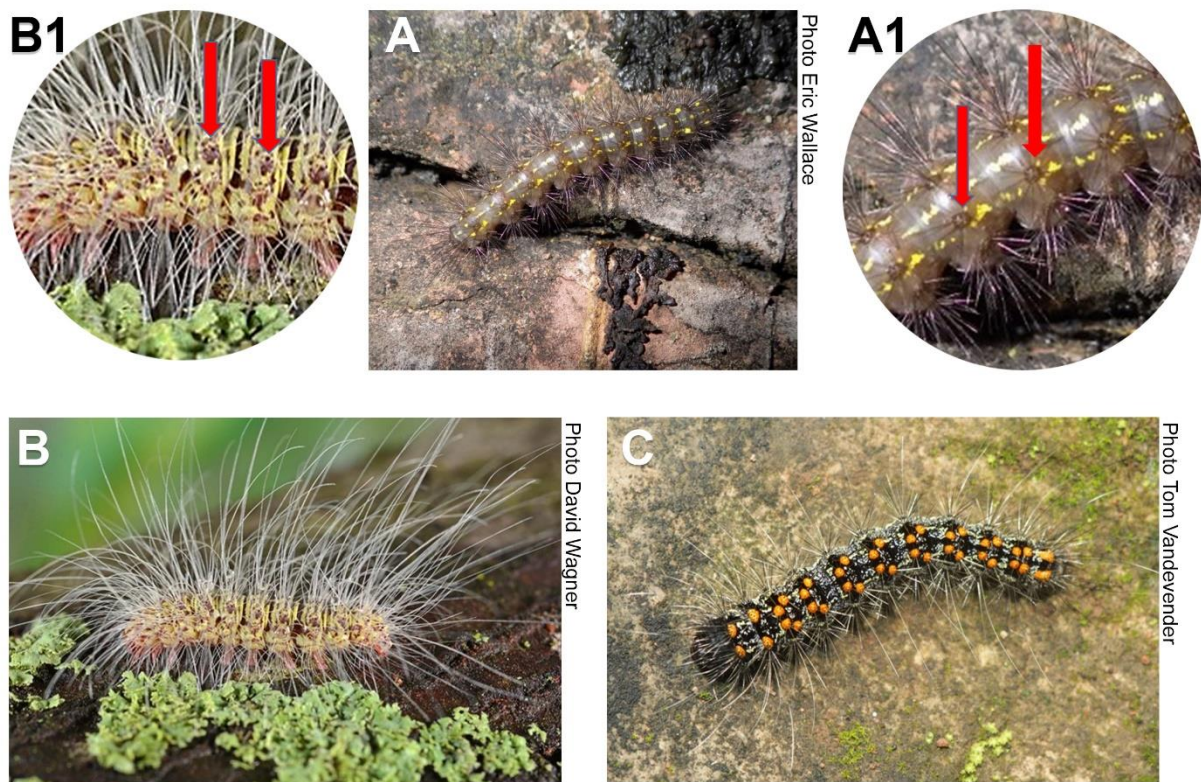
All Photos by R. Nagle

The genus *Gardinia*, represented here by a single American species found in southeastern Arizona, *Gardinia anopla* Hering, 1925, offered another surprise in our analysis. This neotropical genus was placed among the Lithosiina by both Bendib and Minet (2000) and Scott and Branham (2012) in a phylogeny based on morphological characters. While it resembles many of them in both size and color, our analysis indicates it is not a member of this subtribe. *G. anopla* is a relatively large moth (with an average wingspan of 4.5 cm), in fact, the largest lithosiine among our American fauna. Our molecular data (along with additional sequences from GenBank) place this species in the Cisthenina, a true giant among the group (most other members of which have a wingspan 2 cm or less). In Scott et al. (2014) the genus was placed among a polyphyletic Cisthenina in what they called “clade A,” which included putative members of the Cistheniina as well as Nudariina. Zenker et al. (2016) included a different

species of *Gardinia*, *G. paradoxa* Hering, 1925, in their molecular phylogeny of neotropical tiger moths, and it also placed among other Cisthenina rather than among the Lithosiina.

In support of this new placement, we report the second abdominal sternite of *Gardinia* adults (Fig. 2B) exhibits the long apodemes associated with Cisthenina, Eudesmiina and Nudariina, and not the reduced apodemes associated with Lithosiina and other arctines (Bendib and Minet 2000). In addition, the living adults of *G. anopla* hold their wings “tent-like” over their backs and not flat like most Lithosiina. Other aspects of the biology of *Gardinia* lend support to its placement among the Cisthenina. One is that the genus *Gardinia* is neotropical, with *G. anopla* being the only member making it into the US. While the subtribe Lithosiina is primarily a northern group, occurring in NA, Europe and Asia, the Cisthenina is a strictly new world with the center of diversity in the Neotropics proper. Imagos of two other new world genera (from GenBank), *Balbura* Walker 1854 and *Chrysochlorosia* Hampson, 1900, bear a resemblance to *Gardinia* and also fall within the Cisthenina. Another aspect of this placement that correlates is the use of acoustic aposematism by *Gardinia*, which is common among the Cisthenina and not known among the Lithosiina. When captured alive, *Gardinia anopla* produces surprisingly loud clicks, audible to most human ears because of its unusual size. Among the genera *Cisthene* and *Hypoprepia*, researchers have found the adult moths produce clicks in response to bat echolocation (Acharya and Fenton 1992, Conner 2009), thus one can surmise that the loud clicks of *Gardinia* are used in the same manner, to warn bats of their distastefulness. The larvae of *Gardinia* (Fig. 5A) possess verrucae bearing sprays of short, stiff setae. While the overall appearance and coloring is reminiscent of *Hypoprepia*, the presence of verrucae presents another challenge to this being a synapomorphy of Cisthenina.

Figure 5



The uniquely American genus *Bruceia* posed some challenges for Scott et al. (2014), the two members of the genus being polyphyletic in their original molecular analysis. In a later paper (Scott-Chialvo et al. 2018) they place both in a group they call Cisthenina NW (finding the Cisthenina polyphyletic), while in an earlier paper (Scott and Branham 2012) they placed it among the Cisthenina based on morphological characters. In our analysis both *B. hubbardi* and *B. pulverina* place squarely among a monophyletic Cisthenina. The overall aspect of the imagos certainly agrees with this, although the somber coloring is quite different from most other Cisthenina. The larval stage of *B. hubbardi*, reared as part of this study is rather *Cisthene*-like, lacking verrucae, although more flattened, with a distinctive lateral bulge along the thoracic and extending into the first two abdominal segments, giving it the appearance of a miniature *Tolyte* Hubner, 1820 (Lasiocampidae). While Bendib and Minet (2000) did not specifically assign *Bruceia* to a subtribe, we assign it here to the Cisthenina. Our analysis also suggests the existence of an additional undescribed Mexican *Bruceia*, much smaller than either *B. pulverina* and *B. hubbardi* occurring near the US border in the Sierra Del Tigre of Sonora, Mexico. Being a rather drab genus compared to other Cisthenina, additional undescribed taxa likely have been overlooked in Mexico.

Similarly, Bendib and Minet did not specifically assign the neotropical genus *Ptychoglène* to a subtribe. Our molecular data for *P. concinna* H. Edwards, 1886 places it in the Cisthenina, closely allied to *Hypoprepia*. This agrees with Scott and Branham (2012), Scott et al. (2014) and Scott-Chialvo et al. (2018), which included the same species. Like most of the Cisthenina, the larval stages lack verrucae. A recent rearing by R. Nagle of *P. phrada* Druce, 1889 also demonstrated that, like *Hypoprepia*, the immature stages overwinter as fully-grown larvae. This presumably gives them some advantage come spring when they pupate and emerge as adults.

Hypoprepia lampyroides (Palting and Ferguson, 2018) group together as a distinct taxa from other *Hypoprepia* in our concatenated analysis. In the published description, using C01 alone did not provide convincing evidence of this taxa being unique from the more widely distributed *H. inculta* H. Edwards, 1882, despite obvious morphological differences in the size, color, genitalia and antennal flagellomere structure (Palting et al. 2018). While widely used as the “barcode” of life, mitochondrial C01 represents only a tiny fraction of the total organismal genome. It is known among certain other arctiids, notably the genus *Grammia* Rambur, 1866, that C01 alone is of little taxonomic utility, apparently being decoupled from the rest of the genome and lacking the necessary interspecific variation among taxa that are morphologically distinct (Schmidt and Sperling 2008). Even with three genes, however, the position of one *H. inculta* specimen from the type locality of *H. lampyroides* suggests some hybridization events might be occurring where these species are sympatric.

An enigmatic lithosiine group that Bendib and Minet gave *insertae sedis* status to is the genus *Agylla*, represented in the US by *A. septentrionalis* Barnes and McDunnough, 1911. This species is known in the US only from extreme SE Arizona and only from high elevations of the Huachuca and Chiricahua Mountains. Other members of this large neotropical genus occur from Mexico to northern S. America. While Bendib and Minet stated the genus is likely part of the subtribe Lithosiina, larval characteristics (arrangement of the thoracic verrucae) were inconsistent with other members where the larvae are known. In addition, we confirm here that the apodemes of the second abdominal sternite of *A. septentrionalis* (Fig. 4D) are long, like Cisthenina, in contrast to other Lithosiina which have short apodemes. The Neotropical distribution of the genus is also notable, as already mentioned the center of diversity of the Lithosiina is northern, but the phylogeny of Zenker et al. (2016) found other neotropical members of the Lithosiina (including *Agylla*) that suggest the subtribe colonized the neotropics one or

more times in the past. The similarity between the European Lithosiina *Atolmis rubricollis* Linnaeus 1758 and the neotropical *Apistosia judas* Hubner, 1827 is striking and provides morphological evidence that corroborates this. In the case of *Agylla septentrionalis*, there is a striking resemblance to some European members of the genus *Lithosia* Fabricius 1798, such as male *L. quadra* Linnaeus, 1758. Like Zenker (2016), our molecular phylogeny places *Agylla* in the Lithosiina, which creates issue with the proposed Lithosiina apomorphies of the larvae and adult second abdominal sternite. The adults of *Agylla* are predominantly white, fairly large (for a lithosiine), with elongate wings that are held tent-like over the body, another feature they share with the Cisthenina, and not with one wing partially covering the other as in the other Lithosiina.

Two additional genera of uncertain placement that occur in the American southwest are *Inopsis* (Felder 1874) and *Gnamptonychia* Hampson, 1900. *Gnamptonychia ventralis* Barnes and Lindsey, 1921 is found in SE Arizona to New Mexico at higher elevations, while *Inopsis modulata* Edwards, 1884 is a Mexican taxa that makes occasional incursions into the mountains of SE Arizona. The two taxa look remarkably similar, but side-by-side *I. modulata* is a much smaller moth with shorter, more rounded wing shape than *G. ventralis*. Both are evidently part of a Mullerian mimicry complex that includes the arctiine *Pygotenucha terminalis* Walker, 1854, included here as an outgroup, which is similarly colored and a toxic milkweed-feeder in the larval stages. Despite it being less common, the larval stages of *I. modulata* are known, while that of *G. ventralis* is not. *Inopsis* larvae (**Fig. 5C**) are conspicuous feeders on lichens growing on tree branches and bear distinctive orange to red verrucae against a dark body. The second abdominal sternite of the imagos (**Fig. 4E and 4F**) as well as their resting posture is typical of the Lithosiina in both taxa.

Members of the North American genus *Crambidia* are unmarked and mostly white, tan or grey. Because of this, imagos of some species that occur sympatrically are often misidentified. Included in our analysis were specimens identified as representatives of nearly all *Crambidia* found in North America. A recent publication added a new member to the genus east of the Mississippi that was long confused with the widespread Western *C. cephalica* Grote and Robinson, 1820. Our analysis included new and published sequences of this new eastern *C. xanthocorpa* Lewis, 2014 as well as samples of Western *C. cephalica*, and lends support to *C. xanthocorpa* being a unique taxa separate from *C. cephalica*. While there has been some speculation that current *C. cephalica* might be a species complex harboring more cryptic taxa (C. Schmidt, pers. comm), our molecular data suggests it is a single taxa that occurs at many different elevations in the West (from desert to boreal environments) often with great disparity in the size and color of the imagos, some being uniformly white, while other having more grey hindwings. There is also variation in the extent and brightness of the namesake yellow head in this species, with specimens from Texas having much brighter and more extensive yellow than examples from Arizona.

The poorly known Mexican taxa *C. myrlosea* Dyar, 1917 occurs along the southern US border, from Texas to Arizona, the imagos being the same size as *C. cephalica*, but tan rather than white, and lacking the yellow head. Worn specimens of *C. cephalica* lose their bright white scales as well as many of the yellow head scales and have been misidentified as *C. myrlosea*. In Arizona, *C. myrlosea* is very rare along the border, but it can be common in adjacent Sonora, Mexico. Like *C. cephalica*, there is widespread variation in the size of the imagos, and they occur in habitat ranging from desert to pine forests, raising the possibility that there were cryptic taxa included under this name. Multiple adults were included in our molecular analysis with origins from Arizona, several localities in Sonora and from Tamaulipan scrub habitat of south Texas (courtesy of David Wagner). All grouped together, suggesting they are a single,

widespread taxa. Other than size, the uniform tan coloring of both the forewing and hindwing is consistent among them.

The taxa *C. impura* Barnes and McDunnough, 1913 and *C. pura* Barnes and McDunnough, 1913 are often confused with each other and with other *Crambidia*. Specimens of *C. impura* from Arizona and Sonora all group as a single taxa in our analysis. This species can occur sympatrically with *C. cephalica* in the Southwest, but lacks the yellow head and is more drab and larger, particularly the surface area of the hindwing. The flight time of *C. impura* is also restricted to late fall (Sept.-Nov.) while *C. cephalica* can be taken during most of the warmer months. *C. impura* is also sometimes confused with *C. myrlosea* as both lack the yellow on the head, but the latter is always smaller and uniform tan rather than drab white. Specimens identified as *C. pura* from Colorado (ex B. Bartell) actually appear to be *C. impura*, while published sequences of *C. pura* from the eastern US appear as a separate taxa.

Two species of *Crambidia* stand out due to the unusual rounded shape of their wings and the fact, noted by Forbes (1960), that they lack a forewing accessory cell found in all other members of the genus. Both *C. uniformis* Dyar 1898 and *C. pallida* Packard, 1864 are Eastern U.S. species. While such a significant morphological difference such as the absence of the accessory cell may indicate they belong in a genus distinct from *Crambidia*, the molecular data here supports their current placement amongst the *Crambidia*, and suggests that they lost this cell at some point in their evolutionary trajectory as part of this genus. The larvae look very much like other *Crambidia* reared by the author (JDP).

While we were unable to get fresh DNA of the genus *Clemensia*, we included published sequences in our analysis and found that the genus falls outside of the Cisthenina, where they were tentatively placed by Bendib and Minet (2000). This is the same result first reported by Zenker et al. 2016 as “Lithosiini Group 1,” suggesting *Clemensia* + *Pronola* + *Garudinia* may require creation of a new subtribe. In our analysis, *Garudinia*, a S.E. Asian genus, is not part of this clade, which includes just the neotropical genera, *Clemensia* and *Pronola*. Potential apomorphies for this new subtribe have already been defined by Bendib and Minet (2000), based on *Clemensia*, but it remains to be determined if members of *Pronola* possess the same traits. If they do, then the subtribe Clemensiita (type genus: *Clemensia*), previously proposed by Bendib and Minet (2000) as Clemensiiti, may take nomenclatural precedence as a subtribe separate from the Cisthenina.

Acsala anomala occurs on a long branch by itself, confirming the placement of this species in a subtribe of its own, the Ascaliina.

As part of this study, we collected fresh specimens of *Afrida exegens* Dyar, 1922, the northernmost representative of the tribe which lives in very few high elevation meadows in southeastern Arizona. The genus *Afrida* Moschler, 1886 is a Neotropical one with a confusing history. Several authors have considered it a lithosiine (Hampson 1900, Dyar 1913). Even more recently, Lafontaine and Schmidt (2010) retained the genus under the former family Arctiidae but regarded it as *insertae sedis*. While the larvae do feed on lichens, they are morphologically distinct, particularly the shape of their cocoon and the fact they weave bits of lichen into the structure (Wagner et al. 2011), something no other lithosiine is known to do. Several authors have proposed to move this genus from the Erebidae to the family Nolidae, subfamily Afridinae (Holloway 1998, Kitching and Rawlins 1998). Zahiri et al. (2012) performed a molecular phylogenetic study of the family Nolidae, however the Afridinae was not included in their taxon sampling, and thus the phylogenetic placement of this tribe has not been tested within the context of a molecular-based analysis. We initially included it as a potential outgroup in our analysis but

found it to cause long branch issues and removed the sequence from the phylogeny of the other erebids.

Discussion and Conclusion:

The enormous radiation of the noctuoid moths and their relatives has been a formidable target for morphological systematists for a very long time, the confounding loss and change in characters having led to numerous relational configurations. Jacobson and Weller (2002) provides an excellent comparative table of these proposed historical relationships among tiger moths as a group, while Scott and Branham (2012) provides one for the Lithosiini in particular. It was not until Zahiri et al. (2011) applied molecular phylogenetic techniques using mitochondrial COI and seven nuclear genes that a potentially stable taxonomy for the Noctuoidea was realized, one that included moving a large portion of Noctuidae into the resurrected family Erebidae and subordinating Arctiinae as a subfamily under this. This was followed by a molecular phylogenetic analysis of the Erebidae (Zahiri et al. 2012) where they defined the Arctiinae as consisting of three tribes: Lithosiini, Syntomimi and Arctiini. Further work using molecular techniques to elucidate subtribal taxonomic relationships among the tribe Lithosiini has met with results that were sometimes inconsistent and confounding, with the first large molecular analysis of the subtribe being performed by Scott et al. 2014, followed by Zenker et al. 2016 and Scott-Chialvo et al. 2018. These papers added tremendously to our evolving understanding of how these moths are related using molecular data, however, there were some placements that suggested additional work was warranted.

We took a more focused approach using molecular techniques to investigate the diverse lithosiine fauna of the US using fresh specimen DNA with the hope of clearing up some of the inconsistencies and contribute to our body of knowledge about these organisms and their relationships. As with the greater Noctuoidea, we find trying to correlate these results with morphological characters a challenge, although we feel that many of the subtribal placements elucidated here using molecular characters provide a convincing framework for future work. We find support for the monophyly of the lithosiines as well as some of the subtribal classification of Bendib and Minet, with the exception of the Eudesmiini. Excluding *Acsala* and *Clemensia*, we find all the taxa known to occur in the Western Hemisphere belong to two subtribes, the monophyletic, neotropical Cisthenina and the circumpolar Lithosiina. We find the previously proposed apomorphy for the Cisthenina, the lack of larval verrucae, to not hold with the inclusion here of *Gardinia* and *Eudesmia*, thus a new apomorphy is needed to unite them. All Cisthenina examined have long apodemes on the second abdominal sternite, a morphological trait they share with the old world Nudariina (Bendib and Minet 2000). In addition to *Gardinia* and *Eudesmia*, we propose that the genera *Bruceia* and *Ptychoglène* be included in the Cisthenina. We unfortunately were not able to obtain fresh specimen DNA of *Haematomis* Hampson, 1900 for inclusion in our phylogeny, but speculate based on placement of the closely related genus *Rhabdatomis* that it also belongs among the Cisthenina.

The genus *Clemensia* falls outside the Cisthenina, something also seen in Zenker et al. (2016). In our analysis it forms a clade with high bootstrap support with the small neotropical genus *Pronola* (5 species), the adults of which are similarly sized and have a similar peculiar rounded wing shape.

Additional taxon sampling is needed to determine the extent of this clade and hopefully resolve the polytomy seen in this area of our tree.

Agylla septentrionalis is found to have high support as a member of the Lithosiina, despite concerns expressed by Bendib and Minet (2000) that their larvae have a different verrucal configuration from other Lithosiina. We add to this the observation that living adults of *A. septentrionalis* hold their wings “tent-like” over the body rather than flat against it and that the adult possess a Cisthenina-like second abdominal sternite, both features inconsistent with other Lithosiina. Thus, the placement of *Agylla* within the Lithosiina, means that these morphological characteristics are more labile than previously thought.

Less impactful but interesting results of this study confirm the recently named *C. xanthocorpa* as a separate species, and that both *C. myrlosea* and *C. cephalica* are widespread species with significant variation in size and coloring, and that there are not cryptic species among them (at least not among the samples of these taxa which we sequenced here). We also find *C. uniformis* and *C. pallida* to be correctly placed among the other *Crambidia*, despite the lack of an accessory cell in the forewing found in other members of the genus (Forbes 1960). This absence may be why these taxa appear to have shorter, more rounded forewings than other members of the genus. Data for the subtribal placement of the small Mexican genera *Gnamptonychia* and *Inopsis* among the Lithosiina are presented here.

Finally, we confirm that *Afrida exgens* of SE Arizona, long considered by some an arctiid based on the hindwing venation, is in fact an outgroup from them. This supports the findings of Kitching and Rawlins (1998) and Holloway (1998) who regarded the Afridinae a subfamily of the family Nolidae. Zahiri et al. (2103) applied molecular phylogenetics to examine the major lineages of the Nolidae but lacked fresh DNA for members of the Afridinae. Here we filled in that gap by comparing *Afrida exgens* from SE Arizona to Zahiri et al. (2013) published sequences for other nolid subfamilies. The highly diverse Nolidae is another group where morphology alone did not recognize their correct evolutionary relationships and molecular data has played a major role in defining them as a natural familial group.

With a tribe as large as the Lithosiini, it is surprising that a subtribal classification was neglected for so long, but understandable given their worldwide diversity and confounding variation in morphological characters. Beginning with Bendib and Minet (2000), we started to conceptualize how these moths might be related. Some of our placements here, such as *Gardinia* among the Cisthenina, certainly show that the appearance of the imagoes does not necessarily belie their phylogenetic relatedness. With the apparent lack of morphological synapomorphies identified thus far that unite subtribal alliances, molecular techniques provide a useful tool for understanding how their diversity evolved. As additional molecular data is published and made available, their evolutionary relationships will become more apparent and hopefully lead to secondary identification of new morphological apomorphies in both larvae and imagoes. Presently the whole life history is currently known for only a small percentage of species. Thus, we have barely scratched the surface in understanding these remarkable lepidopterans and their unique relationship to their lichen hosts and each other.

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Figure Legends:

Figure 1: Larvae of Arctiini are typically conspicuous diurnal feeders on vegetation, while the larvae of Lithosiini are cryptic nocturnal feeders on lichens: A) *Lerina incarnata* last instar larva feeding on *Asclepias linaria* B) *Lerina incarnata* adult C) *Estigmene albida* last instar larva D) *Estigmene albida* adult E) *Lycomorpha regulus* adult F) *Lycomorpha regulus* last instar larva G) *Crambidia cephalica* adult H) *Crambidia cephalica* larva on lichen.

Figure 2: mandibles of various lithosiines compared to an arctiine, arrows point to the scissorial region modified in the lithosiines: A) *Lerina incarnata* mandible (Arctiini) B) *Lycomorpha fulgens* C) *Crambidia myrlosea* D) *Crambidia cephalica* E) *Hypoprepia lampyroides* F) *Agylla septentrionalis* G) *Eudesmia arida* H) *Cisthene kentuckiensis* (all Lithosiini).

Figure 3: Phylogeny of concatenated dataset generated with IQtree.

Figure 4: Second abdominal sternites from various lithosiines with an arctiine comparator: A) *Cisthene barnesi* B) *Gardinia anopla* C) *Eudesmia arida* D) *Agylla septentrionalis* E) *Inopsis modulata* F) *Gnamptonychia ventralis* (all Lithosiini) G) *Pygarctia roseicapitus* (Arctiini).

Figure 5: Larvae of select lithosiines: A) *Gardinia anopla* last instar (A1 closeup of verrucae) B) *Eudesmia arida* last instar (B1 closeup of verrucae) C) *Inopsis modulata* last instar.

Supplementary Table 1. Taxon sampling within the family Erebiidae and GenBank numbers used in this study.

[Note to reviewers: Entries in shaded boxes are voucher numbers; they will be replaced by GenBank numbers after the manuscript has been accepted.]

		COI	RpS5	28S rDNA
Subfamily Aganainae	<i>Asota heliconia</i>	KC571044	KC571142	KC570976
	<i>Asota orbona</i>	GWORG305-08	KC571143	KC570977
	<i>Peridrome orbicularis</i>	JN401280	JN401903	
	<i>Neochera dominia</i>	JZAGA909-12	KC571144	KC570978
	<i>Pygoctenucha terminalis</i>		3914	3914
Subfamily Arctiinae	Tribe Arctiini			
	<i>Phragmatobia amurensis</i>	KF533492	KF533679	KF533419
	<i>Cynia tenera</i>	KF533458	KF533651	KF533380
	<i>Halysidota tessellaris</i>	KF533469	KF533658	
	<i>Leucanopsis setosa</i>	KJ723706	KJ723700	KF533400
	<i>Virbia costata</i>	MF923392	3538	3538
	Tribe Syntomiini			
	<i>Amata phegea</i>	HQ006238	HQ006749	KF533352
	<i>Apisa canescens</i>	HQ006146	HQ006663	
	<i>Automolis ferrigera</i>	KF533447	KF533641	
	Tribe Amerilini			
	<i>Amerilla brunnea</i>	KX300223	KX300895	
	Tribe Lithosini			
	Zenker Group 1			
	<i>Garudinia simulana</i>	JN401283	JN401905	KF533392
	<i>Clemensia marmorata</i>	KX300245	KX300811	
	<i>Clemensia albata</i>			KF533374
	<i>Pronola magniplaga</i>	KX300312	KX300812	
	unplaced			
	<i>Heliosia jucunda</i>	KC571077	KC571160	KC571014
	Subtribe Acsalina			
	<i>Acsala anomala</i>	KJ378646	KC571145	KC570980
	Subtribe Nudariina			
	<i>Asura cervicalis</i>	KC571050		KC570983
	<i>Asura polypila</i>	KC571051	KC571146	KC570984
	<i>Barsine</i> sp.	JN401286	JN401878	KF533364
	<i>Cyana</i> sp.	JN401285	JN401876	KF533379
	<i>Cyana meyericki</i>	KC571061	KC571151	KC570998
	<i>Lyclene pyraula</i>	KC571084	KC571165	KC571022
	<i>Lyclene reticulata</i>	KC571085	KC571166	KC571023
	<i>Lyclene</i> sp. 1	KC571086	KC571168	KC571024
	<i>Lyclene</i> sp. nr. 1	KC571087	KC571167	KC571025
	<i>Lyclene quadrilineata</i>	KC571093	KC571172	KC571035
	<i>Miltochrista miniata</i>	KC571090	KC571170	KC571031
	<i>Miltochrista</i> sp. 1	KC571091	KC571171	KC571032
	Subtribe Endrosini			
	<i>Stigmatophora micans</i>	KF704470		
	<i>Setina irrorella</i>	KX050282	KX050605	
	<i>Eugoa bipunctata</i>	JN401906	JN401906	KF533390
	<i>Trischalis</i> sp.	HM906475		
	Subtribe Eudsmia			
	<i>Eudsmia arida</i> , Mesa Tres Rios 3910	3910	3910	3910
	<i>Eudsmia arida</i> , Mesa Tres Rios 3911	3911	3911	3911
	<i>Eudsmia arida</i> , Mesa Tres Rios 3912	3912	3912	3912
	<i>Eudsmia arida</i> larva 3907			3907
	<i>Eudsmia arida</i> larva 3987			3987
	Subtribe Cisthenina			
	<i>Abrochocis esperanza</i>	KC571047		KC570979
	<i>Balbura dorsisigna</i>	KC571053		KC570986
	<i>Balbura intervenata</i>	KC571052		KC570987
	<i>Bruceia</i> (large) Sierra del Tigre 3510	3510	3510	3510
	<i>Bruceia</i> (large) Sierra del Tigre 3511	3511		3511
	<i>Bruceia</i> (small) Sierra del Tigre 3512	3512	3512	3512
	<i>Bruceia</i> (small) Sierra del Tigre 3513	3513	3513	3513
	<i>Bruceia</i> (tiny) Sierra del Tigre 4178		4178	4178
	<i>Bruceia hubbardi</i>	KC571054		
	<i>Bruceia hubbardi</i> Santa Catalinas 3502	3502	3502	3502
	<i>Bruceia hubbardi</i> Santa Catalinas 3503	3503	3503	3503
	<i>Bruceia</i> sp. larva 3562	3562	3562	3562
	<i>Bruceia pulverina</i>	KC571055		
	<i>Bruceia pulverina</i> Sierra del Tigre 4165		4165	4165
	<i>Bruceia pulverina</i> Sierra del Tigre 4179		4179	4179

Supplementary Table 1. Taxon sampling within the family Erebiidae and GenBank numbers used in this study.

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	COI	RpS5	28S rDNA
<i>Bruceia pulverina</i> Sierra del Tigre 4180		4180	4180
<i>Chrysochlorosis magnifica</i>	KC571057		KC570996
<i>Cisthene angelus</i> MF924026	MF924026		
<i>Cisthene angelus</i> Tucson Mtns. 3262	3262		3262
<i>Cisthene barnesii</i> HQ543289	HQ543289		
<i>Cisthene barnesii</i> MF923765	MF923765		
<i>Cisthene barnesii</i> MF924314	MF924314		
<i>Cisthene barnesii</i> Ray Nagle 3504	3504	3504	3504
<i>Cisthene barnesii</i> Ray Nagle 3505	3505	3505	3505
<i>Cisthene deserta</i> HQ543389	HQ543389		
<i>Cisthene dorsimacula</i> MF924146	MF924146		
<i>Cisthene faustinula</i> MF922356	MF922356		
<i>Cisthene faustinula</i> MF923287	MF923287		
<i>Cisthene juanita</i>	KC571058		KC570991
<i>Cisthene juanita</i> Huachuca Mnts. 3275	3275		
<i>Cisthene juanita</i> KC571058	KC571058		
<i>Cisthene juanita</i> MF922471	MF922471		
<i>Cisthene juanita</i> MF923887	MF923887		
<i>Cisthene kentuckiensis</i> MF924091	MF924091		
<i>Cisthene liberomacula</i> MF922790	MF922790		
<i>Cisthene liberomacula</i> MF924014	MF924014		
<i>Cisthene martini</i> Huachuca Mnts. 3263	3263		3263
<i>Cisthene martini</i> MF923225	MF923225		
<i>Cisthene packardii</i> MF922694	MF922694		
<i>Cisthene packardii</i> MF923495	MF923495		
<i>Cisthene perrosea</i> HQ543391	HQ543391		
<i>Cisthene picta</i> GU800239	GU800239		
<i>Cisthene picta</i> MF922794	MF922794		
<i>Cisthene plumbea</i>	KC571059		KC570992
<i>Cisthene polyzona</i> BLPDD935-09	BLPDD935-09		
<i>Cisthene</i> sp. larva 3563	3563	3563	3563
<i>Cisthene subjecta</i> MF922463	MF922463		
<i>Cisthene subjecta</i> MF922778	MF922778		
<i>Cisthene subrufa</i> LPYPB681	LPYPB681		
<i>Cisthene subrufa</i> LPYPC078	LPYPC078		
<i>Cisthene tenuifascia</i>	HQ543372		KC570994
<i>Cisthene tenuifascia</i> Peppersauce 3299	3299		3299
<i>Cisthene tenuifascia</i> Santa Catalina Mnts 3264	3264		3264
<i>Cisthene tenuifascia</i> Sawmill 3298	3298		3298
<i>Cisthene unifascia</i> HQ543401	HQ543401		
<i>Cloesia digna</i>	JQ561796		KC570995
<i>Dolichesia falsimonia</i>	KC571062		KC571000
<i>Euthyone grisescens</i>	KC571073		KC571010
<i>Hypoprepia cadaverosa</i> Benny Creek 3901			3901
<i>Hypoprepia cadaverosa</i> Benny Creek 3902			3902
<i>Hypoprepia cadaverosa</i> Benny Creek 3903			3903
<i>Hypoprepia fucosa</i>	KC571078	KC571162	KC571017
<i>Hypoprepia fucosa tricolor</i>	KC571079	KC571163	KC571018
<i>Hypoprepia inculta</i> Barfoot, Chiricahua Mnts. 4168		4168	4168
<i>Hypoprepia inculta</i> Barfoot, Chiricahua Mnts. 4169		4169	4169
<i>Hypoprepia inculta</i> Barfoot, Chiricahua Mnts. 4170	MH337839		4170
<i>Hypoprepia inculta</i> Barfoot, Chiricahua Mnts. 4171		4171	4171
<i>Hypoprepia inculta</i> Chiricahua Mnts. 3259	MH337840		
<i>Hypoprepia inculta</i> Rose Peak, White Mnts. 3573	MH337833		
<i>Hypoprepia inculta</i> Rose Peak, White Mnts. 3574	MH337841		
<i>Hypoprepia inculta</i> Sierra del Tigre 4177		4177	4177
<i>Hypoprepia lampyroides</i> Rose Peak, White Mnts. .	MH337834		3566
<i>Hypoprepia lampyroides</i> Rose Peak, White Mnts. .	MH337835		
<i>Hypoprepia lampyroides</i> Rose Peak, White Mnts. .	MH337836		3568
<i>Hypoprepia lampyroides</i> Rose Peak, White Mnts. .	MH337837		3569
<i>Hypoprepia lampyroides</i> Rose Peak, White Mnts. .	MH337838		3570
<i>Hypoprepia miniata</i> GU089464	GU089464		
<i>Hypoprepia miniata</i> GU089465	GU089465		
<i>Hypoprepia miniata</i> MF922329	MF922329		
<i>Hypoprepia miniata</i> MF922441	MF922441		
<i>Hypoprepia miniata</i> MF923793	MF923793		
<i>Lycomorpha fulgens</i> Rose Peak, White Mnts 3594			3594
<i>Lycomorpha fulgens</i> Hannigan Meadow 3904			3904

Supplementary Table 1. Taxon sampling within the family Erebiidae and GenBank numbers used in this study.

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	COI	RpS5	28S rDNA
<i>Lycomorpha grotei</i> Greer 3913		3913	3913
<i>Lycomorpha regulus</i> Sierra del Tigre 4166		4166	4166
<i>Lycomorpha regulus</i> Summerhaven 3905			3905
<i>Lycomorpha regulus</i> White Mts 3527	3527	3527	3527
<i>Lycomorphodes correbioides</i>	KC571088		KC571027
<i>Lycomorphodes sordida</i>	KC571089		KC571028
<i>Ptychoglene coccinea</i>	HQ918634		KC571036
<i>Ptychoglene coccinea</i> Rancho El Aribabi, Rio Cocospera 3261			3261
<i>Rhabdatomis cora coroides</i>	KC571094		KC571037
<i>Rhabdatomis laudamia</i>	KC571095		KC571038
<i>Rhabdatomis laudamia</i> Sierra La Madera 3274	3274		3274
<i>Rhabdatomis melinda</i>	KC571096		KC571039
<i>Talara cara</i>	KC571098		KC571041
<i>Talara lepada</i>	KC571099		KC571042
<i>Talara nr. mona</i>	KC571100		KC571043
Subtribe Lithosiina			
<i>Agylla argentifera</i>	KC571048		KC570981
<i>Agylla septentrionalis</i>	KC571049		KC570982
<i>Agylla septentrionalis</i> Barfoot 4167		4167	4167
<i>Atolmis rubricollis</i>	ABOLA126-14	KC571147	KC570985
<i>Brunia antica</i>	HQ006193	HQ006706	KF533366
<i>Calamidia hirta</i>	KC571056	KC571148	KC570990
<i>Crambidia casta</i> 3556	3556		
<i>Crambidia casta</i> KJ375095	KJ375095		
<i>Crambidia casta</i> KJ378514	KJ378514		
<i>Crambidia cephalica</i> "giant form" Showlow 3576	3576		
<i>Crambidia cephalica</i> "giant form" Showlow 3577	3577	3577	3577
<i>Crambidia cephalica</i> "giant form" Showlow 3578	3578		3578
<i>Crambidia cephalica</i> "giant form" Showlow 3588	3588		3588
<i>Crambidia cephalica</i> "giant form" Showlow 3589	3589		3589
<i>Crambidia cephalica</i> El Yeso summit 3515	3515		3515
<i>Crambidia cephalica</i> Empelotos 3519	3519	3519	3519
<i>Crambidia cephalica</i> Empelotos 3520	3520		3520
<i>Crambidia cephalica</i> HM423638	HM423638		
<i>Crambidia cephalica</i> HQ543338	HQ543338		
<i>Crambidia cephalica</i> HQ543423	HQ543423		
<i>Crambidia cephalica</i> JF847004	JF847004		
<i>Crambidia cephalica</i> MF922652	MF922652		
<i>Crambidia cephalica</i> MF922757	MF922757		
<i>Crambidia cephalica</i> MF923164	MF923164		
<i>Crambidia cephalica</i> MF923216	MF923216		
<i>Crambidia cephalica</i> MF923823	MF923823		
<i>Crambidia cephalica</i> MF924192	MF924192		
<i>Crambidia cephalica</i> Rancho Los Pinitos 3281	3281	3281	3281
<i>Crambidia cephalica</i> Santa Catalina Mt 3273	3273		
<i>Crambidia cephalica</i> Showlow 3579	3579		
<i>Crambidia cephalica</i> Showlow 3580	3580	3580	3580
<i>Crambidia cephalica</i> Showlow 3590	3590	3590	3590
<i>Crambidia cephalica</i> Sierra La Madera 3271	3271		3271
<i>Crambidia cephalica</i> Sierra La Madera 3282	3282		3282
<i>Crambidia cephalica</i> Tucson Mtn 3266	3266		3266
<i>Crambidia cephalica</i> TX DLW reared 4162		4162	4162
<i>Crambidia cephalica</i> TX DLW reared 4163		4163	4163
<i>Crambidia cephalica</i> TX DLW reared 4164		4164	4164
<i>Crambidia cephalica</i> TX DLW Wagner Camp Wood 3500	3500	3500	3500
<i>Crambidia cephalica</i> TX DLW Wagner Camp Wood 3501	3501		3501
<i>Crambidia dusca</i> MF923036	MF923036		
<i>Crambidia dusca</i> MF923859	MF923859		
<i>Crambidia impura</i> Gila Co., N. of Winkelman 3270	3270	3270	3270
<i>Crambidia impura</i> Golden, CO 3530	3530		3530
<i>Crambidia impura</i> Golden, CO 3531	3531	3531	3531
<i>Crambidia impura</i> Golden, CO 3532	3532	3532	3532
<i>Crambidia impura</i> Golden, CO 3533	3533		
<i>Crambidia impura</i> Golden, CO 3534	3534		
<i>Crambidia impura</i> Golden, CO 3535		3535	3535
<i>Crambidia impura</i> HM423637	HM423637		
<i>Crambidia impura</i> Huachuca Mts 3267	3267		3267
<i>Crambidia impura</i> MF923147	MF923147		

Supplementary Table 1. Taxon sampling within the family Erebidae and GenBank numbers used in this study.

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	COI	RpS5	28S rDNA
<i>Crambidia impura</i> MF923583	MF923583		
<i>Crambidia impura</i> MF923816	MF923816		
<i>Crambidia impura</i> New Mexico 3545	3545		
<i>Crambidia impura</i> Showlow 3575	3575		
<i>Crambidia lithosioides</i> MF923153	MF923153		
<i>Crambidia lithosioides</i> MF924003	MF924003		
<i>Crambidia myrlosea</i> HM426606	HM426606		
<i>Crambidia myrlosea</i> HQ543416	HQ543416		
<i>Crambidia myrlosea</i> HQ543417	HQ543417		
<i>Crambidia myrlosea</i> large ex Wagner Texas, Came	3278	3278	3278
<i>Crambidia myrlosea</i> larva 3561	3561	3561	3561
<i>Crambidia myrlosea</i> Sierra La Purica 3268	3268		3268
<i>Crambidia myrlosea</i> small ex Wagner Texas, Came	3277		3277
<i>Crambidia myrlosea</i> Mesa Tres Rios Sonora 3595			3595
<i>Crambidia myrlosea</i> Mesa Tres Rios Sonora 3596			3596
<i>Crambidia myrlosea</i> Mesa Tres Rios Sonora 3597			3597
<i>Crambidia nr. pallida</i> HM423629	HM423629		
<i>Crambidia nr. pallida</i> MF922594	MF922594		
<i>Crambidia pallida</i> 3547	3547		3547
<i>Crambidia pallida</i> 3549	3549		3549
<i>Crambidia pallida</i> 3550	3550		3550
<i>Crambidia pallida</i> 3551	3551		3551
<i>Crambidia pallida</i> MF923729	MF923729		
<i>Crambidia pallida</i> MF923904	MF923904		
<i>Crambidia pallida</i> MF923906	MF923906		
<i>Crambidia pallida</i> reared N. Carolina 3509	3509	3509	3509
<i>Crambidia pallida</i> reared Ohio 3508			3508
<i>Crambidia pura</i> HM423630	HM423630		
<i>Crambidia pura</i> KJ375112	KJ375112		
<i>Crambidia uniformis</i> HQ918617	HQ918617		
<i>Crambidia uniformis</i> MF922930	MF922930		
<i>Crambidia xanthocorpa</i> GU089387	GU089387		
<i>Crambidia xanthocorpa</i> MF923203	MF923203		
<i>Crambidia xanthocorpa</i> Purdue, IN 3536	3536	3536	3536
<i>Crambidia xanthocorpa</i> Purdue, IN 3537	3537	3537	3537
<i>Cybosia mesomella</i>	ABOLA124-14		KC570999
<i>Eilema auriflua</i>	KC571065		KC571003
<i>Eilema bicolor</i>	KC571063	KC571152	KC571001
<i>Eilema bicolor</i> , Gilpin CO 3598			3598
<i>Eilema bicolor</i> , Gilpin CO 3599			3599
<i>Eilema bicolor</i> CO 3586	3586	3586	3586
<i>Eilema bicolor</i> CO 3587	3587		3587
<i>Eilema bicolor</i> Golden, CO 3528		3528	
<i>Eilema complanum</i>	KC571064	KC571153	KC571002
<i>Eilema complanum</i> 3291	3291		
<i>Eilema complanum</i> 3581	3581		3581
<i>Eilema complanum</i> 3582	3582		
<i>Eilema griseolum</i>	TRUXA012-17	KC571154	KC571004
<i>Eilema plana</i>	KC571067		KC571005
<i>Eilema</i> sp. 3	KC571070	KC571157	KC571008
<i>Eilema</i> sp. 4	KC571071		KC571009
<i>Eilema</i> sp. Romania 3584	3584		
<i>Gardinia anopla</i>	KC571075	KC571159	KC571012
<i>Gardinia anopla</i> Summerhaven, Santa Catalina Mr	3250		3250
<i>Gardinia anopla</i> Sierra del Tigre 4172			4172
<i>Gnamptonychia flavicollis</i>	KC571076	KC571158	KC571013
<i>Gnamptonychia ventralis</i> Sierra del Tigre 4173		4173	
<i>Gnamptonychia ventralis</i> Sierra del Tigre 4174		4174	4174
<i>Gnamptonychia ventralis</i> Sierra del Tigre 4176			4176
<i>Hiera gyge</i>		KC571161	KC571015
<i>Inopsis modulata</i>	KC571082	KC571164	KC571020
<i>Lithosia quadra</i>	KC571083		KC571021
<i>Lithosia quadra</i> 3564	3564		3564
<i>Lithosia quadra</i> 3585	3585		3585