Molecular phylogeny of Lymantriinae (Lepidoptera, Noctuoidea, Erebidae) inferred from eight gene regions

Houshuai Wang[a,b], Niklas Wahlberg[c], Jeremy D. Holloway[d], Johannes Bergsten[e], Xiaoling Fan[a], Daniel H. Janzen[f], Winnie Hallwachs[f], Lijun Wen[a], Min Wang[a,*] and Sören Nylin[b]

[a]*Department of Entomology, College of Natural Resources & Environment, South China Agricultural University, 510642 Guangzhou, China;* [b]*Department of Zoology, University of Stockholm, SE-10691 Stockholm, Sweden;* [c]*Department of Biology, Laboratory of Genetics, University of Turku, 2014 Turku, Finland;* [d]*Department of Life Sciences, Natural History Museum, Cromwell Road, London SW7 5BD, UK;* [e]*Department of Zoology, Swedish Museum of Natural History, SE-10405 Stockholm, Sweden;* [f]*Department of Biology, University of Pennsylvania, Philadelphia, PA 19104, USA*

Abstract

To understand the evolutionary history of Lymantriinae and test the present higher-level classification, we performed the first broad-scale molecular phylogenetic analysis of the subfamily, based on 154 exemplars representing all recognized tribes and drawn from all major biogeographical regions. We used two mitochondrial genes (*cytochrome c oxidase subunit I* and *16S ribosomal RNA*) and six nuclear genes (*elongation factor-1α, carbamoylphosphate synthase domain protein, ribosomal protein S5, cytosolic malate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase* and *wingless*). Data matrices (in total 5424 bp) were analysed by parsimony and model-based evolutionary methods (maximum likelihood and Bayesian inference). Based on the results of the analyses, we present a new phylogenetic classification for Lymantriinae composed of seven well-supported tribes, two of which are proposed here as new: Arctornithini, Leucomini, Lymantriini, Orgyiini, Nygmiini, Daplasini trib. nov. and Locharnini trib. nov. We discuss the internal structure of each of these tribes and address some of the more complex problems with the genus-level classification, particularly within Orgyiini and Nygmiini.

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Noctuoidea are the largest superfamily of Lepidoptera, containing just over one-quarter of the known species: 42 407 according to van Nieukerken et al. (2011). As such, they have presented a formidable challenge to obtaining a satisfactory, predictive, hierarchical classification that reflects evolutionary history (Kitching, 1984; Kitching and Rawlins, 1998; Fibiger and Lafontaine, 2005; Lafontaine and Fibiger, 2006; Zahiri et al., 2011). As for most groups of organisms, early classifications were developed in the 18th and 19th centuries primarily from regional treatments with a small amount of serendipitous input from collections made on global voyages. However, the very high diversity of the noctuoids tended to inhibit attainment of a global consensus from these regional efforts well into the cladistic era, particularly between those of the Old and New Worlds (cf. checklists and reviews by Franclemont and Todd, 1983; Kitching, 1984; Fibiger and Hacker, 1991; Poole, 1995; Fibiger and Lafontaine, 2005).

While cladistic methodology provided a means to revise groups that had been based on plesiomorphic characters (e.g. Noctuidae), the importance of taxon sampling representing a wide range of taxa from diverse lineages soon became apparent (Hillis, 1996; Graybeal, 1998; Pollock et al., 2002; Zwickl and Hillis, 2002; Heath et al., 2008). Consequently, recent attempts based on multiple nuclear markers to reconstruct the evolutionary history of noctuoids and unify

*Corresponding author:
E-mail address*: minwang@scau.edu.cn

the classification have put strong emphasis on comprehensive taxon sampling in conjunction with targeting type-genera of higher-level taxa (Mitchell et al., 2006; Regier et al., 2009; Zahiri et al., 2011, 2012). Despite these efforts, some large and economically important clades such as the cosmopolitan tussock moths, long recognized as a monophyletic taxon but variously treated at family or subfamily level (see below), were in these studies only partially included and we are still lacking a higher-level classification of Lymantriinae based on a comprehensively sampled phylogenetic analysis. The present study aims to fill this gap, motivated by the need to test and reconcile regional classifications and provide a phylogenetic framework for studies of host feeding evolution (foraging behaviour, host range), biogeographical history and diversification.

The subfamily Lymantriinae, commonly referred to as tussock moths, contains more than 2500 described species placed in approximately 360 genera, but the phylogenetic affinities within the group are very poorly understood (see below). The subfamily is cosmopolitan in distribution with most species diversity in the Old World (Kitching and Rawlins, 1998). Species of the subfamily are predominantly arboreal defoliators in the larval stage and frequently polyphagous (Holloway, 1999). Some are major forest and agricultural pests such as the gypsy moth *Lymantria dispar* Linnaeus, *L. monacha* Linnaeus (black arches or nun moth), *Orgyia antiqua* Linnaeus (vapourer or rusty tussock moth), *O. pseudotsugata* McDunnough (Douglas-fir tussock moth), *Leucoma salicis* Linnaeus (white satin moth) and *Arna pseudoconspersa* Strand (tea tussock moth) (Wallner, 1988; Chao, 2003; Pederson and Munson, 2006; Pogue and Schaefer, 2007; Ziemnicka, 2008; Uhlikova et al., 2011). The larvae of some species of the tribe Nygmiini, such as *Euproctis chrysorrhoea* Linnaeus (brown-tail moth) and *Sphrageidus similis* Fuessly (yellow-tail moth), possess urticating setae that can be hazardous to human health (Holloway et al., 1987; Common, 1990). The genus *Toxoproctis* Holloway is also notorious in this respect (Holloway, 1999). In total, 154 species were listed as economically important by Zhang (1994), and over half the genera in which these are now placed are included in our analysis. This total represents about 10% of economically important Noctuoidea species, these representing one-quarter of the total species of Lepidoptera listed by Zhang. A better understanding of the evolutionary relationships within the group will help us understand these important aspects of their biology.

Lymantriinae are clearly noctuoid moths as they possess a metathoracic tympanum, which is considered a synapomorphy for the group. However, the taxonomic rank of this group has varied in different noctuoid classification systems. It was traditionally assigned family rank within Noctuoidea, alongside, for example, Noctuidae and Erebidae, on the basis of morphological characters (Miller, 1991; Scoble, 1992; Kitching and Rawlins, 1998; Fibiger and Lafontaine, 2005), and was only recently downgraded to subfamily rank, first within Noctuidae (Lafontaine and Fibiger, 2006) and later within the newly revised family Erebidae based on support from molecular data (Zahiri et al., 2011, 2012). The larvae have a single and eversible mid-dorsal gland on each of the sixth and seventh abdominal segments; these glands are suggested to represent an apomorphy supporting the monophyly of Lymantriinae (Yela and Zahiri, 2011). Other distinctive features are: bipectinate antennae in the male with one to three spinules at the terminal of each branch; strongly reduced or absent proboscis; a prespiracular counter-tympanal hood; and males with paired, pocket-like tymbal organs on the third abdominal sternite (Holloway, 1999).

Previous studies have identified two primary hypotheses regarding the sister-group and wider relationships of Lymantriinae (Speidel and Witt, 2011). One suggested that Arctiinae are the sister group of Lymantriinae (Mitchell et al., 2006; Regier et al., 2009), morphologically supported by synapomorphies such as a prespiracular counter-tympanal hood, general similarities of the male genitalia structures, and the terminal spinules of the bipectinate male antennae, although such spinules only occur on some arctiines. A sister-group relationship of Lymantriinae and Pantheinae is the alternative hypothesis (Kuznetzov and Stekolnikov, 2004; Stekolnikov and Speidel, 2009), proposed on the basis of the following characters: the presence of secondary setae in first instars; the well-developed pupal cremaster; the interapophysal position of the retractor muscles of the apophyses of the ninth segment; and the absence of depressor muscles of the anal papillae in the adult female terminalia.

However, the first hypothesis is not clearly supported by recent molecular studies (Zahiri et al., 2011, 2012). These indicate, but without strong support, a sister-group relationship within the highly diverse Erebidae between Lymantriinae and a clade consisting of Pangraptinae, Aganainae, Herminiinae and Arctiinae. The second hypothesis is rejected by the clear placement of Pantheinae in a relatively basal position within a more restricted concept of Noctuidae (Zahiri et al., 2013).

Lymantriinae stand out amongst Erebidae in having non-feeding adults, in contrast to other groups of large physical size such as Erebinae and Calpinae where adult feeding is commonplace (Zaspel et al., 2012; Holloway et al., 2013). Holloway et al. (2013) noted parallels between these extremes in Erebidae and the alternative life strategies for tropical large moths proposed by Janzen (1984) with a focus on the

Bombycoidea. Non-feeders tend to be short-lived and sexually dimorphic, with males searching for relatively static females calling with pheromone plumes; oviposition is in masses, with larvae aposematic or cryptic, often with urticating spines. Feeders tend to be long-lived, with sexual dimorphism weak at most, both sexes being active and often migratory; they oviposit singly, and the larvae are generally unspined and cryptic. Janzen also noted general larval host plant differences between the two categories, with non-feeders favouring hosts with foliage low in nutrients but rich in phenolics, and feeders favouring nutrient-rich foliage that is low in phenolics but may contain toxic alkaloids.

The classification within Lymantriinae remains quite fragmentary, and a comprehensive and global systematic study is still lacking. Ferguson (1978) treated Nearctic taxa of Lymantriidae (the previous concept as family-rank) as being within one single subfamily (Lymantriinae) that included two tribes, Lymantriini and Orgyiini, but felt that African and Indo-Australian taxa would probably represent additional subfamilies. Holloway (1999) followed Ferguson's concept of one subfamily, but recognized three additional tribes (Arctornithini, Leucomini and Nygmiini) based on Indo-Australian taxa. Benkhelil (1999) redefined Ferguson's Lymantriini and Orgyiini as two subfamilies based mainly on European taxa, with the genus *Euproctis* Hübner being transferred to Orgyiinae from Lymantriinae. More recently, Speidel and Witt (2011) supported Lymantriinae as a subfamily of Erebidae following the arrangement of Zahiri et al. (2011), and separated European Lymantriinae species into two tribes, Lymantriini and Orgyiini. These were further divided into five subtribes corresponding to five tribal groups of Holloway (1999): Lymantriini with Lymantriina, Arctornithina and Leucomina, and Orgyiini with Orgyiina and Nygmiina. However, Lafontaine and Schmidt (2013) commented that the concept of Orgyiini proposed by Speidel and Witt (2011) seemed not to be monophyletic. All of these studies are regional systematic overviews, and Neotropical taxa as well as the diverse African species have barely featured in the existing classification.

To date, little molecular information has been applied to elucidate the phylogenetic relationships within Lymantriinae. Mitchell et al. (2006) used two nuclear genes from a sample of four lymantriine genera, with the results indicating that *Euproctis* is more closely related to *Lymantria* Hübner than to *Orgyia* Ochsenheimer + *Dasychira* Hübner. Based on the five type genera representing separately the five lymantriine tribes of Holloway (1999) and the phylogenetic analysis of eight gene regions, Zahiri et al. (2011, 2012) proposed a phylogenetic hypothesis that Arctornithini are sister to the remaining four tribes, and that these are

divided into two pairs: Orgyiini + Nygmiini and Lymantriini + Leucomini.

Here, we offer the first broad-scale molecular phylogenetic analyses of lymantriine moths using molecular data from eight gene regions to investigate the phylogenetic relationships and revise the existing classification within the subfamily.

Materials and methods

Taxon sampling

We selected 154 exemplars from 55 genera representing all the currently recognized lymantriine tribes and drawn from almost all biogeographical regions, together with ten representatives of other Erebidae as outgroups. Note that although this is the most comprehensive investigation of Lymantriinae so far, it is still possible that there are additional major lineages to be found, in particular when the African and Neotropical regions can be better sampled. For identification, the types of most of the species sampled were examined, but in some cases illustrations of type specimens in the literature were used. A list of taxa with the localities, voucher codes and GenBank accession numbers is provided in Table S1.

DNA extraction, amplification and sequencing

We extracted total genomic DNA from adults, using one or two legs that had been dried or freshly preserved in 96% ethanol, using the Tiangen™ DNA extraction kit (Beijing, China) or the Qiagen DNeasy tissue extraction kit (Hilden, Germany) following the protocol suggested by the manufacturers. For molecular markers, we selected *cytochrome c oxidase subunit I* (*COI*) and the more slowly evolving *16S ribosomal RNA* (*16S rRNA*) from the mitochondrial genome, and *elongation factor-1α* (*EF-1α*), *carbamoylphosphate synthase domain protein* (*CAD*), *ribosomal protein S5* (*RpS5*), *cytosolic malate dehydrogenase* (*MDH*), *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) and *wingless* (*WNT*) from the nuclear genome. All these markers have been used in previous studies of Lepidoptera, and they have been found to be highly informative in revealing phylogenetic relationships (Wahlberg and Zimmermann, 2000; Wahlberg et al., 2009; Zahiri et al., 2011, 2012, 2013). DNA amplification and sequencing protocols followed Wahlberg and Wheat (2008) and Wang et al. (2014).

Sequence alignment

The sequences obtained were edited and aligned using BioEdit version 7.2.0 (Hall, 1999) or MEGA

version 5.0 (Tamura et al., 2011), except that *16S* was aligned using the online version of MAFFT (http://mafft.cbrc.jp/alignment/server/). For the *16S* sequences, ambiguously aligned regions were identified and excluded using Gblock version 0.91b (Castresana, 2000; Talavera and Castresana, 2007), with parameters allowing for smaller final blocks, gap positions within the final blocks and less strict flanking positions. Neighbour-joining and maximum-likelihood (ML) trees were separately constructed for each gene to reduce errors in alignments and avoid the risk of confusion during sequencing.

Phylogenetic analyses

The data were analysed using non-model-based (maximum-parsimony, MP) and model-based (ML and Bayesian inference (BI)) phylogenetic methods. For MP analysis, we performed New Technology heuristic searches (Goloboff, 1999) developed for large datasets using a "driven search" until minimum length was hit ten times by means of a combination of Tree Fusion, Ratchet, Tree Drifting and Sectorial searches under default parameters in the program TNT version 1.1 (Goloboff et al., 2008). All characters were treated as unordered and equally weighted. Nodal support for each clade was estimated by Bremer support (Bremer, 1988, 1994) using a script (Pena et al., 2006).

For the model-based analyses, we used two different partitioning strategies: by gene regions (eight partitions) and by codon positions separated by genome source (see Miller et al., 2009; seven partitions: the first, second and third positions for nuclear genes, same for mitochondrial COI, and with ribosomal *16S* as a separate partition). Sequence alignments were carefully examined manually to ensure proper assignment of codons to positions. The GTR+G+I model was selected as the most appropriate model of sequence evolution for each gene and codon partition using the Akaike information criterion (AIC) implemented in MrModeltest2 (Nylander, 2004). However, MrModeltest only evaluates a subset of the 203 possible and reversible four by four substitution rate models. When evaluating all by using reversible-jump Markoc chain Monte Carlo (MCMC), commonly less complex models (three or four rate parameters) than the GTR (six) are selected (Huelsenbeck et al., 2004). We therefore used the reversible-jump MCMC setting across model space in MrBayes 3.2 (Ronquist et al., 2012) but implemented the suggested gamma-distributed rate variation across sites (G) and the proportion of invariant sites (I). Based on Bayes factor tests on initial BI analyses, we decided to use the partitioning scheme by gene regions. Partitions were allowed to evolve at different rates and all other parameters of the model (four by four substitution rate matrix, state

frequencies, among-site rate variation and proportion of invariant sites) except branch length and topology were unlinked and estimated separately for each partition.

The BI analysis was executed using MrBayes version 3.2.2 (Ronquist et al., 2012) on the CIPRES Science Gateway (Miller et al., 2010), with the default parameters and four independent runs for 50 million generations, each with one cold chain, sampled every 1000th generation, and three heated chains. Mixing, convergence and a suitable burn-in were assessed with the statistics provided by the program and with Tracer v. 1.6 (Rambaut et al., 2014). Post burn-in samples from the four runs were merged prior to the calculation of a majority-rule consensus tree. ML analysis was carried out with the web-server RAxML (Stamatakis et al., 2008) on CIPRES, using 1000 bootstrap replicates. Due to the debate about the correlation between parameters I and G (see Ren et al., 2005; Kelchner and Thomas, 2007; Zahiri et al., 2011) and whether G adequately models also invariant sites in the absence of I, we ran ML analysis under both GTR+G and GTR+I+G for each partition. This merely affected a few poorly supported nodes and we report only on the result from the analysis using GTR+G.

Results

The analyses of this study are based on the sequence data from two mitochondrial gene regions (670 bp of *COI* and 596 bp of *16S*) and six nuclear gene regions (820 bp of *CAD*, 1240 bp of *EF-1a*, 691 bp of *GAP-DH*, 407 bp of *MDH*, 600 bp *RPS5* and 400 bp of *WNT*). The final aligned data matrix contained 5424 nucleotide sites, of which 193 for the *16S* gene were excluded from subsequent analyses due to alignment ambiguities. Some genes failed to amplify for some taxa (Table S1). In the following, the robustness of clades found is (depending on method) presented as either bootstrap values (BP, for the ML analysis), posterior probabilities (PP, for the Bayesian analysis) or Bremer support values (BS, for the MP analysis).

The three phylogenetic analyses (MP, ML and BI) of the combined datasets for eight gene regions yield very similar topologies (Fig. 1, Fig. S1), and the monophyly of Lymantriinae is strongly supported by the model-based analyses (BP = 99, PP = 1.00). Despite the relatively large number of genes included in the dataset, support for clades in the phylogeny was often low and many internal branches were very short, indicating rapid diversification in the subfamily. However, all the topologies are characterized by seven major clades, although there are slight differences in the most parsimonious topological arrangement (Fig. S1). In the classification below we give the seven clades
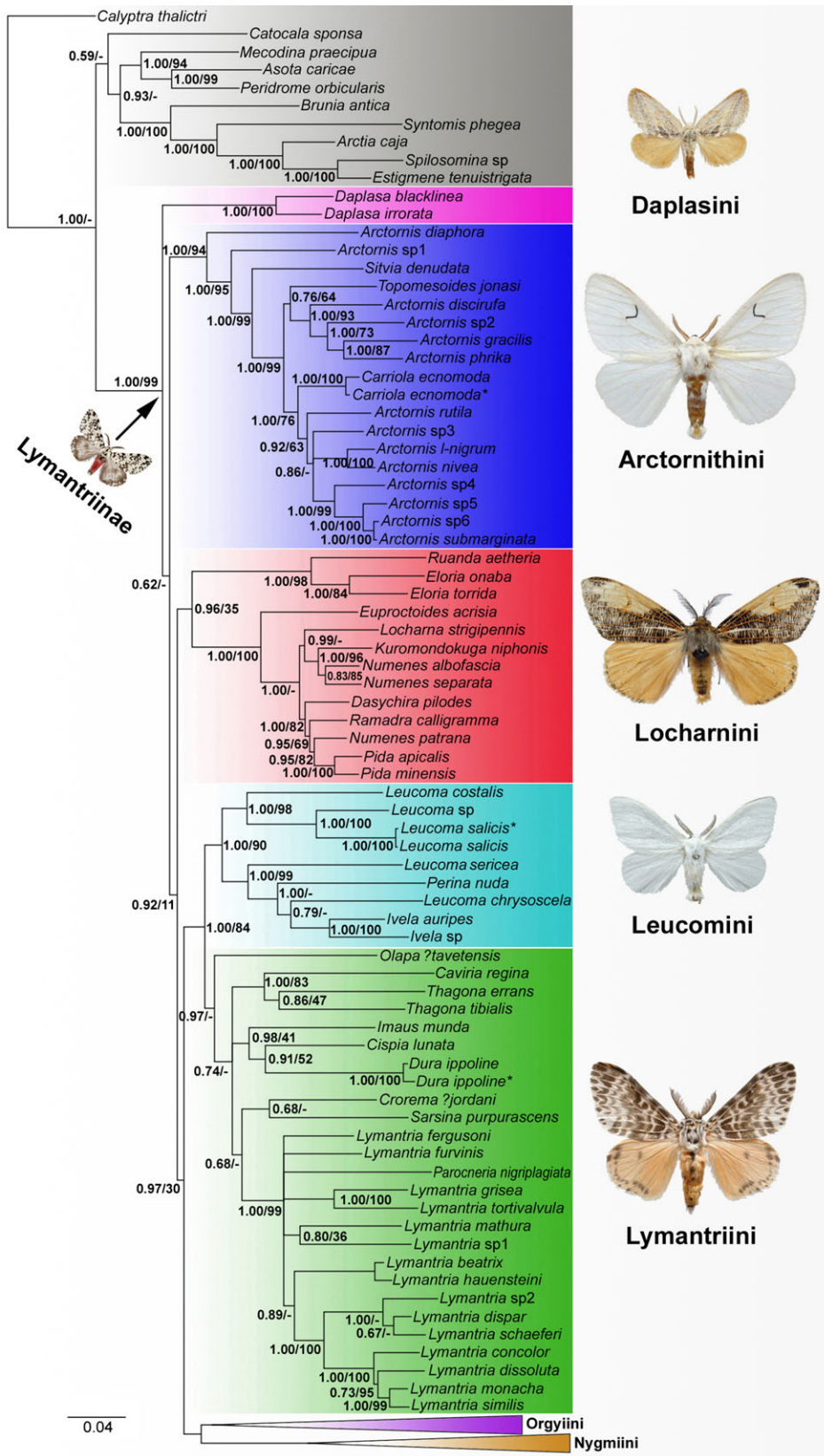
Fig. 1. Majority rule consensus tree from the BI analysis of the full dataset. Posterior probabilities from BI analysis and bootstrap support values from the ML analysis are indicated at the nodes as PP/BP.
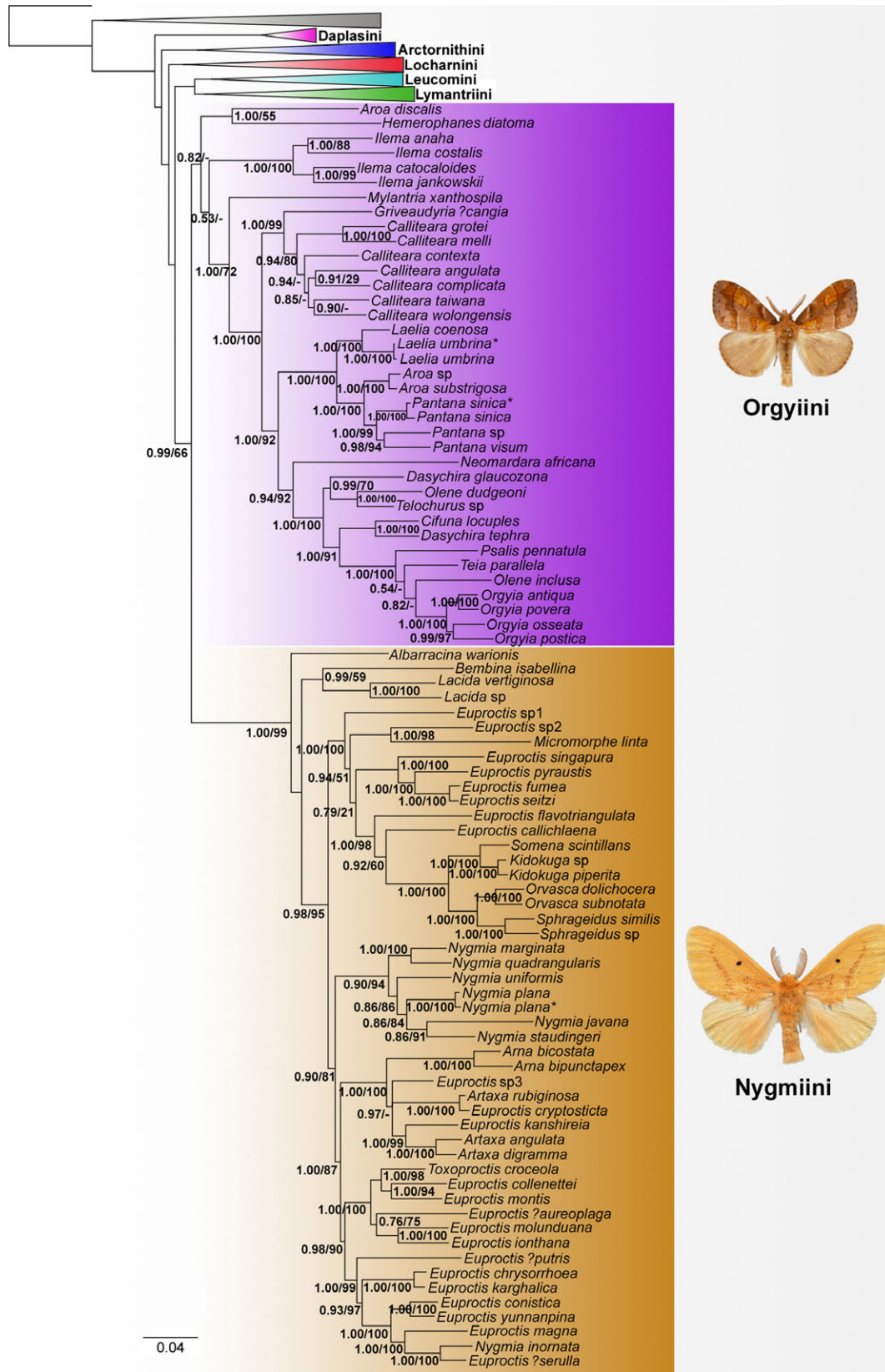
Fig. 1. Continued.

tribe status, two of which are proposed as new. The tribes are Daplasini trib. nov., Arctornithini, Locharnini trib. nov., Leucomini, Lymantriini, Orgyiini and Nygmiini.

Daplasini are fully supported by our analyses as a monophyletic clade consisting of a single genus, *Daplasa* Moore (PP = 1.00; BP = 100; BS = 10). The Arctornithini clade is highly supported by the model-based analyses (PP = 1.00; BP = 94). *Carriola* Swinhoe, *Topomesoides* Strand and *Sitvia* Walker, with their type species sampled, render the genus *Arctornis* Germar paraphyletic with good support; the first two share its morphological features, but the status of *Sitvia* requires further study. MP analysis suggests that the African taxon *Crorema ?jordani* Collenette could also be included in Arctornithini, with only moderate support (BS = 6), but the model-based analyses assign it to Lymantriini.

The monophyly of the Locharnini clade is well supported by the Bayesian analysis (PP = 0.96). Within the clade, the African genus *Euproctoides* Bethune-Baker, four Oriental genera, *Locharna* Moore + *Kuromondokuga* Kishida + *Ramadra* Nye + *Pida* Walker, and species currently misplaced in *Numenes* Walker and *Dasychira* come out as a strongly supported subclade (PP = 1.00; BP = 100; BS = 9), in which *Dasychira pilodes* Collenette, *Ramadra calligramma* Walker and *Numenes patrana* Moore form a clade with *Pida*, and *Numenes albofascia* Leech and *N. separata* Leech are assigned to *Kuromondokuga*. The subclade appears as sister to a pairing of the African genus *Ruanda* Strand and the New World tropical genus *Eloria* Walker in the model-based analyses, but the latter two genera are not associated with the remaining Locharnini in the MP analysis.

The Leucomini clade is composed of *Leucoma* Hübner, *Perina* Walker and *Ivela* Swinhoe with high support in ML and BI analyses (PP = 1.00; BP = 90). The genus *Leucoma* is found to be polyphyletic, as *L. sericea* Moore and *L. chrysoscela* Collenette form an assemblage together with *Perina* and *Ivela*. The parsimony consensus tree also includes an African genus, *Olapa* Walker, but with very low support (BS = 2).

Lymantriini are redefined to accommodate two African genera (*Olapa, Crorema* Walker), three New World ones (*Sarsina* Walker, *Caviria* Walker + *Thagona* Möschler), an Oriental clade (*Imaus* Moore + *Cispia* Walker + *Dura* Moore) and *Lymantria* on the basis of the model-based analyses. The Bayesian tree provides strong support for the monophyly of Lymantriini (PP = 1.00). The genus *Parocneria* Dyar is associated with *Lymantria* with high support (PP = 1.00), but its position is unresolved with regard to *Lymantria* species.

The Oriental genus *Ilema* Moore and three African genera, *Aroa* Walker, *Hemerophanes* Collenette and *Mylantria* Aurivillius, constitute basal clades within Orgyiini, but the precise relationships lack good support. The remainder of the Orgyiini clade is strongly supported as a monophyletic group (PP = 1.00; BP = 72; BS = 16). However, some current generic concepts within the group are polyphyletic. For example, the type species of *Aroa*, *A. discalis* Walker, is the species that is sister to *Hemerophanes*, while two Oriental exemplars, *A. substrigosa* Walker and an unidentified taxon, are grouped as sister to *Pantana* Walker with full support (PP = 1.00; BP = 100; BS = 36) and are therefore not congeneric with the type. *Olene inclusa* Walker appears as sister to the members of *Orgyia*, but *O. dudgeoni* Swinhoe pairs with a representative of *Telochurus* Maes. The two *Dasychira* species are scattered across the distal clades of Orgyiini; a clade with four *Orgyia* species, including the type species *O. antiqua*, is strongly supported (PP = 1.00; BP = 100; BS = 17).

Our results robustly recover the monophyly of the Nygmiini clade (PP = 1.00; BP = 99), but confirm that *Euproctis* and possibly *Nygmia* Hübner are polyphyletic groups. In Nygmiini, *Albarracina* Staudinger is the most basal taxon followed by the *Bembina* Walker + *Lacida* Walker pair in both model-based trees, but this trio is broken up in the parsimony consensus tree. *Somena* Walker + *Kidokuga* Kishida and *Orvasca* Walker + *Sphrageidus* Maes form a strongly supported clade (PP = 1.00; BP = 100; BS = 29). *Arna* Walker is a sister group to *Artaxa* Walker (PP = 1.00; BP = 99; BS = 28), with *Euproctis kanshireia* Wileman, *E. cryptosticta* Collenette and *E.* sp3 included in the latter genus (PP = 0.97; BS = 6).

Resolution of the inter-tribal relationships within Lymantriinae remains limited in our analyses. Daplasini are found at the sister position to all other lymantriine lineages in the BI and MP trees, but branch off after Arctornithini in the ML tree. The model-based analyses indicated that Locharnini form the sister group to a large clade formed by Leucomini + Lymantriini (PP = 1.00; BP = 84) and Orgyiini + Nygmiini (PP = 0.99; BP = 66), but this lacks good support, while the MP analysis suggests that Locharnini branch off after Daplasini, followed by Orgyiini, with the remaining four tribes forming a polytomy.

Discussion

The multi-gene phylogeny in this study, with extensive taxon sampling, recovers seven major lymantriine lineages that we define here as tribes, five of which are roughly concordant with the previous morphology-based concepts of five tribes recognized by Holloway (1999). With regard to the phylogenetic relationships among these five lineages, our results

from the model-based analyses indicate that Arctornithini are a sister group to Orgyiini + Nygmiini together with Lymantriini + Leucomini, supporting the findings of Zahiri et al. (2011, 2012), although the latter dealt with higher taxonomic levels and sampled only a single species per tribe. The fact that a denser taxon sampling recovered the same topology is reassuring and could indicate that it reflects the true phylogenetic relationships. Yet, there are no obvious morphological synapomorphies in support of this topology, and such traits would be desirable before it can be accepted with more confidence. The seven proposed tribes will be discussed in the following sections.

Tribe Arctornithini

Morphologically, the signum of the female genitalia is a broad, sparsely but uniformly scobinate plate, which has been thought to be an apomorphic character of Arctornithini (Holloway, 1999). Speidel and Witt (2011) downgraded this tribe to subtribe, placing it in their redefined Lymantriini based on larval characters. This taxonomic arrangement, however, is not supported by our molecular evidence, as Arctornithini appear in a more basal position in our phylogeny. Larval morphology in *Arctornis* has been found to be strikingly variable in Malaysian species in the form and distribution of hair-tufts (H. S. Barlow, pers. comm.).

Holloway (1999) originally proposed the tribe Arctornithini to accommodate two genera, *Arctornis* and *Carriola*. He mentioned that the two genera share many genital features. He also discussed the similarity of another unplaced genus, *Sitvia*, to *Arctornis* in some features of the genitalia and pupae. The Asian monotypic genus *Topomesoides* was previously assigned by Chao (2003) to his Orgyiinae concept, but the male genitalia of this genus illustrated by him show the definitive features of those of *Arctornis*. In this study, we found that *Carriola*, *Topomesoides* and *Sitvia* Walker (represented by their type species) fell within *Arctornis* with very strong support, and thus transfer them to that genus (syns. nov.), although the morphological justification for this in the case of *Sitvia* is weaker.

Arctornis diaphora has undergone a series of taxonomic changes after it was described by Collenette (1934) in *Leucoma* and then transferred to *Redoa* Walker, a synonym of *Arctornis* established by Holloway (1999). This species was then placed in *Carriola* by Chao (2003), but with illustration of male genitalia that differ strikingly from those of *Arctornis*. Thus, *A. diaphora*, with distinct male genital characters and unusual discal spots on the hindwing similar to those of the forewing, has little in common with the species of *Arctornis*, and thus stands apart within Arctornithini. The basal phylogenetic position of this species in our analyses suggests that it needs further study, particularly with regard to the female.

The tribe has the highest species richness in the Oriental tropics, attenuating eastwards to New Guinea, and has been recorded feeding on a wide range of plant families. The genus *Arctornis* shows particular affinity for Dipterocarpaceae at the larval stage, which may explain the conspicuous radiation of this group in south-east Asian forests that are dominated by this plant family (Holloway, 1999; H. S. Barlow, unpubl. data).

Tribe Daplasini Holloway and Wang trib. nov

Type-genus. *Daplasa* Moore (Holloway, 1999: figs 43–47 of plate 5, figs 235, 237, 242, 245; Chao, 2003: fig. 99 of Plate VI, figs 123–125).

Description. An areole is present in the forewing venation, with R5 branching from Rs more distally than R2. M3 and CuA1 separate in the hindwing. Male abdomen has tymbals. Male genitalia with uncus widely bifid, gnathos present. Female genitalia with signum consisting of an elongate, somewhat oval, marginally dentate plate.

Daplasini are proposed here as a new, currently monobasic (type genus *Daplasa*) tribe. *Daplasa* was previously included by Chao (2003) as a genus in his concept of Orgyiinae, on the basis of having an areole in the forewing. In our phylogeny, the Daplasini exemplars (two rather different species, including the type species, of *Daplasa*) formed a distinct group that fell into a basal position in the Lymantriinae clade. In the male genitalia the uncus consists of two widely separated processes, and there is a well-developed gnathos, extremely large in the type species but only moderate in others. This feature is atypical of the quadrifid Noctuoidea families, and it is unclear whether the socii seen widely in the two trifid ones are homologous with this structure (Miller, 1991). A gnathos is not evident in other Lymantriinae tribes except for Orgyiini where some structures ventral to the uncus in genera such as *Clethrogyna* Rambur, *Olene* Hübner and *Teia* Walker indicate a vestigial presence. Some nygmiine genera such as *Artaxa* Walker have socii. The widely bifid uncus is similar to that seen in some Nygmiini such as *Euproctis singapura* Swinhoe of our analysis, but the wing venation is plesiomorphic relative to that of nygmiines as described by Holloway (1999), who placed two related Bornean species, *lyclene* Swinhoe and *albolyclene* Holloway, under "*Euproctis*" in the tribe Nygmiini, but these now appear to be Daplasini. The venation features that set them apart from typical ny-

gmiines are that R5 branches from Rs more distally than R2, rather than more basally, in the forewing, and M3 and CuA1 are separate in the hindwing, rather than stalked. The tribe is currently restricted to the Oriental Region and exhibits two distinct forewing pattern types: the typical one of *irrorata* Moore with diffuse, oblique banding; and the more sharply marked *blacklinea* Chao type with a black postmedial and tornal streak, and a basal ring of red or yellow spots. The latter includes species such as *variegata* Moore and *lyclene* in which the male has a dark suffusion to the forewing, and the female is white as in both sexes of *blacklinea* and *albolyclene* (*postincisa* Moore may be the female of *variegata*). Males of *irrorata* have a simple aedeagus with no cornutus but those of the *blacklinea* group have an aedeagus that is much longer, basally bulbous and with a single cornutus in the vesica. The female genitalia of the *blacklinea* group have a long ductus bursae with a prominent sclerotized collar distally, whereas *irrorata* has a shorter, wider ductus without a collar, but with a short, broad appendix bursae at the base of the corpus bursae; all have the typical signum. In the *blacklinea* group, *blacklinea* was originally described in *Daplasa* by Chao (2003) and *variegata* was placed there by Kishida (1993). Therefore, the taxa *postincisa* Moore, *lyclene* Swinhoe and *albolyclene* Holloway are here formally transferred to *Daplasa* combs nov.

Tribe Locharnini Holloway and Wang trib. nov

Type-genus. *Locharna* Moore (Holloway, 1999: figs 21, 22 of plate 12, fig. 387; Chao, 2003: figs 91–93 of plate V, fig. 113).

Description. Adults usually show obvious sexual dimorphism. Forewing venation has an areole from which R2 branches off near the apex, R5 somewhat connate or slightly stalked with R3 + 4. Rs stalked with M1 in the hindwing. Tymbals are present in the male abdomen. Male genitalia have apically sclerotized valves.

On the basis of our results, we erect the Locharnini as a new tribe, represented by two sublineages. One comprises two genera, *Ruanda* (Africa) and *Eloria* (Neotropics), and the other one encompasses the remainder of the sampled Locharnini. However, a sister relationship between the two sublineages is not well supported in our analyses. Therefore, we tentatively incorporate both in this tribe until their phylogenetic structure is better resolved, but typify the tribe on *Locharna* in the larger and more cohesive sublineage. The tribe retains a more plesiomorphic condition, such as forewing venation with an areole. Species in the typical sublineage tend to have a short, robust uncus and relatively simple, apically sclerotized valves in the

male genitalia. The type genus and two other Oriental genera, *Pida* and *Kuromondokuga*, are strikingly sexually dimorphic (Chao, 2003), and our results suggest that other taxa sequenced could be incorporated within one or the other of these genera, as follows.

Numenes was classified in Orgyiini by Holloway (1999), with the suggestion that placement of some Chinese species in this genus was problematic, as their male genitalia features were quite different from those of the type species, *N. siletti* Walker. Our phylogeny indicates that *Numenes* is not a monophyletic group, even within Locharnini. This supports the morphological evidence, although no member of the typical group was available for sequencing. Here we transfer *N. patrana* to the genus *Pida* comb. nov., which then also includes *Dasychira pilodes* comb. nov. and *Ramadra calligramma* comb. nov., bringing *Ramadra* (= *Mardara* [praeocc.]) into synonymy with *Pida*, syn. nov. We transfer *N. albofascia* and *N. separata* into *Kuromondokuga* combs nov., a genus segregated by Kishida (2011) from *Pida*.

Tribe Leucomini

Members of the tribe Leucomini were morphologically defined by Holloway (1999) as sharing pronounced asymmetry in the male genitalia, although this feature is not seen in the type species of *Leucoma*. *Leucoma* currently consists of a group of many satiny white species with a reddish orange tinge to the forelegs, and has been suggested to be paraphyletic (Holloway, 1999). This is confirmed by our molecular phylogeny, indicating that further revision of this genus is needed, possibly with revival of current synonyms such as *Charala* Moore (type species *sericea*) and *Candidata* Toxopeus (type species *subargentea* Felder). *Leucoma* ranges from the Palaearctic to Africa, New Guinea and Australia, with highest species diversity in the Oriental tropics. *Perina* is an Oriental genus that displays extreme sexual dimorphism, the much smaller males being black with transparent areas in the wings; the larvae have only been recorded feeding on *Ficus* Röding (Moraceae). The Asian genus *Ivela*, now included in Leucomini, shares the definitive male genital characters with the other genera of the tribe.

Tribe Lymantriini

The tribe Lymantriini was defined by Ferguson (1978) based on the following features: adults lack an accessory cell (areole) in the forewing and the larvae do not have hair pencils or dense dorsal tufts on the first four abdominal segments. The tribe was subsequently redefined by Holloway (1999), with diagnostic features such as forewing facies with zig-zag or lunulate fasciation, a V-shaped discal spot and an orbicular

spot. Speidel and Witt (2011) proposed a broader concept of this tribe that includes three subtribes (Lymantriina, Arctornithina and Leucomina), on the basis of larval characters that they considered to be apomorphic, as follows: seta L1 replaced by a verruca in a postero-dorsal position from the spiracle; and verrucas L2 and L3 approximate to each other on abdominal segments 1–6. This concept is not supported by our results, so we follow Holloway's (1999) taxonomic treatment, but add two African genera (*Olapa* and *Crorema*), three New World genera (*Caviria*, *Thagona* and *Sarsina*) and the genus *Cispia* to Lymantriini. The tribe thus has a global distribution, but still with its greatest diversity in the Oriental Region; this picture may change as further African genera are sequenced.

The genus *Lymantria* is the most species-rich genus, and there is sexual dimorphism in size and pattern. In our phylogeny, the internal structure within the genus accords quite closely with the morphological subgeneric phylogeny suggested by Schintlmeister (2004), except for the pairing of *Parocneria* with *Lymantria*. However, many of the subgenera remain to be sequenced. Sutrisno (2014) also noted extensive, but not complete, support for the subgenera of Schintlmeister (2004) in his analysis of *COI* gene data from a sample of just over 40 *Lymantria* species (most of the sequences originating from deWaard et al. (2010)).

Tribe Orgyiini

Orgyiini are most clearly defined by the dorsal brushes on the first four larval abdominal segments (Holloway, 1999). The presence of an areole in the forewing was another diagnostic character suggested for this tribe by Ferguson (1978), but this feature is also found in Daplasini, Arctornithini and Locharnini, so is probably plesiomorphic.

The African sister genera *Aroa* and *Hemerophanes* and the genus *Ilema*, sharing the above larval features, are included as basal lineages in Orgyiini in our phylogeny, but lack good support for the precise positions. Further sampling is therefore needed to clarify their taxonomic placement.

Holloway (1982) revived the genus *Calliteara* Butler from synonymy with *Dasychira* and divided it into seven species groups. The monophyly of this genus is moderately supported by our analyses, with the African genus *Griveaudyria* Viette placed as sister to it. A high proportion of the species are montane, and all are robust with relatively narrow forewings. The larva is typically orgyiine, but with an additional, shorter, dorsal brush on the eighth segment, a feature also seen in *Ilema* and *Cifuna* Walker.

Our results provide strong support for the sister relationship of *Laelia* Stephens and *Pantana*, with

Aroa substrigosa transferred here to the latter genus, comb. nov. Both genera were discussed by Holloway (1999). Their larvae are considered to be specialists on Poaceae and Cyperaceae, and the adults are day-flying (Schaefer, 1989). *Psalis pennatula* Fabricius (see below) also shows some preference for Poaceae.

The genus *Dasychira* as historically defined was thought to be a very complex group (Schaefer, 1989); this is further confirmed by our study, although *Calliteara* had already been excluded, as noted above. The type species, *D. tephra* Hübner, was considered by Ferguson (1978) to be part of an exclusively North American group and he suggested that *Dasychira* should be restricted to this group. It is placed in our study as sister to *Cifuna*. This pair is one of three subclades of a well-supported distal clade of the Orgyiini that is sister to the African genus *Neomardara* Hering. Many generic names are represented in this clade, often by their type species, including *Orgyia*, leading Holloway (1999) to attempt to find an acceptable classification that excluded *Dasychira* and retained *Orgyia*, but in the process he broadened the concept of *Olene* Hübner to include the species *dudgeoni*. Our results indicate this to have been a mistake because *dudgeoni* is placed in the most basal subclade, but the more typical *Olene inclusa* is part of the third subclade that also includes *Psalis* Hübner, *Teia* Walker and four *Orgyia* species. The basal subclade includes a species thought to be a *Telochurus* and *Dasychira glaucozona* Collenette as well as *dudgeoni*. The oldest generic name available for this subclade is *Pseudodura* Strand, based on a current junior synonym of *dudgeoni*. More extensive sampling will be needed across this complex to resolve the generic classification satisfactorily.

Tribe Nygmiini

The tribe Nygmiini was described by Holloway (1999), comprising 16 genera. Most of these genera were segregated from the old concept of *Euproctis*. Kishida (2011) recognized another distinct genus, *Kidokuga*. Speidel and Witt (2011) transferred *Albarracina* to Nygmiini, a move supported by our analyses. The tribe is best defined by possession of a strong corethrogyne on an expanded seventh segment of the female. Holloway (1999) also noted characters that might be definitive as discussed earlier in relation to Daplasini; an areole is absent from most genera, another potential apomorphy. Our phylogeny with 13 genera sampled strongly supports the monophyly of the tribe, agreeing with the previous morphological classification.

In our results, the old broad concept of *Euproctis* remains extremely polyphyletic, and is in great need of revision, although the results do provide some reassurance that recent attempts to identify valid generic concepts within the *Euproctis* complex have some validity

whilst at the same time emphasizing the scale of the task remaining.

The revival of the genus *Bembina* by Holloway (1999) is supported, but it is paired with *Lacida*; these genera do not appear to be close morphologically, with *Bembina* lacking tymbals and both genera having distinctive male genitalia.

The next large clade is well supported but mixes many species currently in *Euproctis* with representatives of *Micromorphe* Felder, *Somena + Kidokuga* and *Orvasca + Sphrageidus*, the last four sharing loss of vein M2 in the hindwing (Holloway, 1999).

Somena and *Kidokuga* form a well-supported lineage. The type species of both genera share a bicornute signum in bursa of the female, providing morphological support for the lineage, although their male genitalia structures show great differences (Kishida, 2011; Wang et al., 2011).

The lineage of *Orvasca* and *Sphrageidus* is also well supported. *Orvasca* has a major representation in Australia, referred to under *Chionophasma* Butler by Common (1990), and many of the smaller species in New Guinea and the south-west Pacific may also be referrable to this genus (Holloway, 1999). *Orvasca dolichocera* Collenette in our analysis is an example of this from Vanuatu.

Also in this clade, the four Oriental species, *E. singapura* Swinhoe, *E. pyraustis* Meyrick, *E. fumea* Chao and *E. seitzi* Strand, share similar male genitalia structures, with the following diagnostic features: broadly bifid uncus, rather square valves and single cornutus in the aedeagus vesica (Schintlmeister, 1994; Chao, 2003). They form a well-supported clade (PP = 1.00; BP = 100), which may represent a potential genus. Some allies such as *E. wilemani* Collenette and *E. subfasciata* Walker were noted by Holloway (1999).

All representatives of *Nygmia* sequenced except one are recovered in the next clade with moderate support. Probably many of the groups of larger species in Australasia will be found to belong here, as Holloway (1999) transferred several species to *Nygmia* from that region that typified some of the major species complexes.

The most distal clade in the Nygmiini, itself well supported, has three well-supported subclades. The first suggests a sister relationship between *Arna* Walker and *Artaxa*. However, *E. kanshireia*, *E. cryptosticta* and *E.* sp3 are mixed in the genus *Artaxa*.

The second includes a representative of *Toxoproctis* Holloway, counter to the suggestion by Holloway (1999) that this genus might be related to *Sphrageidus*. This subclade also includes species such as *E. collenettei* Chao and *E. montis* Leech that have similar male genitalia to *Choerotricha biflava* Holloway (Holloway, 1999; Chao, 2003). Three of the four African *Euproctis* sequenced fall into this clade as a trio that forms a sister group to the species just mentioned. The fourth, tentatively identified as *E. putris* Hering, occupies a basal position in the next clade.

The third subclade includes the type species of *Euproctis*, *E. chrysorrhoea*, paired with the Palaearctic *E. karghalica* Wileman.

Nygmiini occur in the Old World with major higher level diversity in the Oriental tropics, but have yet to be identified in the New World. Most species appear to be polyphagous, although there are indications of some specialization on Loranthaceae by species groups in *Nygmia* (Holloway et al., 2001).

Biogeography

To conclude, we place these revised and globally based tribal concepts within Lymantriinae in a biogeographical context. All seven tribes are found in the Oriental Region, Daplasini uniquely so. If the basal position of Daplasini found by the model-based approaches is correct, the most parsimonious interpretation of the biogeographical patterns would thus be that the subfamily originated in the Oriental Region. However, as can be seen in the following descriptions of the remaining tribes, this conclusion is weakened not only by remaining phylogenetic uncertainty but also by wide distributions.

Arctornithini are highly diverse in the Oriental Region but extend into tropical Australasia and the Palaearctic. Leucomini are similar but relatively less diverse in the Oriental Region. The type genus and species, *Leucoma salicis*, extends to the Nearctic. The status of both tribes in Africa is unclear, but the genus *Leucoma* as currently constituted is represented by several species there.

Locharnini are more widespread, with significant pattern at a higher taxonomic level. The type lineage is restricted to the Oriental Region, but has a sister genus in Africa. This group is in turn sister to a small but intriguing lineage of two genera: the African *Ruanda* and the Neotropical *Eloria*.

Lymantriini are the only other tribe to include Neotropical genera: *Caviria*, *Sarsina* and *Thagona*. Otherwise, this tribe is widespread and diverse in the Old World, more so in the tropics. One species, *Lymantria dispar*, occurs in the Nearctic as an introduction.

Nygmiini are essentially an Old World group with only the type species of *Euproctis* and *Sphrageidus* extending to the Nearctic. Again, the greatest diversity is Oriental, but the group is relatively rich in Australasia, and there appear to be distinct lineages in Africa.

Orgyiini have a similar distribution to Nygmiini but with a significant Nearctic representation consisting of the genus *Dasychira* and several species of *Orgyia*, one of which extends to Central America. Only Nygmiini

and Orgyiini extend into the Pacific, but not further than Fiji and New Caledonia.

The lowest diversity at all levels appears to be in the New World, with little overlap between the Nearctic and the Neotropics. The genus *Desmoloma* Felder is of interest in the latter, as DNA barcoding results suggest that it is distinct from genera assigned in this analysis to the Locharnini and Lymantriini (data from http://www.boldsystems.org). There are still a few unsequenced Oriental genera unplaced to tribe (Holloway, 1999), and there are many genera in Africa left to sequence; it is therefore possible that the synopsis above will see changes as these gaps are filled.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Strict consensus of four equally parsimonious trees from the combined dataset of all eight genes (length 29 824, consistency index 0.145, retention index 0.542). Numbers given above branches are Bremer support values.

Table S1. List of taxa with voucher codes and GenBank accession numbers (TS, type species).