Systematics and origin of moths in the subfamily Arctiinae (Lepidoptera, Erebidae) in the Neotropical region

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The availability of standard protocols to obtain DNA sequences has allowed the inference of phylogenetic hypothesis for many taxa, including moths. We here have inferred a phylogeny using maximum-Likelihood and Bayesian approaches for a species-rich group of moths (Erebidae, Arctiinae), with strong emphasis on Neotropical genera collected in different field campaigns in the Atlantic Forest of Brazil, eastern Amazon and southern Ecuador. A total of 277 species belonging to 246 genera were included in the analysis. Our main objectives were to shed light on the relationships between suprageneric groups, especially subtribes, and hypothesize colonization events in and out of the Neotropics. The monophyly of Arctiinae and its four tribes (Lithosiini, Amerilini, Syntomini and Arctiini) was recovered in the ML and Bayesian trees. Three Lithosiini subtribes previously found and two additional species groups were recovered monophyletic in both phylogenetic estimation methods. In Arctiini, the monophyly of Spilosomina and Arctiina was highly supported in the ML and Bayesian trees, but the monophyly of Ctenuchina and Echromiina was weakly supported in the ML tree and absent in the Bayesian tree; the remaining subtribes were paraphyletic and, in the case of Phageopterina, formed several species groups. The mapping of species occurrence in our ML tree suggests that Arctiinae have an Old World origin and that the Neotropical region was colonized at least six times independently. Our analysis also suggests that a number of species that occur in Neotropical and other zoogeographic regions may have originated in the Neotropics, although further taxon sampling is required to support this hypothesis. To our knowledge, this is the first time that a highly speciose group of tropical moths is well covered in a phylogeny, and it seems plausible that the results reported here may be extendable to other species-rich tropical undersampled moth taxa.

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As the field of phylogenetic systematics became formalized as a science in the mid-twentieth century, it has been developed into a multidisciplinary field that utilizes concepts and methods from a wide range of disciplines. The availability of genomic data in conjunction with paleontological and morphological evidence has been helping to resolve the classification of insects (Trautwein *et al.* 2012). Examples of congruent results between morphology and molecules are the recent and widely accepted phylogeny of Holometabola (Beutel *et al.* 2010; Misof *et al.* 2014) and the discovery that Hexapoda is very likely a crustacean lineage (Giribet *et al.* 2001; Regier *et al.* 2010). Although an integrative approach is considered the most effective way to do systematics, it is usually highly dependent on fossil records, which may not be available, and/or on thorough morphological analysis that can be very time-consuming if a large number of taxa are analysed (Scotland *et al.* 2003; Heikkilä *et al.* 2015). The rapid rate with which genomic data can be produced for phylogenetic analysis and the usually high congruence with the results based on other sources of biological variation have allowed DNA-based phylogenies to become the standard in systematics (Hillis 1987; Pisani *et al.* 2007).

The rapid increase in genomic data being produced has made it possible to infer relationships for most insect orders, including Lepidoptera (Mutanen *et al.* 2010; Regier *et al.* 2013). Higher-level classifications based on molecular phylogenies have been proposed for, among others, the most species-rich (Noctuoidea, Zahiri *et al.* 2011) and conspicuous (butterflies and skippers, Heikkilä *et al.* 2012) groups of Lepidoptera, as well as many enigmatic groups such as metalmark moths (Choreutidae, Rota & Wahlberg 2012). The growing interest in the phylogenetic relationships of Lepidoptera based on molecular data has been accompanied by the development of new analytical methods and discussions on analytical strategies. Perhaps one of the most debated issues is whether increasing the number of characters and/or taxa in the data matrix would also increase the accuracy of phylogenetic estimation (Poe & Swofford 1999; Rosenberg & Kumar 2001; Hillis *et al.* 2003; Heath *et al.* 2008; Nabhan & Sarkar 2011). The alignment length and the accuracy of the phylogenetic estimation have been increased considerably with the development of phylogenomics; for example, Misof *et al.* (2014) used 1478 genes to infer a robust phylogeny for insects. Furthermore, it has been shown that the accuracy of tree estimation is also usually improved by increasing the number of taxa in the data matrix, although issues can occur when taxa share a large number of homoplastic characters erroneously interpreted as being homologous (*i.e.* long-branch attraction LBA, Poe & Swofford 1999; Hillis *et al.* 2003; Heath *et al.* 2008). Fortunately, this is of less concern when groups such as Lepidoptera are considered (but see Wahlberg *et al.* 2003) and when less sensitive methods to LBA (*i.e.* maximum-likelihood and Bayesian inference) are used to infer the tree (Heath *et al.* 2008).

A careful sampling of taxa within a group/clade is part of any well-designed phylogenetic study, but the large number of undescribed/unknown species makes a complete taxon sampling very difficult or even impossible. A total of ca. 160 000 species of Lepidoptera are known to science (van Nieukerken *et al.* 2011), but the actual number is estimated at ca. 500 000 (Kristensen *et al.* 2007). Most of this undocumented biodiversity is concentrated in tropical countries, which have a shortage of systematists and are under an intense and uncontrolled deforestation process (Duarte *et al.* 2012). Therefore, our capacity to infer evolutionary relationships among taxa is not only constrained by methodological issues such as the number of characters and taxa in the data matrix, especially if we consider the latest breakthroughs in that area (Lemmon & Lemmon 2013), but also by the limited time and resources spent recording and analysing biodiversity in tropical regions.

The subfamily Arctiinae is a taxon of moths found in all zoogeographic regions and, as in many arthropod groups, its diversity peaks within the tropics (Brehm 2009; Weller *et al.* 2009). With more than 11 000 described species, arctiines have been studied in many contexts, from chemical ecology to adaptive coloration and mimicry, including acoustic mimicry (Conner *et al.* 2009; Conner & Corcoran 2012). Perhaps one of the most interesting topics is the relationship between insects and their host plants. Several arctiines are known to be 'specialized generalists' (Singer & Bernays 2009), meaning that they can feed on a wide variety of plants but also depend on specific plants. The specific plants usually contain chemical compounds such as pyrrolizidine alkaloids that make moths unpalatable and are thus avoided by predators (Singer & Bernays 2009). This has allowed arctiines to form various mimicry rings with other moths, butterflies, cockroaches, lycid beetles and other insects. The alpha- and beta-diversity patterns of arctiines along the environmental gradients have also been studied (*e.g.* Hilt & Fiedler 2005, 2006; Zenker *et al.* 2015), particularly in the Neotropical region which is regarded as the most species rich of all regions (Brehm 2009).

The classification of Arctiinae has always been contentious, and species have been placed in different superfamilies and in as many as six separate families before a proper phylogenetic analysis was available (Weller *et al.* 2009). The first cladistic analysis was based on adult and immature morphological characters and recognized three groups (*i.e.* Arctiinae, Syntominae and Lithosiinae) within the former family Arctiidae (Jacobson & Weller 2002). More recently, Zahiri *et al.* (2011) proposed a new

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classification for Noctuoidea, and Arctiidae along with other families were ranked at subfamily level and included in Erebidae. Two recent molecular studies resulted in valuable insights into the evolution of the Arctiinae. In the first molecular phylogenetic study of the tribe Lithosiini, Scott *et al.* (2013) found that four of the seven subtribes proposed by Bendib & Minet (1999) based on morphological information could be recovered, although three of them had low branch support. The most in-depth study and the only molecular phylogeny of the whole Arctiinae to date (Zaspel *et al.* 2014) corresponded largely to that of Jacobson & Weller (2002), recovering the monophyly of the tribes, although neither phylogeny could completely resolve the relationships between subtribes. Moreover, they used different sets of taxa: for example, only one species of the tribe Lithosiini was represented in the analysis of Jacobson & Weller (2002), whereas 15 were included in the tree of Zaspel *et al.* (2014).

Although the two abovementioned studies have included species of all known subtribes and have sampled a diversity of zoogeographic regions, the number of tropical species was underrepresented in their tree, and the monophyly of several subtribes could not be established. We here add a large number of neotropical genera to the phylogeny of Zaspel *et al.* (2014) to test whether additional clades occur and whether these clades correspond to existing tribes and subtribes. In addition to better understand the classification of Arctiinae, especially at the subtribe level, the phylogenetic framework obtained here was used to formulate a basic biogeographic hypothesis for Arctiinae in the Neotropical region. Our phylogenetic hypotheses can be further tested by including taxa from the Afrotropical, Australasian and Oriental regions which deserve a more complete taxon sampling as now available for the Neotropics. Our study is the result of collaboration between different research groups working on biodiversity surveys in different parts of the Neotropical region (see Material and Methods section).

Material and methods

Taxon sampling

Most of samples used in this study were obtained from collections performed in different parts of the Neotropical region between 2000 and 2013. These collections were performed independently by different research groups, aiming to record and compare diversity patterns in their study areas. The taxon sampling was designed to cover a broad range of Arctiinae genera from different regions of South America, namely Atlantic Forest of Brazil, eastern Amazon rainforest and Andean rainforest of Ecuador (Fig. 1A). The exact location of the sampling sites, the sampling procedures employed and the complete lists of taxa found in the three abovementioned regions can be found in Zenker *et al.* (2015, 2016), Freitas (2014) and Süßenbach (2003), respectively. An updated list of Arctiinae species of southern Ecuador is in preparation (G. Brehm, unpublished data).

A total of 268 species representing 235 genera of arctiines were included in the analysis along with nine species, representing three subfamilies of Erebidae (*i.e.* Calpinae, Lymantrinae and Aganainae), used as out-groups (Appendix S1). We used a total of 80 species representing 65 genera included in the phylogeny of Zaspel *et al.* (2014), and five additional species of five different genera from our database. The gene regions used in this work corresponded largely to the same gene regions used in Zaspel *et al.* (2014), and because of this compatibility, it was possible to combine data sets into one data matrix. To the phylogenetic backbone of Zaspel *et al.* (2014), we added a total of 183 species representing 173 genera. These taxa were Neotropical, except for seven African species provided by LP. An overview of all sampled taxa is provided in Appendix S2.

Taxonomic treatment

The assignment of species into tribes and subtribes obtained with our phylogeny was compared to that of previous works. Arctiini species (subtribes Phaegopterina, Pericopina, Arctiina and Spilosomina) were assigned to subtribe based on the catalogue of Vincent & Laguerre (2014); the remaining Arctiini species (subtribes Ctenuchina and Euchromiina) and Lithosiini species were assigned to subtribes based on Bendib & Minet (1999), Jacobson & Weller (2002), Witt & Ronkay (2011) and Scott *et al.* (2013). Outgroup species were assigned to subtribes based on catalogues covering all moths (Holloway 2001) and Noctuoidea (Lafontaine & Schmidt 2010).

DNA extraction, PCR and sequencing

Two different protocols of DNA extraction were used in two different sets of samples. The DNA of the samples obtained from the Atlantic Forest (100 species) was extracted between 2010 and 2011 at the Biodiversity Institute of Ontario, Canada (BIO), following the standard protocols (Hajibabaei *et al.* 2005; Ivanova *et al.* 2006; deWaard *et al.* 2008). These extracts were subsequently shipped to Finland for further laboratory work. The DNA of the remaining 83 samples was extracted in the laboratory of the Department of Genetics, University of Turku, Finland. We extracted DNA from one dried leg of each specimen using the NucleoSpin® Tissue Extraction Kit (Macherey-Nagel).

The PCR reactions for all samples were done using the MyTaq™ Red Mix (Bioline) in a final volume of 12.5 μl

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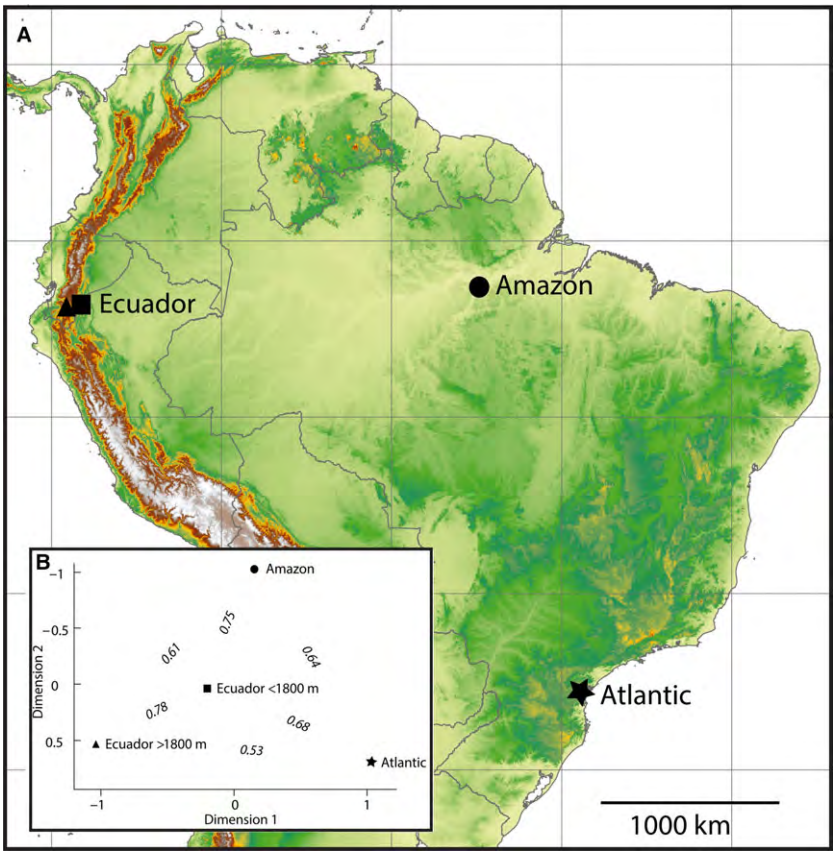

Fig. 1 —A. Map with the major study areas, —B. ordination (two-dimensional scaling) of the occurrence of Arctiinae genera in the study areas. The symbols correspond to the sampling regions: star (southern Atlantic Forest), circle (Eastern Amazon), square (southern Ecuador below 1800 m a.s.l.), triangle (southern Ecuador above 1800 m a.s.l.). Numbers between symbols represent species turnover between sampling sites.

per sample. For each reaction, we used 4 μl of MQ-H_20, 6.25 μl of 2x MyTaq Red Mix, 0.625 μl of both forward and reverse primers and 1 μl of extracted DNA. To facilitate the high-throughput PCR and sequencing, a pair of universal primers was attached to each of the degenerated primers, following the standard procedures of the Nymphalidae Systematics Group (NSG), available at http://www.nymphalidae.net/. We used eight of the 11 genes proposed by Wahlberg & Wheat (2008) which were shown to be robust and stable across different reconstruction methods, namely carbamoylphosphate synthase domain protein (CAD), cytochrome oxidase subunit I (COI), elongation factor 1-α protein (EF1-α), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), isocitrate dehydrogenase (IDH), cytosolic malate dehydrogenase (MDH), ribosomal protein S5 (RpS5) and wingless (Wg). The primers and PCR cycling profiles followed Wahlberg & Wheat (2008) and references therein, and are available at the NSG website. The PCR products were cleaned using the A'SAP™ PCR Clean-up kit (ArcticZymes) and shipped to Macrogen in Holland where they were sequenced using the Sanger sequencing method.

Data analysis

The quality of the sequences was checked with their respective chromatograms, and poor quality and/or very short sequences were excluded from the data set. The sequences were aligned with template sequences of each gene based on their chromatograms using the program Mega 6 (Tamura *et al.* 2013) and uploaded into the VoSeq database (Peña & Malm 2012). All sequences obtained for each species were concatenated, and the data matrix was exported in Phylip and Nexus formats using tools implemented online in the VoSeq database.

We used maximum-likelihood and Bayesian inference as phylogenetic reconstruction methods, which have been shown to outperform the traditional methods of maximum parsimony and neighbor joining (Ogden & Rosenberg 2006) and avoid the occurrence of the long-branch attraction (Heath *et al.* 2008). The maximum-likelihood analyses as well as the bootstraps (1000 replicates) used as branch support values were done using RAxML-HPC2 on XSEDE implemented on CIPRES Science Gateway (Miller *et al.* 2010); the ML analysis was run under the GAMACAT model (Stamatakis 2006). Each gene was considered as a different partition, following the previous studies on

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Noctuoidea (Zahiri *et al.* 2011, 2012; Zaspel *et al.* 2014). The Bayesian inference was done using MrBayes on XSEDE on CIPRES Science Gateway. We performed 10 million generations with sampling every 1000 generation and four chains, one cold and three heated, in two independent runs. The parameters and models of evolution were unlinked across character partitions, and the mixed evolutionary model was used. The convergence of the two runs was ascertained by visual inspection of the log-likelihoods stationary distribution, discarding the first 25% sampled trees, as well as by checking that the final average standard deviation of split frequencies was below 0.05 and that the potential scale reduction factor (PSRF) for each parameter was close to 1.

Biogeography patterns

We used two approaches to identify basic biogeographic patterns. First, we mapped the zoogeographic regions on our maximum-likelihood tree using parsimony in the program Mesquite (Maddison & Maddison 2015); to complement our own (incomplete) occurrence data, we used data retrieved from the Bold Systems database (http://www.boldsystems.org). Secondly, we assessed the genus turnover using an unconstrained ordination method (Brehm & Fiedler 2004) in four sampling regions: a) Atlantic forest, b) eastern Amazon forest, c) Ecuador lowland forest up to 1800 m a.s.l. and d) Ecuador high-elevation forests above 1800 m. The turnover was calculated using EstimateS 9.1.0 (Colwell 2013), and the multidimensional scaling (MDS) ordination was performed using the software Statistica 7.1 (StatSoft, Tulsa, USA).

Results

Systematics

The sampling coverage varied markedly between gene regions, from 95.08% in the barcode region to 28.99% in the CAD region (Table 1). We obtained an average of 60.73% of the total alignment length (Table 1).

The topology obtained with the ML and Bayesian analyses is shown in Fig. 2 and in the Appendix S3, respectively. We chose the ML tree as the basis for our discussion; any differences between topologies are stated in the text. Branch support values obtained with both methods are shown in Fig. 2. The monophyly of Arctiinae and its four tribes was recovered by both methods as previously shown elsewhere (Zaspel *et al.* 2014). The inclusion of two African species of Amerilini (*Amerila brunnea* and *Amerila vitrea*) corroborated the monophyly of this tribe.

Five Lithosiini clades were highly supported: two small groups with three and two genera each, here called Group 1 and Group 2, and three of the seven subtribes proposed by Bendib & Minet (1999), and recovered with low branch support by Scott *et al.* (2013). Two of the three species included in Group 1 (*Clemensia marmorata* and *Pronola magniplaga*) were previously assigned to Cisthenina by Bendib & Minet (1999), and one species (*Garudinia simulana*), to our knowledge, has never been assigned to any subtribe. In Group 2, one of the species (*Eugoa bipuncta*) was previously assigned to Cisthenina (Holloway 2001), and another (*Setina irrorella*) was assigned to Endrosina (Bendib & Minet 1999; Witt & Ronkay 2011). The species included in Cisthenina, Lithosiina and Nudarina had been assigned to these groups before. *Minitopola braziliensis* was found to be closely related to *Agylla*.

The results of ML and Bayesian analyses differed significantly in the tribe Arctiini. Except for Spilosomina and Arctiina, Bayesian inference did not recover any monophyletic subtribe, while two additional subtribes (Ctenuchina and Euchromiina) were monophyletic in the ML analysis, although the last two with low branch supports (Fig. 2). A clade comprising Ctenuchina and Euchromiina was well supported in the ML analysis (bootstrap = 89), but absent in the Bayesian tree. The subtribe Callimorphina was represented by the same taxa included in Zaspel *et al.* (2014), except for the African species *Alytarchia leonina*. Our results show that the genus *Alytarchia* is the closest known relative of *Utetheisa*, the only Callimorphina genus that occurs in the Neotropical region (Weller *et al.* 2009).

The ML and Bayesian analyses revealed that the subtribes Pericopina and Phaegopterina were paraphyletic (see Fig. 2). Both phylogenetic estimation methods supported two clades of Pericopina, although the posterior probability was lower in one of them. A total of three large clades and one lineage leading to the *Anaxita* grade into the well-supported clade Ctenuchina+Euchromiina in the ML tree. The relationships between Phaegopterina and the clade comprising Ctenuchina+Euchromiina could not be established in the Bayesian tree, and only one of the larger clades and the monospecific lineage found in the ML tree were also found in the Bayesian tree. Additionally, the remaining taxa included in Phaegopterina were grouped into different smaller clades or monospecific lineages (Appendix S3).

The classification of four genera assigned to Ctenuchina according to our bibliographic revision conflicts with their current grouping in Euchromiina: *Pseudosphex rubripalpus*, *Pseudaclytia unimacula*, *Demolis albicostata* and *Prosopidia oviplaga*. On the other hand, four genera assigned to Euchromiina in the same bibliographic revision group in the Ctenuchina: *Belemnia ochriplaga*, *Orcynia* sp., *Coystea analis* and *Mevania basalis*. The assignment of these genera should be checked morphologically and be revised in the future systematic work. As previously noted by Zaspel *et al.*

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Table 1 Number of species and sampling coverage of gene regions in the higher taxa used in this study

	Lithosiini	Amerilini	Syntomini	Callimorphina	Spilosomina*	Pericopina	Phaegopterina	Ctenuchina	Euchromiina	Total
N° species	39	4	5	13	26	9	94	37	41	268
CAD	33.88	0.00	39.35	22.77	15.65	44.35	27.09	46.43	21.55	28.99
COI-begin	97.60	99.51	98.69	90.85	96.48	87.31	95.95	92.32	94.45	95.08
COI-end	69.39	74.35	54.32	45.30	33.24	76.73	79.25	79.99	74.36	70.44
EF1α-begin	62.76	67.24	15.42	38.46	34.66	89.40	68.85	63.59	69.07	62.15
EF1α-end	85.42	50.14	58.85	60.04	70.1	60.4	76.8	71.62	87.69	76.26
GAPDH	14.28	34.33	18.87	17.84	25.62	29.01	40.40	41.71	43.39	33.84
IDH	41.35	40.89	37.95	41.07	17.66	59.70	51.91	57.53	47.57	46.47
MDH	33.33	41.40	34.69	28.48	43.21	84.63	65.99	58.97	43.16	52.42
RpS5	86.72	47.64	57.38	93.01	76.59	97.72	87.40	76.79	80.48	83.19
Wingless	70.62	56.68	79.87	80.87	72.97	68.73	66.90	66.55	65.40	68.58
Total	59.18	49.94	79.87	50.61	46.55	67.67	64.71	65.32	62.05	60.73

Sampling coverage of gene regions is given in percentages based on a total number of base pairs expected in each gene region/taxon – CAD: 865 bp, COI-begin: 670 bp, COI-end: 806 bp, EF1α-begin: 541 bp, EF1α-end: 699 bp, GAPDH: 691 bp, IDH: 722 bp, MDH: 407 bp, RpS5: 603 bp, wingless: 475 bp, total alignment length: 6479 bp.
*Arctiina included.

(2014), two Lithosiini/Nudarina genera (*Miltochrista* and *Lyclene*), and a genus belonging to Euchromiina (*Syntomeida*), and another belonging to Phaegopterina (*Melese*) were paraphyletic. Additionally, our results suggest that two Spilosomina genera (*Estigmene* and *Hypercompe*) are also paraphyletic.

Biogeography

The mapping of the species occurrence in the maximum-likelihood tree (Fig. 2) suggests that Arctiinae have an Old World origin and that the Neotropical region was colonized at least six times independently (Fig. 3). Three radiations of different magnitudes may have occurred in Lithosiini: a smaller one in Group 1, a medium one in Lithosiina and a larger one in Cisthenina (Fig. 3A). Several radiations may have occurred in Arctiini, with the most prominent including the four most speciose subtribes of Arctiini in the Neotropics: Phaegopterina, Pericopina, Ctenuchina and Euchromiina (Fig. 3A). Smaller radiations may have occurred in the subtribes Spilosomina and Arctiina, and the genus *Utetheisa* (Callimorphina) which is represented by only a few species on the continent, including Galapagos Islands.

On the other hand, an 'out-of-South America' origin is found in several clades of Arctiinae (Fig. 3B), predominantly in genera occurring in the Nearctic region such as *Euchaetes* (Fig. 2). Several genera such as *Bertholdia* and *Halysidota* occur in the both Nearctic and Neotropical regions, but the mapping of their occurrence in the ML tree clearly suggests that their biogeographic origin is South America. The most remarkable 'out-of-South America' genus is *Euchromia*, the subtribe-defining species of the subtribe Euchromiina. *Euchromia* is the only known genus of this subtribe that occurs in the Old World, with *Syntomeida* being its Neotropical/Nearctic sister group. Our analysis comprises two African (*Euchromia lethe* and *Euchromia schoutedeni*) and an Asian species of *Euchromia* (*E. creusa*). Our taxon sampling does not allow us to make any conclusions about the direction of colonization, and further taxon sampling and study are required to solve this question.

The overlap of genera between the four regions ranges between 0.53 (Atlantic forest *vs.* Ecuador high elevations) and 0.78 (Ecuador low *vs.* high elevations) (Fig. 1B). The composition of genera of Ecuadorian low elevations and the Amazon site (distance > 2500 km) is surprisingly similar (index value: 0.73).

Discussion

Implications for Arctiinae systematics

The availability of a phylogenetic hypothesis representing the diversity of any taxon is of great importance not only in systematics but also in many other fields, such as comparative biology (Sanford *et al.* 2002), conservation biology (Faith 2008) and ecological and evolutionary modelling (Wahlberg *et al.* 2013; Penz *et al.* 2015). Despite the first efforts to produce a comprehensive sampling of taxa to infer the phylogenetic relationships of very speciose groups of moths such as geometrids (Sihvonen *et al.* 2011) and gelechioids (Kaila 2004), several clades may possibly not be represented. This happens because many regions of the world, especially the tropical ones, are undersampled mainly because of the high species richness and secondarily because of the lack of professional systematists (Kristensen *et al.* 2007). Here, we have shown that whether a large number of Neotropical genera of arctiines found in different regions of South America are added to a phylogenetic backbone, representing a broad range of clades, new clades

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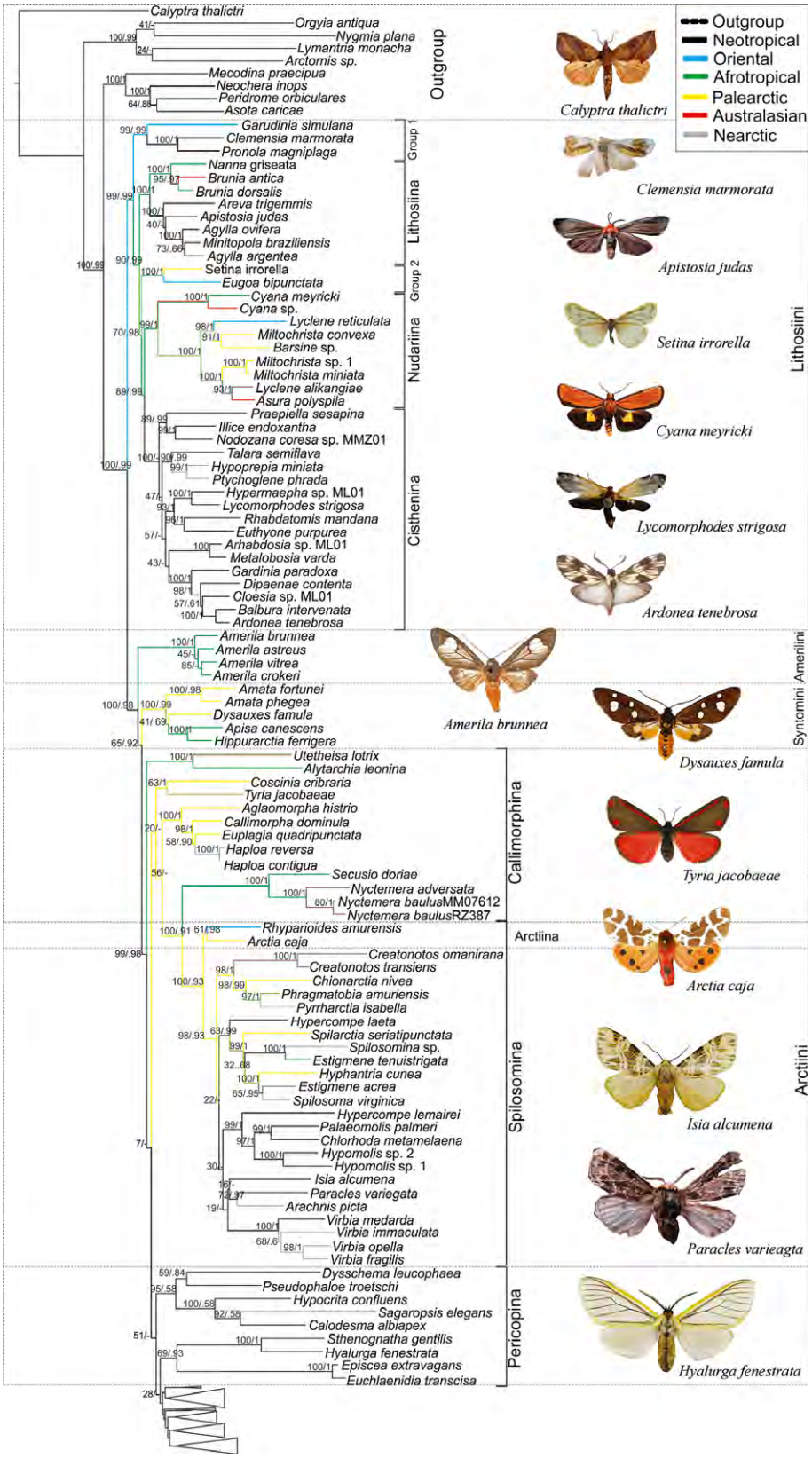

Fig. 2 Maximum-likelihood tree. Numbers next to the branches are bootstrap values and posterior probabilities.

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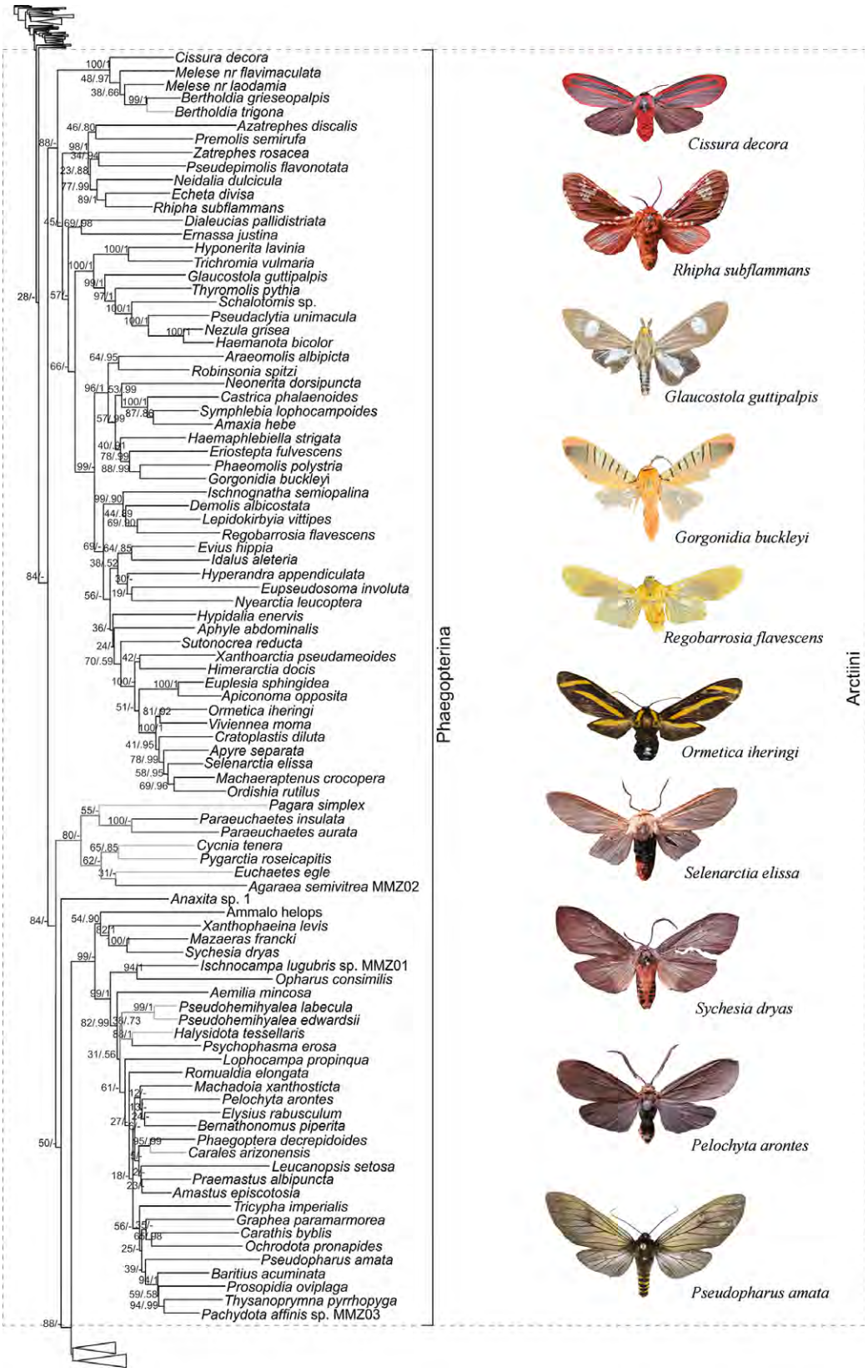

Fig. 2 Continued

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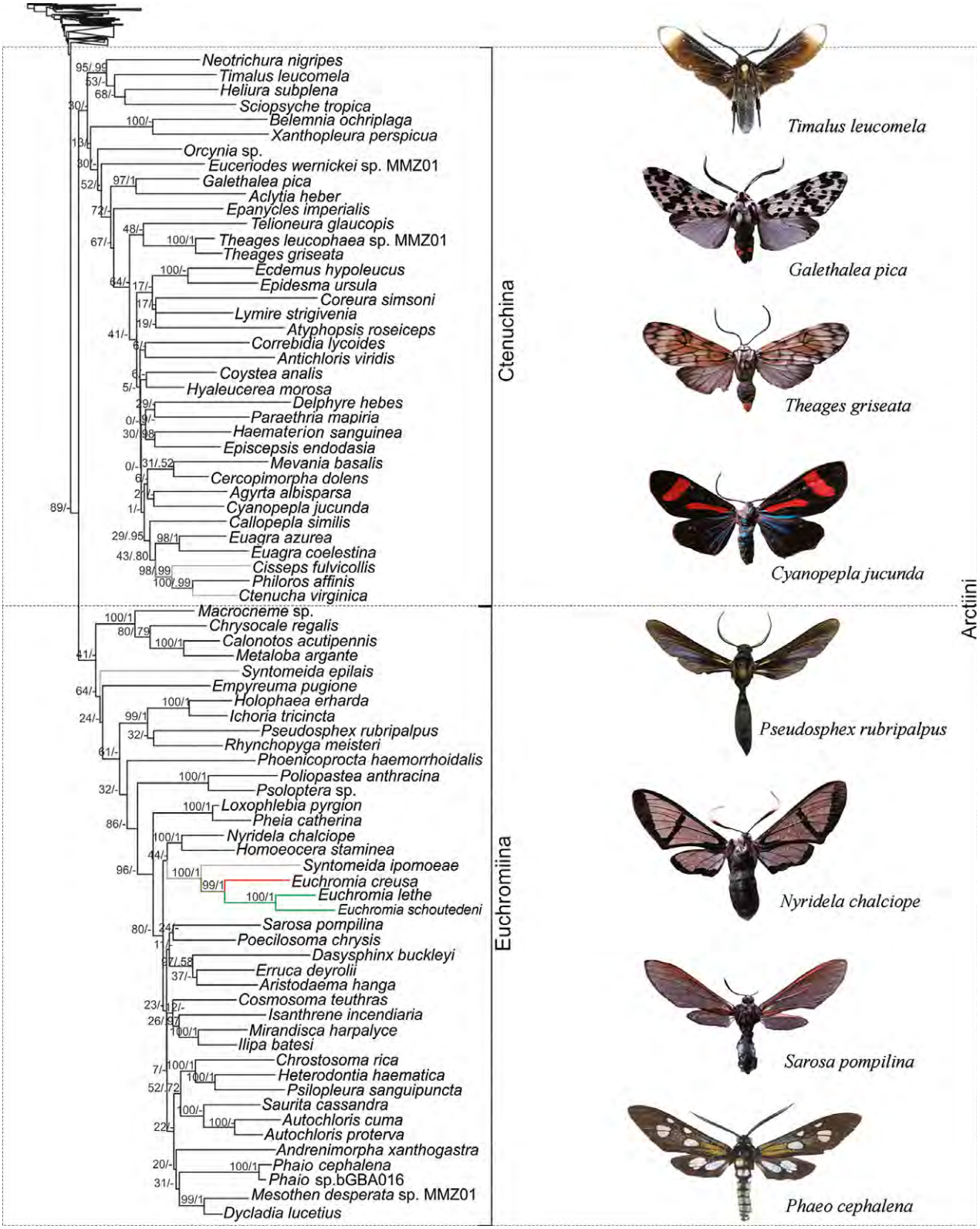

Fig. 2 Continued.

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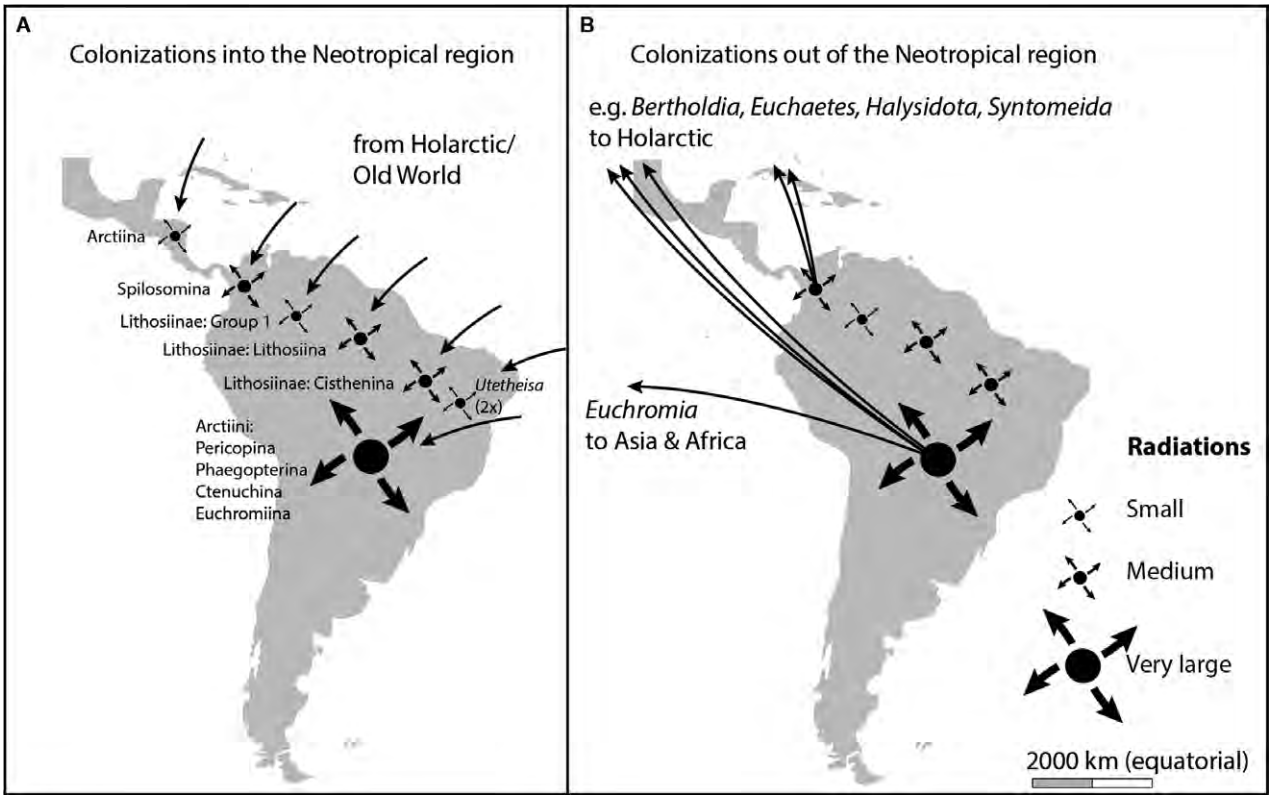

Fig. 3 —A. Hypothesized colonization events of Arctiinae clades in the Neotropical region. —B. Hypothesized 'out-of-South America' colonization events.

can be found that give us new insights into the evolutionary history of the moths. This has important implications not only in systematics but also in testing any phylogenetic dependent hypotheses at the subfamily level. To our knowledge, this is the first large-scale phylogeny of a group of moths covering the Neotropical region.

The sampling coverage of the gene regions (percentage of the total base pairs expected in each gene region) used in this study varied according to gene regions and subtribes (Table 1). However, the results reported in Table 1 should be interpreted only as the amount of information used to infer a phylogeny rather than success of primer amplification. In addition to the primer effectiveness, the age of the samples (Hebert *et al.* 2013) and issues caused during sequencing procedure also can affect the number of expected base pairs. It was not our intention to investigate the causes behind the sampling coverage of the gene regions, and thus, we did not evaluate these issues.

Similar to Zaspel *et al.* (2014), the maximum-likelihood and the Bayesian analyses recovered the monophyly of Arctiinae and its four tribes. Because we used the phylogeny of Zaspel *et al.* (2014) as a phylogenetic framework, to which we added a high number of Neotropical species, the results for Amerilini and Syntomini (non-Neotropical groups) were very similar to those of Zaspel *et al.* (2014). The results for the tribe Lithosiini differed from the phylogeny of Scott *et al.* (2013) and were very similar to those of Zaspel *et al.* (2014). Five highly supported groups were found in Lithosiini, corresponding to three known subtribes (Cisthenina, Lithosiina and Nudarina) and two additional groups (Fig.2). The high number of Neotropical species analysed in this work suggests that the subtribe Cisthenina is a primarily Neotropical clade with a few Nearctic representatives. As a corollary, our phylogeny suggests that the genera included in Cisthenina, Lithosiina and Nudarina should remain in these subtribes, except for the species included in groups 1 and 2 previously assigned to Cisthenina and Endrosina. Additional genera from the Oriental and Palearctic regions should be investigated to find out whether these two groups actually represent undescribed subtribes.

Although there was a high correspondence between maximum-likelihood and Bayesian results in the tribes Lithosiini, Amerilini and Synthomini, the Arctiini subtribes remained largely unresolved in the Bayesian tree. The most species-rich subtribes of Arctiini in the Neotropics

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(Pericopina, Phaegopterina, Ctenuchina and Euchromiina) and Callimorphina were paraphyletic in both phylogenetic estimation methods, except for the poorly supported Ctenuchina and Euchromiina in the ML analysis. Although the assumptions of the ML and Bayesian methods are different from each other, we believe that their discrepant results are related to the size of our data matrix and not to the inference method itself. It is well known that the accuracy of a phylogenetic estimation is directly related to the length of the analysed alignment (Rosenberg & Kumar 2001), and thus, convergent results could be found whether further informative sequences were added to our matrix. To this particular purpose, new methods of phylogenomics are being developed (see Lemmon & Lemmon 2013 for a review), and they certainly should be used to resolve the deeper nodes of Arctiinae in the future.

Although the most species-rich clades of Arctiini in the Neotropics were paraphyletic, interesting and new phylogenetic hypotheses could be drawn from our results. The number of analysed species in Arctiini, except Callimorphina, Arctiina and Spilosomina, increased dramatically compared to the two previous phylogenies of Arctiinae, from 30 in Jacobson & Weller (2002) and 34 in Zaspel *et al.* (2014) to 181 in this work (Table 1). The representativeness of Arctiini genera (except Ctenuchina and Euchromiina) based on the catalogue of Vincent & Laguerre (2014) also increased substantially, especially in the subtribe Phaegopterina where 78 out of 151 genera reported in the catalogue are now represented. Our results for the subtribe Pericopina include nine compared to only two genera included in Zaspel *et al.* (2014) and corroborate a recent cladistic study (Moraes 2014). Similar to our results, a morphological phylogeny comprising 48 species of *Dysschema* and 43 other Pericopina species showed that this subtribe is not monophyletic (Moraes 2014). However, in contrast to our results, Moraes (2014) could not establish the position of Pericopina in relation to the other subtribes used as out-groups. The close proximity of *Euchlaenidia transcisa* and *Episcea extravagans* is worth mentioning. Our results suggest that *Episcea* could be a junior synonym of *Euchlaenidia* because both investigated taxa are type species of their respective genera. Watson & Goodger (1986) (followed by Vincent & Laguerre (2014)) treated both genera relatively far from each other and depicted them on different colour plates and thus considered the two genera as distantly related to each other. Further morphological study is required to investigate the relationship between *Euchlaenidia*, *Episcea* and other Pericopina.

The monophyly of the subtribe Phaegopterina could not be recovered. Zaspel *et al.* (2014) found two clades, with one being the sister clade of a larger one comprising Ctenuchina, Euchromiina and two Phaegopterina sister clades. Our results are similar to those of the available phylogenies of Arctiinae, that is three clades of Phaegopterina grade into a larger clade comprising Ctenuchina and Euchromiina (see also Simmons *et al.* 2012). These three clades and their species composition corroborate the coarse affinities among Phaegopterina genera proposed in the catalogue of Watson & Goodger (1986) (followed by Vincent & Laguerre (2014)) with the exception of three species (*Dialeucias pallidistriata*, *Haemaphlebiella strigata* and *Xanthophaeina levis*). However, the previous authors did not explicitly evaluate the morphological characteristics in a phylogenetic context (autapomorphies) that could be used to back up clades established in our study. In detail, the order of species presented in the available catalogues only infrequently reflects relationships as inferred from our phylogeny. An example is represented by the 'possible jamming clade' (Zaspel *et al.* 2014) composed of *Melese*, *Bertholdia* and *Cissura* that is present in our analysis and that has been previously acknowledged by the previous authors. We suggest that morphology should also be investigated to corroborate (or not) whether Phageopterina indeed should be split into different subtribes.

Our results confirm the position of *Virbia* (represented by four species) in Spilosomina as found by Zaspel *et al.* (2014) and recently by Rönkä *et al.* (2016). The placement of *Virbia* in Arctiina as suggested by Vincent & Laguerre (2014) likely needs to be revised.

Because the species of Arctiinae have been traditionally assigned to genera based on colour patterns, several genera are expected to be paraphyletic (Weller *et al.* 2009; Rönkä *et al.* 2016). Therefore, it is not surprising that additional two genera (*Hypercompe* and *Estigmene*) were also found to be paraphyletic in our analysis.

Insights into the biogeography of the Neotropical region and beyond

Our knowledge on biogeographic patterns of the Neotropical Arctiinae is still poor compared to better studied groups such as Neotropical butterflies (e.g. Wahlberg & Freitas 2007; Elias *et al.* 2009), vascular plants (e.g. Segovia & Armesto 2015) or birds (*e.g.* Ericson 2011), because reliable species distribution maps are not available. However, an increased knowledge of the phylogenetic relationships at the genus level and coarse distribution data from four regions in South America give some first and preliminary ideas of historical biogeographic events of Neotropical arctiines that might have occurred.

Our ML tree (Fig. 2) suggests that Arctiinae have an Old World origin and that South America has been colonized independently by at least six (likely more) Arctiinae lineages. We cannot state when these colonization events have occurred because a calibration of the molecular clock

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is impeded by the lack of suitable arctiine fossils, particularly from South America (see Sohn *et al.* (2012) for a list of fossil Lepioptera of the world).

The number of colonization events could actually be higher due to incomplete taxon sampling. However, sampling coverage of Neotropical genera has greatly improved due to 183 new taxa analysed in our study, and we would not expect a great increase in such possible events with future studies. On the other hand, the knowledge about 'out-of-South America' colonization is likely to increase with better taxon sampling outside the Neotropics, because many lineages and regions are still clearly undersampled. So far, the remarkable example of *Euchromia* is to our knowledge unique in Arctiinae. It is distributed in the Oriental, Australasian and African regions, but has Neotropical ancestors. A better taxon sampling of Oriental, Australasian and African *Euchromia* species could shed more light into the biogeographic patterns of this clade.

A parallel case (but with opposite signs) is represented by the genus *Utetheisa*, the only member of the subtribe Callimorphina that occurs in the New World, as mentioned before (Weller *et al.* 2009; DaCosta 2010). *Utetheisa* is found in tropical and subtropical habitats worldwide and has an Old World origin (Forbes 1941; DaCosta 2010). Only one species (*Utetheisa ornatrix* (Linnaeus, 1758)) is widely distributed in the New World, and a small radiation of five species is endemic to the Galapagos Islands, located 900 km off Ecuador's west coast (Roque-Albelo & Landry 2009; DaCosta 2010). *Utetheisa* moths feed, for example, on *Crotalaria* (Fabaceae) species that are found on many Pacific islands, and they may have thus been able to colonize the New World using these islands as stepping stones. A better taxon sampling is also required in this group, including more Australasian species, to clarify its biogeographic patterns.

The richness and diversity of Neotropical Arctiinae generally decrease with elevation (Fiedler *et al.* 2008; Brehm 2009; Zenker *et al.* 2015), and this appears to be true for the majority of each of the Arctiini and Lithosiini lineages. We hypothesize that colonization in South America has usually started in the large lowlands and that the colonization of mountains was impeded by environmental filtering, such as the availability of certain host plants that are not present at higher elevations in the Andes or other Neotropical mountain ranges, as suggested, for example, for Neotropical geometrid moths (Brehm *et al.* 2013). Some genera of arctiines also occur at high elevations or are even restricted to high-elevation habitats. However, such examples in the Andes are rare, and information on host plants is unavailable; one is the Euchromiina genus *Chrysocale* that appears to occur exclusively in high-elevation Andean elfin forests (GB own observations, Piñas & Manzano 2003). One clade of Neotropical and Nearctic Spilosomina possibly has a biogeographic origin at cooler climates because most of its members are found at high-elevation and/or in temperate regions. This clade includes genera such as *Hypomolis*, *Chlorboda* and *Palaeomolis*. Ancestors of this clade have possibly directly colonized high-elevation habitats in the Andes from cool temperate regions. The phylogeny suggests that only a few taxa of this clade have secondarily entered the tropical lowlands with the lowland genus *Virbia* as one such example. The subtribe Arctiina is mainly distributed in the Holarctic region (see Rönkä *et al.* 2016) and only represented by very few species that (also) occur in the Neotropics (Vincent & Laguerre 2014). The genus *Virbia* belongs to Spilosomina. Watson & Goodger (1986) and Vincent & Laguerre (2014) also list the genus *Pseudalus* Schaus in Arctiina, but their distribution (South America) and their external appearance suggest that *Pseudalus* could actually belong to Phaegopterina – further morphological and molecular study is also required here.

Closing remarks

In this work, we have shown that our knowledge on the evolution of tropical arctiines is still incipient. Although we have sampled a high number of taxa in the Neotropical region, other species-rich tropical regions of the world remain undersampled. Unfortunately, remaining natural areas are often poorly protected and have been highly exploited in the last decades, and thus, many species or even genera could be at risk of disappearing before we even know that they exist. The collaboration between different research groups from institutions in Brazil, Europe and North America has produced valuable information on biodiversity patterns (Zenker *et al.* 2015) and taxonomy (Zenker *et al.* 2016) of arctiines in the highly threatened Brazilian Atlantic Forest. In this work, we take a step further by producing a broad-scale phylogeny covering different areas in the Neotropical region that can be used to test a broad range of ecological and evolutionary questions, including the complex chemical ecology of Arctiinae (Hawkins 2010; Zaspel *et al.* 2014).

We hope that the taxon coverage of arctiines in other undersampled regions of the world will soon increase and that a consistent biogeographic framework and classification for this exciting group of moths will emerge.

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Supporting Information

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

Appendix S1. Taxonomic information, sampling location, number of base pairs sequenced in each gene fragment and GenBank access numbers of the genes obtained in this study.

Appendix S2. Further taxonomic information on the samples used in this study. Illustration of the taxa included in the analyses and type species of the genera.

Appendix S3. Bayesian tree. Numbers next to the branches are posterior probabilities.

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