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Putting *Parasemia* in its phylogenetic place: a molecular analysis of the subtribe Arctiina (Lepidoptera)

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Abstract. Despite being popular among amateur and professional lepidopterologists and posing great opportunities for evolutionary research, the phylogenetic relationships of tiger moths (Erebidae: Arctiinae) are not well resolved. Here we provide the first phylogenetic hypothesis for the subtribe Arctiina with the basic aim of clarifying the phylogenetic position of the Wood Tiger Moth *Parasemia plantaginis* Hübner, a model species in evolutionary ecology. We sampled 89 species in 52 genera within Arctiina s.l., 11 species of Callimorphina and two outgroup species. We sequenced up to seven nuclear genes (CAD, GAPDH, IDH, MDH, Ef1 α , RpS5, Wingless) and one mitochondrial gene (COI) including the barcode region (a total of 5915 bp). Both maximum likelihood and Bayesian inference resulted in a well-resolved phylogenetic hypothesis, consisting of four clades within Arctiina s.s. and a clade comprising spilosomine species in addition to Callimorphina and outgroups. Based on our results, we present a new classification, where we consider the *Diacrisia* clade, *Chelis* clade, *Apantesis* clade, *Micrarctia* Seitz and *Arctia* clade as valid genera within Arctiina s.s., whereas *Rhyparia* Hübner **syn.n.** and *Rhyparioides* Butler **syn.n.** are synonymized with *Diacrisia* Hübner; *Neoarctia* Neumoegen & Dyar **syn.n.**, *Tancrea* Püngeler **syn.n.**, *Hyperborea* Grum-Grshimailo **syn.n.**, *Palearctia* Ferguson **syn.n.**, *Holarctia* Ferguson **syn.n.**, *Sibirarctia* Dubatolov **syn.n.** and *Centrarctia* Dubatolov **syn.n.** are synonymized with *Chelis* Rambur; *Grammia* Rambur **syn.n.**, *Orodemnias* Wallengren **syn.n.**, *Mimarctia* Neumoegen & Dyar **syn.n.**, *Notarctia* Smith **syn.n.** and *Holarctia* Smith **syn.n.** are synonymized with *Apantesis* Walker; and *Epicallia* Hübner **syn.n.**, *Eucharia* Hübner **syn.n.**, *Hyphoraia* Hübner **syn.n.**, *Parasemia* Hübner **syn.n.**, *Pericallia* Hübner **syn.n.**, *Nemeophila* Stephens **syn.n.**, *Ammobiota* Wallengren **syn.n.**, *Platarctia* Packard **syn.n.**, *Chionophila* Guenée **syn.n.**, *Eupsychoma* Grote **syn.n.**, *Gonerda* Moore **syn.n.**, *Platyrepria* Dyar **syn.n.**, *Preparctia* Hampson **syn.n.**, *Oroncus* Seitz **syn.n.**, *Acerbia* Sotavalta **syn.n.**, *Pararctia* Sotavalta **syn.n.**, *Borearctia* Dubatolov **syn.n.**, *Sinoarctia* Dubatolov **syn.n.** and *Atlantarctia* Dubatolov **syn.n.** are synonymized with *Arctia* Schrank, leading to 33 new genus-level synonymies. Our focal species *Arctia plantaginis* **comb.n.** is placed as sister to *Arctia festiva* **comb.n.**, another widespread aposematic species showing wing pattern variation. Our molecular hypothesis can be used as a basis when adding more species to the tree and tackling interesting evolutionary questions, such as the evolution of warning signalling and mimicry in tiger moths.

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Introduction

Tiger moths are a highly diverse group consisting of about 11 000 species worldwide. Of these, approximately 4000 species in 113 genera belong to the subtribe Arctiina (Erebidae: Arctiinae: Arctiini: Arctiina s.l.) (see Weller *et al.*, 2009 and references therein). Their visual appearance and diverse ecology have made them popular among amateur lepidopterists and some species are studied intensively [e.g. the Ornate Moth *Utetheisa ornatrix* (Linnaeus), Garden Tiger Moth *Arctia caja* (Linnaeus) and the Wood Tiger Moth *Parasemia plantaginis* (Linnaeus)], but in general our knowledge of their diversity and phylogenetic relationships is surprisingly limited (Bendib & Minet, 1998; Conner, 2009). New species are still found, perhaps because many are relatively rare, difficult to observe, or may occur in small numbers in remote places (e.g. *Micrarctia kautti* Saldaitis & Pekarsky, 2015). The present classification of Arctiina s.l. is based mainly on detailed studies based on morphological characters (Dubatolov & de Vos, 2010; Lafontaine & Schmidt, 2010; Fibiger *et al.*, 2011; Vincent & Laguerre, 2014). However, these data have not been subjected to rigorous phylogenetic analyses.

Most tiger moths are chemically defended, advertise their unpalatability with spectacular warning colours and take part in several Müllerian mimicry rings (Conner, 2009). High morphological variability in Arctiinae means that it is difficult to determine unequivocal synapomorphies [shared derived characters that support monophyletic groups (clades)], which makes it challenging to trace the evolutionary relationships within the group (Schmidt, 2007; Weller *et al.*, 2009). As mimicry is very likely to occur within Arctiinae, another phenomenon that can potentially obscure our understanding of the systematics of this group is incomplete lineage sorting. This is likely to be common in many systems, such as mimetic butterflies, resulting from rapid radiation or adaptive introgression facilitated by strong selection on adaptive loci (Kozak *et al.*, 2015). In addition, the tendency of researchers to describe each colourful and uniquely patterned species in its own genus has led to a less informative classification, in which many tiger moth genera are species-poor, monotypic and, in some cases, probably paraphyletic (Weller *et al.*, 2009).

Parasemia plantaginis is the only species in its nominal genus *Parasemia* Hübner. The species occurs in the Holarctic, forming two distinct clades, one of which corresponds to *P. plantaginis* ssp. *caucasica* (Ménétries), with both male and female moths expressing 'interrupted' forewing pattern (Hegna & Mappes, 2014; Honma *et al.*, 2015) and hindwing coloration varying from yellow to red (Fig. 1D). The other clade comprises all other forms of *P. plantaginis* with various patterns and polymorphic hindwing coloration (Fig. 1A–C; Hegna *et al.*, 2015). The effects of variation in both larval and adult coloration of *P. plantaginis* on their predation risk and other fitness measures, as well as population genetics, have been intensively studied (e.g. Ojala *et al.*, 2005, 2007; Lindstedt *et al.*, 2011; Nokelainen *et al.*, 2011; Hegna *et al.*, 2013 & Galarza *et al.*, 2014) and the species has great potential for becoming a model system in the study of the evolution of warning coloration (Stevens & Ruxton, 2012) and colour polymorphism.

Thus, to further investigate interesting evolutionary questions in this system, such as the evolution of warning signal polymorphism or convergent evolution in mimicry rings, a well-resolved phylogeny of Arctiina is crucially needed (Simmons, 2009; Hegna *et al.*, 2015). With a phylogenetic hypothesis available, it will be possible to determine when colour polymorphisms have evolved in the group and to study the occurrence of mimetic patterns in detail (Simmons, 2009).

The higher classification of tiger moths (Lepidoptera: Erebidae: Arctiinae) was recently studied with molecular methods by Zaspel *et al.* (2014), but this study had sparse sampling of the species-rich subtribe Arctiina. Zaspel *et al.* (2014) sampled only *Arctia caja* from the diverse *Arctia* group and did not include *Parasemia*. *Parasemia* is thought to be closely related to *Arctia*, with some evidence that it may, in fact, be within the genus (Fibiger *et al.*, 2011). Schmidt's (2007) tree, with combined evidence from barcode and morphology, placed *Parasemia* in the same clade with *Arctia*, *Platyprepia*, *Platarctia* and *Pararctia*. With the broadest sampling of related genera so far, Dubatolov (2008) placed *Parasemia* closest to *Hyphoraia*, which consists of three species [*Hyphoraia aulica* (Linnaeus), *H. dejeani* (Godart) and *H. testudinaria* (Geoffroy)], and *Epicallia* (= *Arctia*) *villica* (Linnaeus), a monotypic genus, based on morphological characters.

In this study, we infer a molecular hypothesis of the phylogenetic relationships of species in the subtribe Arctiina, aiming to clarify the position of *Parasemia* within the subtribe. Based on our results, we revise the classification of Arctiina s.s. By doing this we contribute to establishing the relationships among many monotypic genera, stated by Weller *et al.* (2009) as the next big challenge in arctine systematics.

Material and methods

Sampling

Many Palearctic Arctiina species are rare and/or occur in areas that are not easily accessible to collectors. However, with the aid of several amateur lepidopterologists and fellow scientists (see the Acknowledgements) we were able to sample many of the species in the subtribe putatively related to *Parasemia*. The selection of taxa was based on previous studies (Jacobson & Weller, 2002; Schmidt, 2007; Dubatolov, 2008, 2009; Zaspel *et al.*, 2014) and available checklists relevant to our taxon sampling (Dubatolov & de Vos, 2010; Lafontaine & Schmidt, 2010; Fibiger *et al.*, 2011; Vincent & Laguerre, 2014). Within the tribe Arctiini we sampled 11 species representing nine genera of the subtribe Callimorphina and 89 species representing 52 genera of the subtribe Arctiina, but excluded the mostly tropical subtribes Pericopina, Ctenuchina, Euchromiina and Phaegopterina. As outgroups we used *Setina* sp. (Erebidae: Arctiinae: Lithosiini) and *Amata* sp. (Arctiinae: Syntomini), which are closely related to Arctiini according to Zaspel *et al.* (2014).

Our focal study species, *P. plantaginis*, is placed in Arctiini: Arctiina. To our knowledge, *Parasemia* together with other genera putatively related to *Arctia* belong to Arctiina s.s., and,

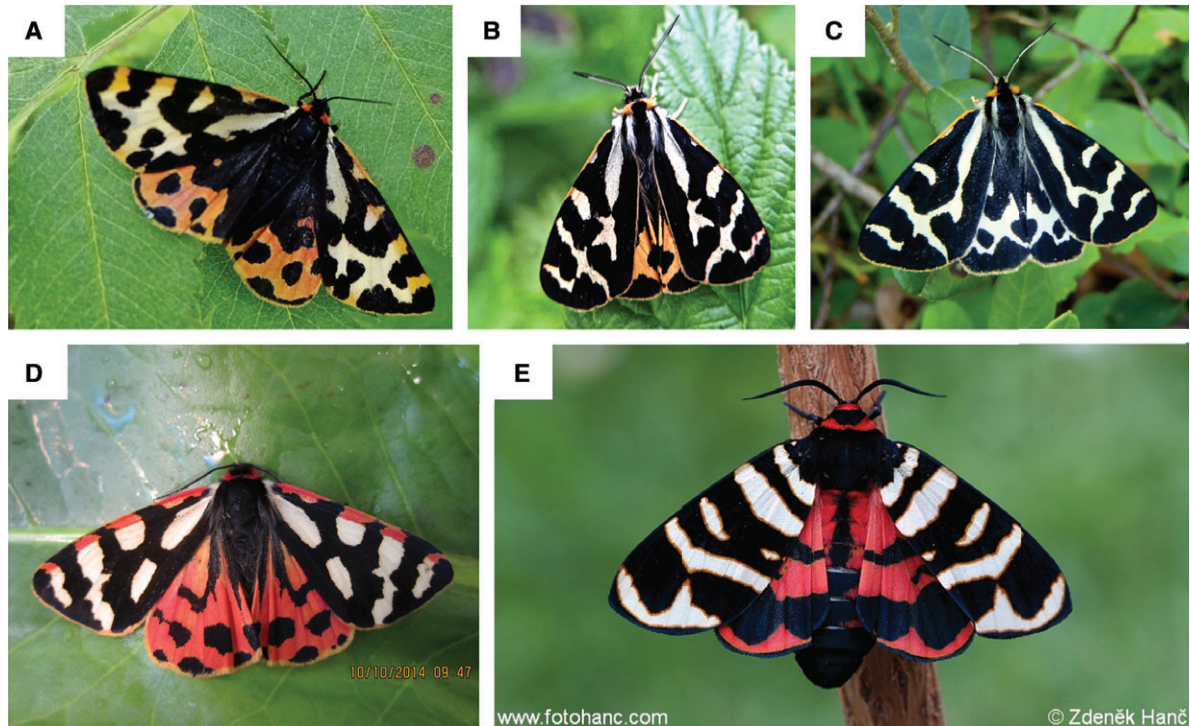


Fig. 1. (A–E) Female *Arctia plantaginis* (A), male *A. plantaginis* colour variants (B, C), male *A. plantaginis* ssp. *caucasica* (D) and female of the sister species *Arctia festiva* (E) on natural backgrounds. [Photographs were taken by Bibiana Rojas (A–C), KR (D) and Zdenek Hanc (E).]

within that, to a lineage that has a Holarctic distribution (Weller *et al.*, 2009). Sampling within Arctiini was thus limited to the Holarctic region, with most species having a Palaearctic distribution, although eight species occurring only in the Nearctic were also included. For species with a wide distribution range we aimed to sample at least two individuals representing different populations to avoid possible bias caused by local adaptive evolution. As we focused our sampling to Arctiina s.s. in the hope of finding the closest relatives of *Parasemia*, the so-called spilosomine genera and other mainly tropical lineages of Arctiini were left more sparsely sampled. However, including the sequences of Arctiina used by Zaspel *et al.* (2014) in our analysis broadened our coverage to tropical regions for the spilosomine genera.

We used samples that were as fresh as possible, with the oldest ones sampled successfully being up to 10 years old, stored dry, in alcohol or frozen at -20°C . For DNA extraction we used either one to two legs of adult specimen or a small piece of tissue (e.g. anal prolegs) from larvae. The barcode (COI) sequences of our samples were cross-checked in the Barcode of Life Data System (Ratnasingham & Hebert, 2007) for those species that already had a reference barcode provided. All our sampled taxa, genes and GenBank accession numbers are provided in Appendix S1.

DNA markers and laboratory protocols

The eight genetic markers used in this study have proven useful in resolving evolutionary relationships between species

above and below the family level (e.g. Wahlberg & Wheat, 2008; Zahiri *et al.*, 2011, 2012; Zaspel *et al.*, 2014). We amplified the mitochondrial cytochrome oxidase (COI), including the barcode region, as well as the nuclear gene regions carbamoylphosphate synthase domain protein (CAD), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), isocitrate dehydrogenase (IDH), cytosolic malate dehydrogenase (MDH), elongation factor 1- α protein (E1 α), ribosomal protein subunit S5 (RpS5) and wingless (WGS).

DNA extraction was conducted using the DNeasy Blood + Tissue extraction kit (Qiagen, Hilden, Germany) both in Turku and Jyväskylä according to the manufacturer's protocols, but assisted by a robot (Kingfisher, Waltham, MA, U.S.A.) in Jyväskylä. Washing and eluting DNA in Jyväskylä was thus done using MagAttract tubes and the Kingfisher robot with the programme Qiagen Blood. For polymerase chain reaction (PCR) and primer pairs we followed the laboratory protocols of Wahlberg & Wheat (2008). However, for some older samples processed in Jyväskylä, in cases where we did not obtain enough product to be visualized and purified from agarose gel during the first PCR, we did a second PCR using the first PCR product as a template with the same primers. PCR products were sent to MacroGen Europe in the Netherlands for sequencing, except for part of the barcode region (the 5' half of COI) samples, which were sequenced in Jyväskylä with Big-Dye terminator v3.1, Cycle Sequencing kit (Applied Biosystems, Carlsbad, CA, U.S.A.) and run on an ABI 3130xl Genetic Analyzer (Applied Biosystems). Finally, we aligned DNA sequences manually using MEGA version 5.2.2 (Tamura

et al., 2011) or BIOEDIT (Hall, 1999) and stored them on the web-based VOSEQ database software (Peña & Malm, 2012).

Phylogenetic analysis and checking for errors

To check for erroneous sequences, we performed neighbour-joining and Bayesian analyses on single-gene alignments. These analyses were compared with the combined analysis of all genes, and if the species were placed in a radically different relationship between these two, the original sequence data for the differing gene were examined, and, in cases of possible contamination or low-quality sequence, omitted from further analysis.

We performed both maximum likelihood (ML) and Bayesian inference (BI) analyses on the combined dataset of a minimum of two successfully sequenced gene regions (min. of approximately 1000 bp). The Bayesian information criterion using PARTITION FINDER v. 1.1.1 (Lanfear *et al.*, 2012) was used to determine the best-fit partitioning scheme and evolutionary model for the dataset, which was partitioned into each codon position for each gene region. For ML analysis we used RAXML-HPC2 (Stamatakis, 2014) on XSEDE (Townes *et al.*, 2014) and ran 1000 replicates of bootstrapping to calculate support for ML nodes using the Cipres science gateway (Miller *et al.*, 2010). The BI analyses were carried out using MRBAYES v3.2.3 (Ronquist *et al.*, 2012) on the Cipres science gateway. We performed 10 million generations, with sampling every 1000 generations 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% of 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 for each parameter was close to 1. Resulting trees for both ML (Fig. 2) and BI analyses (Appendix S2) were visualized using FIGTREE v.1.4.2. (Rambaut, 2014).

Results

The most optimal partitioning scheme found by PARTITION FINDER had 16 partitions (out of a total of 24). Most codon positions of each gene were kept in their own partition, except for the following, which were combined: position 3 of CAD and position 3 of MDH; position 2 of CAD and position 2 of IDH; position 3 of GAPDH, position 3 of IDH and position 3 of WGS; position 2 of GAPDH, position 2 of MDH; and position 1 of MDH, position 1 of RpS5 and position 1 and 2 of WGS.

Both ML and BI analyses resulted in well-resolved topologies with nearly identical branching patterns (Fig. 2, Appendix S2). The topologies are rooted with Lithosiini (*Setina* sp.) and the sample representing Syntomini (*Amata* sp.) is positioned as sister to all other clades [bootstrap (BS) = 100, Bayesian posterior probability (BP) = 1.0]. Our 11 species formally placed in Callimorphina are divided into two strongly supported clades,

eight species forming Callimorphina (BS = 99, BP = 1.0) and three species of *Nyctemera* + *Secusio* forming another clade (BS = 100, BP = 1.0). The latter is sister to Arctiina with strong support. Within Arctiina s.l., we find strong support for the monophyletic group of spilosomine genera (BS = 100, BP = 1.0) separate from Arctiina s.s. (BS = 49, BP = 0.94).

Within Arctiina s.s., several clades are formed, but the relationships between and within some of these groups are not clear. The first clade comprises *Diacrisia*, *Rhyparia* and *Rhyparioides* (the *Diacrisia*-clade), which form a strongly supported monophyletic group (BS = 100, BP = 1.0). *Hyperborea*, *Sibirarctia*, *Chelis* and *Neoarctia* + *Holarctia* + *Paelearctia* + *Tancrea* + *Centrarctia* also form a clade with strong support (the *Chelis* clade; BS = 99, BP = 1.0) as do *Holarctia*, *Grammia*, *Apantesis* and *Notarctia* (the *Apantesis* clade; BS = 99, BP = 1.0).

Micrarctia trigona (Leech) is placed alone as a sister to the monophyletic grouping of 'Arctia' species (the *Arctia* clade; BS = 86, BP = 0.99), which is divided in two subclades, which we term the 'Northern *Arctia*' (BS = 98, BP = 1.0) and the 'Mediterranean *Arctia*' (BS = 100, BP = 1.0). Six species of *Arctia* form a monophyletic '*Arctia caja* group', of which *A. intercalaris* (Eversmann) + *A. thibetica* Felder are placed as sister to *A. caja* + *A. martinhoneyi* Dubatolov & Gurko + *A. brachyptera* Troubridge & Lafontaine + *A. opulenta* (H. Edwards), which show very little difference in the molecular data. We consider the '*Arctia caja* group' as part of the sister 'Northern *Arctia*' subclade, where *Platyrepia* and *Oroncus* form the most basally arising branches, with some support for a monophyletic grouping of *Preparctia* [including *Sinoarctia sieversi* (Grum-Grshimailo)] + *Gonerda* + *Platarctia souliei* (Oberthür) placed as sister to *Orontobia secreta* (Draudt) + *Acerbia seitzii* (Bang-Haas) + *Arctia rueckbeili* Püngeler and a grouping of *Pararctia* + *Acerbia alpina* (Quensel), *Platarctia parthenos* (Harris), *Pericallia matronula* (Linnaeus), *Borearctia menetriesii* (Eversmann) and *Arctia flavia* (Fuessly) with a non-resolved branching structure. The other subclade of the monophyletic group of 'Arctia' is the 'Mediterranean *Arctia*', which comprises our focal study species *P. plantaginis* placed as sister to *Eucharia* (= *Ammobiota/Arctia*) *festiva* (Hufnagel) (BS = 94, BP = 1.0), next to all three *Hyphoraia* species, which in turn form the sister clade of *Atlantarctia ungemachi* (Le Cerf), *Atlantarctia* (= *Arctia*) *tigrina* (Villers) and *Epicallia* (= *Arctia*) *villica* (Linnaeus).

Discussion

A molecular hypothesis of Arctiina phylogenetic relationships

We were able to sample a wide range of Arctiina species throughout their distribution ranges in the Holarctic, while aiming to find all the potential relatives of *Parasemia*. Our sampling is the most comprehensive to date of the subtribe Arctiina and brings many species that have been difficult to place in a phylogenetic context for the first time. The resolution of our hypothesis could well be further improved by adding samples

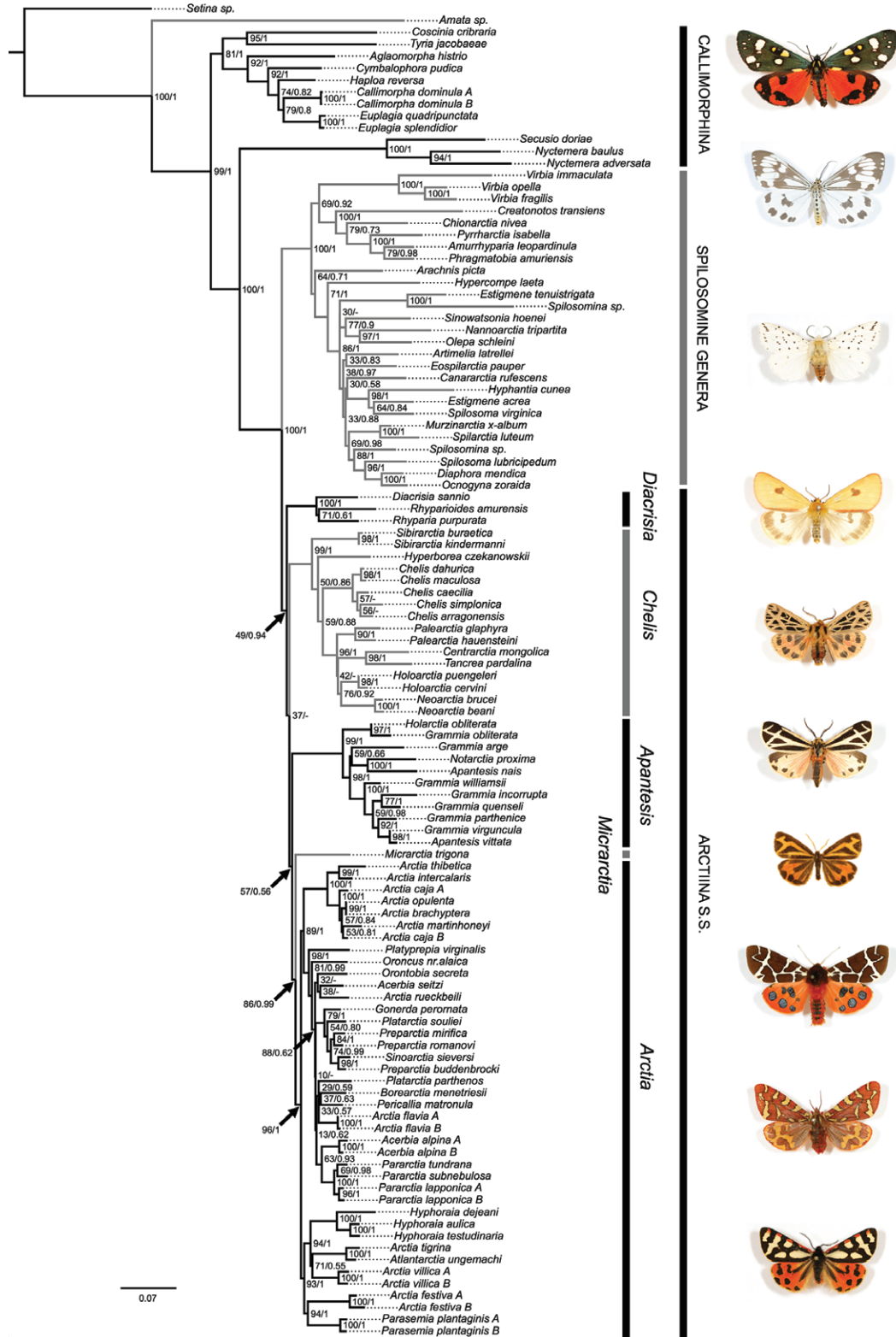


Fig. 2. Phylogram of the potentially closest relatives of *Arctia plantaginis*. Bootstrap/Bayesian posterior probability support values are given next to the nodes. Lines on the right delimit the revised genera and other monophyletic groupings formed. Tiger moths illustrated in the pictures from top down are *Callimorpha dominula*, *Nyctemera adversata*, *Spilosoma lubricipedium*, *Diacrisia sannio*, *Chelis daturica*, *Apantesis vittata*, *Micrarctia trigona*, *Arctia caja*, *Arctia lapponica* **comb.n.** and *Arctia plantaginis* ssp. *caucasica* **comb.n.**

Table 1. Formal generic revision of Arctiina s.s.

Valid genera	Synonymized genera
<i>Apantesis</i> Walker, 1855	<i>Grammia</i> Rambur, 1866 syn.n. <i>Orodemnias</i> Wallengren, 1885 syn.n. <i>Mimarctia</i> Neumoegen & Dyar, 1894 syn.n. <i>Notarctia</i> Smith, 1938 syn.n. <i>Holarctia</i> Smith, 1938 syn.n.
<i>Chelis</i> Rambur, 1866	<i>Neoarctia</i> Neumoegen & Dyar, 1893 syn.n. <i>Tancrea</i> Püngeler, 1898 syn.n. <i>Hyperborea</i> Grum-Grshimailo, 1900 syn.n. <i>Palearctia</i> Ferguson, 1984 syn.n. <i>Holoarctia</i> Ferguson, 1984 syn.n. <i>Sibirarctia</i> Dubatolov, 1987 syn.n. <i>Centrarctia</i> Dubatolov, 1992 syn.n.
<i>Diacrisia</i> Hübner, [1819] 1816	<i>Rhyparia</i> Hübner [1820] 1816 syn.n. <i>Rhyparioides</i> Butler, 1877 syn.n.
<i>Micrarctia</i> Seitz, 1910 <i>Arctia</i> Schrank, 1802	<i>Eyprepia</i> Ochseneheimer, 1810 junior objective synonym of Arctia Schrank, 1802. <i>Epicallia</i> Hübner, [1820] 1816 syn.n. <i>Eucharia</i> Hübner, [1820] 1816 syn.n. <i>Hyphoraia</i> Hübner, [1820] 1816 syn.n. <i>Parasemia</i> Hübner, [1820] 1816 syn.n. <i>Zoote</i> Hübner, [1820] 1816 junior objective synonym of Arctia Schrank, 1802. <i>Pericallia</i> Hübner [1820] 1816 syn.n. <i>Nemeophila</i> Stephens, 1828 syn.n. <i>Ammobiota</i> Wallengren, 1855 syn.n. <i>Callarctia</i> Packard, 1864 junior objective synonym of Arctia Schrank, 1802. <i>Platarctia</i> Packard, 1864 syn.n. <i>Chionophila</i> Guenée 1865 syn.n. <i>Eupsychoma</i> Grote, 1865 syn.n. <i>Gonerda</i> Moore, 1879 syn.n. <i>Platyprepia</i> Dyar, 1897 syn.n. <i>Preparctia</i> Hampson, 1901 syn.n. <i>Oroncus</i> Seitz, 1910 syn.n. <i>Acerbia</i> Sotavalta, 1963 syn.n. <i>Pararctia</i> Sotavalta, 1965 syn.n. <i>Borearctia</i> Dubatolov, 1984 syn.n. <i>Sinoarctia</i> Dubatolov, 1987 syn.n. <i>Atlantarctia</i> Dubatolov, 1990 syn.n.

of the rarer species, e.g. from the small genera *Atlantarctia* Dubatolov, *Divarctia* Dubatolov, *Ebertarctia* Dubatolov, *Lep-tarctia* Stretch, *Ocnogyna* Lederer, *Oroncus* Seitz, *Orontobia* de Freina, *Palerontobia* Dubatolov, *Sonorarctia* Ferguson, *Allan-watsonia* Ferguson and *Pseudalus* Schaus. However, many of the missing species are described from only a few specimens, or from the type series only, and fresh samples are thus extremely difficult to obtain.

Both ML and BI analyses resulted in nearly identical topologies. Within Arctiini, the selected 11 species of Callimorphina are segregated into the Callimorphina clade and *Nyctemera* + *Secusio*, forming a clade sister to Arctiina. Whether reinstating Nyctemerina as a separate subtribe would be necessary, as discussed in Zaspel *et al.* (2014), is beyond the scope of this study. We find strong support for a large monophyletic grouping of the spilosomine genera as separate from Arctiina s.s. Within Arctiina s.s., four well-supported clades are recovered. We find it most informative, and probably also most stable, to

consider these clades to represent the generic level within the subtribe. Each clade and the implications of our results on the taxonomy of Arctiina are discussed further in the following. Formal taxonomic revision of the genera is given in Table 1.

In the broad sense, our molecular hypothesis of the evolutionary history of *P. plantaginis* and relatives is in concordance with earlier phylogenies by Ferguson (1985), Schmidt (2007) and Dubatolov (2008, 2009), which were based on morphological characters, as well as the COI barcode region in Schmidt (2007). Dubatolov (2008, 2009) divided the Arctiina s.s. into 'Micrarctiini' and 'Arctiini'. Dubatolov's (2009) 'Micrarctiini' comprises mostly same genera as in our *Diacrisia*, *Chelis* and *Apantesis* clades, but with different hypothesized phylogenetic relationships. All of Dubatolov's (2008) 'Arctiini' are placed in *Arctia* as delimited below. Dubatolov (2008) divided 'Arctiini' into two clades, one associated with 'northern and mountainous areas of Asia and North America' and the other with 'plains of moderate altitudes', which correspond largely to our subclades

‘Northern *Arctia*’ and ‘Mediterranean *Arctia*’, but again his tree derived from morphology has a different branching order. Interestingly, *Micrarctia* is placed as sister to our *Arctia*.

Spilosomine genera

The *Spilosoma* group has been considered part of Arctiina s.l. (e.g., Ferguson, 1985) or as a separate tribe or subtribe called Spilosomina (e.g. Schmidt, 2007; Vincent & Laguerre, 2014). Zaspel *et al.* (2014) did not find Spilosomina separate from Arctiina and discussed whether the division has been made in an attempt to categorize moths by similar appearance. In our tree with a larger sampling of Arctiina, the spilosomine genera come out as a well-supported monophyletic group corroborating the preliminary results of Schmidt (2007) – a hypothesis that is also supported by the light wing coloration shared by many species within the group. However, as the spilosomine genera are highly diverse and globally distributed, with hotspots of diversity in the tropical Asia and Africa (Ferguson, 1985), our sampling does not allow substantive interpretation of the interrelationships within the clade. We agree with Fibiger *et al.* (2011) that this species group needs more work and a thorough phylogenetic revision. We thus prefer to retain the spilosomine genera in the subtribe Arctiina s.l. for the time being.

Arctiina s.s.: Diacrisia, Chelis and Apantesis clades

Diacrisia, *Rhyparia* and *Rhyparioides* have been suggested to be closely related in several studies (Ferguson, 1985; Koda, 1987; Dubatolov, 2009). Our analyses corroborate these studies as we also find them to form a monophyletic entity. Species in this clade differ in their adult forewing coloration and pattern from other Arctiina by their bright yellow and red hues. This group has the highest species diversity in Asia. As *Diacrisia* is the oldest available genus name for these, we synonymize *Rhyparia syn.n.* and *Rhyparioides syn.n.* with *Diacrisia*.

The second clade combines the rather large genera *Chelis* and *Palaearctia* together with many smaller genera. Ferguson (1985) noted the close relationship of *Neoarctia*, *Holoarctia*, *Palaearctia* and *Hyperborea*. The internal relationships of this clade are not well resolved and would benefit from adding more samples of species and genera than are included in our analysis. Due to the well-supported monophyly of this clade, all genera in the *Chelis* clade are here combined into *Chelis*.

The third clade comprises almost solely species assigned to *Grammia*, but also *Notarctia proxima* (Guérin-Méneville), *Apantesis nais* (Drury) and *A. vittata* (Fabricius). The close relationship of *Grammia*, *Notarctia* and *Apantesis* has previously been suggested based on morphological characters (Ferguson, 1985). *Arctia* [later in *Grammia*] *obliterata* Stretch was placed in its own genus *Holarctia* by Smith, based on its more variable morphology and wider distribution than other *Grammia* species. Schmidt (2009) considered the species *obliterata* to be related and probably basal to *Grammia*, a view corroborated by our analysis. Contrary to Schmidt (2009), however, we find the

clade consisting of *Grammia syn.n.*, *Holarctia syn.n.*, *Notarctia syn.n.* and *Apantesis* monophyletic with high support, and therefore place all these genera under *Apantesis* (see Table 1). Synonymy of *Holarctia* with *Apantesis* and *Holoarctia syn.n.* with *Chelis* will also clarify the confusion caused by the similar orthography of these two genus names (Ferguson, 1985).

Micrarctia

Micrarctia trigona is an especially interesting case of Arctiinae tiger moths. The tribe Micrarctiini (originally established by Seitz as Micrarctiinae) was used by Dubatolov (1990, 2009) to host many superficially similar arctiine genera that could not be placed elsewhere. Later, most of these genera were moved to other (sub)tribes, leaving *M. trigona* the only genus and species of Micrarctiini. Recently, a second species was described in *Micrarctia* that is sympatric with *M. trigona* (Saldaitis & Pekarsky, 2015). This species, *M. kautti*, is nocturnal, unlike its sister species, and perhaps this is why it had remained unnoticed for so long. It would be intriguing to include *M. kautti* in an analysis to further elucidate the position of *Micrarctia* and thus potentially help to resolve the branching order of all four clades within Arctiina s.s. As the position of *Micrarctia* is not as strongly supported (BS = 86, BP = 0.99) as the other clades (BS = 99–100, BP = 1.0), we prefer to retain it as a valid genus until further work can ascertain its phylogenetic position.

The Arctia clade

The unusually short branching within the *Arctia* clade and low support values for internal nodes suggest rapid radiation. This type of quick speciation leaves little phylogenetic evidence in the nuclear genes to study the species-level branching. ‘*Arctia*’ species (excluding *Micrarctia* at the base of the clade) form a well-supported clade. The superfluous number of monotypic genera that also causes polyphyly of *Arctia* is obviously unwarranted. To render the classification more natural, and also simplify it, we combine all these species under *Arctia* (see Table 1). However, two well-supported subclades can be distinguished – our ‘Northern *Arctia*’ and ‘Mediterranean *Arctia*’.

Northern Arctia and A. caja group

Many Arctiina species, especially in the ‘Northern *Arctia*’ clade, are better adapted to cooler environments than most other noctuid moths (Ferguson, 1985). Adapting to cold environments could be one mechanism behind the apparently rapid diversification that has occurred in this clade. The subclade has been divided into many monotypic genera containing some of the most rarely encountered species with almost mysterious life histories. For example, there was a gap lasting for decades between the observations of the Menetries’s Tiger Moth *Borearctia menetriesii* in Finland and the next discovered sites are not only separated by hundreds of kilometres but are also in different habitats (Bolotov *et al.*, 2013).

The species in this subclade are very distinctive, with their conspicuous wing patterns, bright colours and large size. The Garden Tiger Moth *Arctia caja* is no exception, but is in addition very variable in its patterning. Many species, such as *A. intercalaris*, *A. martinhoneyi*, *A. tibetica*, *A. brachyptera* and *A. opulenta*, have been split from *A. caja* based on appearance, but in our molecular hypothesis all these species group together with high support and very little genetic difference. However, as the molecular markers we used in this study are too conservative for inferring interrelationships between very closely related species, other markers should be used to study patterns and levels of differentiation at the species level. We consider the *A. caja* group to be part of the 'Northern Arctia' clade.

Dubatonov (2008) arranged his 'Northern mountainous clade' to (*Gonerda* + *Preparctia*) + *Sinoarctia* + (*Borearctia* + (*Pararctia* + *Platarctia*)) + (*Orontobia* + (*Oroncus* + (*Acerbia* + *Platyprepia*))). These genera form our 'Northern Arctia' subclade, supplemented with *A. caja* group, *A. flavia*, *A. rueckbeili* and *Pericallia matronula*. There is also some evidence in our dataset (Appendix S1) indicating that *Ebertarctia nordstroemi* (Brandt) could belong to the 'Northern Arctia'. According to our hypothesis the Nearctic genus, *Platyprepia* is closer to the base and not at the tip of the subclade and *Sinoarctia sieversi* is nested within *Preparctia*. Based on the short branching, we combine all these genera under *Arctia* (see Table 1). By so doing, we again move away from the uninformative monotypic genera.

Some other monotypic genera, such as *Leptarctia* and *Palerontobia*, that we were not able to sample or to obtain good-quality sequences of, are likely to belong to this subclade, and including them could help to resolve the internal relationships within the subclade. However, we consider it more likely that the low resolution within this subclade results from rapid diversification rather than sparse sampling, as both morphological and molecular data have repeatedly proved indecisive within this subclade (Ferguson, 1985; Dubatonov, 2008, 2009; Weller *et al.*, 2009).

Mediterranean Arctia

This is another subclade consisting of the equally showy and colourful *Atlantarctia ungemachi*, *Arctia* (= *Epicallia*) *villica*, *Arctia* (= *Atlantarctia*) *tigrina*, *Eucharis* (= *Ammobiota/Arctia*) *festiva*, *Hyphoraia* spp. and *Parasemia*. As their distribution ranges meet at the Mediterranean, we call this group 'Mediterranean Arctia'. This monophyletic group includes only a few species, and several of them are already ascribed to *Arctia*. We combine both this subclade and the 'Northern Arctia' subclade under *Arctia* (see Table 1). The species in the two subclades are also morphologically quite similar to each other, and these clades lack reliable synapomorphies.

Concluding remarks and future applications of the phylogeny of Arctiina

This study stemmed from the need to find the closest relatives of *Arctia plantaginis* to be able to further understand

the evolutionary origins of its peculiar polymorphic warning coloration and also tiger moths in general. *Arctia plantaginis* has been suggested to originate in the Caucasus or south-eastern Europe based on COI, ten microsatellite loci haplotypes and species distribution modelling (Hegna *et al.*, 2015). Hegna *et al.* (2015) hypothesized that, as sexually monomorphic hindwing coloration seems to be ancestral in arctiines, the Caucasian form, *A. plantaginis caucasica*, of which hindwing coloration varies continuously from yellow to red in both sexes, would be ancestral to all other *A. plantaginis*. In other populations, female hindwing coloration still varies continuously from yellow to red, but male hindwing coloration is polymorphic and the ground colour can be white, yellow or black (Fig. 1A–D). Based on our results, the closest relatives of *A. plantaginis*, like *Arctia festiva* (Fig. 1E), are indeed sexually monomorphic in their hindwing coloration, although many species continuously vary in forewing pattern. This comparison implies that the polymorphism in *A. plantaginis* male hindwing coloration is a more recent development.

Another obvious application of our phylogenetic hypothesis is in the study of diversification patterns of Arctiina species. Most Arctiina species are diurnal with polyphagous larvae, feeding on, amongst others, dandelion (*Taraxacum* spp.) and plantain (*Plantago* spp.), including in the Nearctic, where these plants are naturalized European species (Conner, 2009). Dubatonov (2008, 2009) suggests that Arctiina most probably originated in Asia, from where they have spread in multiple occasions to the Western Palearctic and Nearctic. It is also possible, however, that there were some refugia during glaciation periods in the Mediterranean region, which enhanced diversification.

In conclusion, we would like to encourage researchers to study below the surface of these popular, colourful and dazzling species, so as to gain information that escapes our eyes. Our work offers long-awaited clarification of the phylogenetic relationships of Arctiina, especially within Arctiina s.s. – a group of spectacular and popular moths that have been much studied, yet proven difficult to classify with traditional methods. It was beyond our scope to provide a complete systematic revision of Arctiina s.l., with a vast majority of the 4000 species occurring in the tropics, and more work needs to be done to solve the evolutionary relationships between and within clades in this highly diverse and specialized group of moths. We hope that our molecular hypothesis for Arctiina will work as a backbone, where many more tiger moth species can find their relatives. With rigorous phylogenetic hypotheses, it will be possible to tackle many interesting evolutionary questions to come.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/syen.12194

Appendix S1. Taxon sampling table. Letter A or B after the species name refers to the voucher positioned to the trees in Fig. 2 and Appendix S2. Samples with less than two

successfully sequenced gene region (min. of approximately 1000 bp) were not included in the final analysis. Samples marked with an asterisk (*) in collection country are from Zaspel *et al.* (2014).

Appendix S2. Bayesian topology for the same dataset as in the maximum likelihood phylogram in Fig. 2.

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