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ORIGINAL ARTICLE

An insight into the molecular phylogeny of Drepanidae (Lepidoptera) with an emphasis on the European fauna

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Abstract. Drepanidae are one of the families of Lepidoptera that have received little attention in terms of phylogenetic studies. This study aims to elucidate the phylogenetic relationships among Drepanidae using eleven molecular markers, totalling more than 7,500 bp. A phylogenetic tree based on 37 species of Drepanidae was inferred, including 21 of the 22 European drepanids. The results of this study support monophyly of all four subfamilies of Drepanidae. Oretinae and Drepaninae were recovered as sister groups forming one of the two main clades of Drepanidae, whereas Cyclidiinae and Thyatirinae belong to the other clade. This analysis demonstrates that hooked wingtips, a characteristic feature of the Drepaninae + Oretinae lineage, have disappeared repeatedly in the course of evolution. All "winter moths" among the drepanids, i.e. species characterized by their flight period being very early in spring form a distinct well-supported clade within the subfamily Thyatirinae. Moreover, all studied thyatirine genera that are characterized by pink or orange blotches on the forewings also belong to one statistically well-supported clade. The phylogenetic framework presented enhances our understanding of the diversification of Drepanidae and provides the groundwork for future taxonomic and evolutionary studies.

INTRODUCTION

The taxonomic composition and phylogenetic affinities of the moth superfamily Drepanoidea (Lepidoptera: Macroheterocera) has been an object of much debate. The traditional view (Minet, 1991; Minet & Scoble, 1999; Kristensen et al., 2007), primarily supported by morphological synapomophies of the pupal stage, is that Drepanoidea are closely related to Geometroidea, and comprise two families, Drepanidae and Epicopeiidae. Recent molecular studies have, however, refuted both of these hypotheses. First, Mutanen et al. (2010), Kawahara et al. (2019) and Mayer et al. (2021) have convincingly demonstrated that Drepanoidea are sister to all other Macroheteroceran superfamilies. Mitter et al. (2017) and Rota et al. (2022) expanded the concept of Macroheterocera to include Mimallonoidea, but the phylogenetic position of Drepanoidea is nevertheless identical in their studies and those cited above. Second, a substantial number of molecular studies (e.g. Regier et al., 2009, 2013; Heikkilä et al., 2015; Kawahara et al., 2019; Mayer et al., 2021; Rota et al., 2022) have proven that Epicopeiidae do not belong to Drepanoidea, but form a sister clade to Sematuridae in the Geometroidea. On the other hand, Doidae (previously associated with Noctuoidea, see Kitching & Rawlins, 1999) and Cimeliidae (believed to be a rather isolated group, see Minet (1999) as Axioidea) were recently moved to Drepanoidea (van Nieukerken et al., 2011), which was subsequently supported by molecular studies (e.g. Mutanen et al., 2010; Heikkilä et al., 2015; Kawahara et al., 2019; Mayer et al., 2021; Rota et al., 2022).

The family Drepanidae, comprising approximately 650 species worldwide (Minet & Scoble, 1999; van Nieukerken et al., 2011), is by far the largest subdivision of Drepanoidea. Currently Drepanidae are considered to include four subfamilies: Cyclidiinae, Drepaninae, Oretinae and Thyatirinae (Wu et al., 2010; Song et al., 2012; Jiang et al., 2016). Historically, the subfamilies Cyclidiinae and Thyatrinae were often treated as separate families (Thyatiridae and Cyclidiidae) based on morphological evidence (e.g. Smith, 1893; McDunnough, 1938; Nakamura, 1981; de Freina & Witt, 1987; Laszlo et al., 2007), while Oretinae were raised to the rank of subfamily relatively recently (Wu et al., 2010). The reason for such inconsistency between different classifications in a relatively small family lies in the very variable habitus of these moths. Drepaninae and Oretinae possess broad wings, with the forewings typically angular or falcate, leading to their nickname "hook-tip moths". Moths in these two subfamilies often have a reduced or no proboscis. While adults of Drepaninae and Oretinae are primarily active at night, some groups (e.g. *Nidara* Mabille, 1897) fly during the day (Minet & Scoble, 1999). Both Drepaninae and Oretinae are widely distributed in the Old World and Nearctic region, being most diverse in eastern Palaearctic, Afrotropical and Oriental realms (Minet & Scoble, 1999; Song et al., 2012). Unlike Drepaninae and Oretinae, moths of the subfamily Thyatirinae have a stout body and narrow wings. Due to their resemblance to owlet moths (Noctuidae), thyatirines have sometimes been named "false-owlets". The centre of thyatirine diversity is in temperate regions in the Holarctic, but this subfamily is also present in Oriental, Afrotropical and Neotropic regions (Minet & Scoble, 1999; Laszlo et al., 2007). The fourth subfamily, Cyclidiinae, comprises moths with very slender bodies and broad and delicate wings that superficially resemble Geometrids rather than other drepanids (Jiang et al., 2016). This group occurs only in the eastern Palaearctic and Oriental regions (Jiang et al., 2016). Despite such distinct differences in habitus, all drepanids share the unique build of abdominal tympanal organs (Minet & Scoble, 1999; Surlykke et al., 2003). Large-scale molecular studies (e.g. Mutanen et al., 2010; Regier et al., 2013; Kawahara et al., 2019) indicate that these groups are indeed very closely related, which supports treating them as subdivisions of Drepanidae.

While significant attention was recently devoted to determining the phylogenetic relationships between taxa in the lower systematic ranks of various Lepidoptera (e.g. Rönkä et al., 2016; Rota et al., 2016; Wiemers et al., 2020; Õunap et al., 2024), very little is known about the phylogeny of Drepanidae (Davis et al., 2022). Wu et al. (2010) shed some light on the relationships between East Asian species in the subfamilies Drepaninae and Thyatirinae, and Jiang et al. (2016) on species of *Cyclidia* Guenée, 1858 from China, but otherwise there is no phylogenetic information on Drepanidae. No molecular phylogenies exist even for the relatively well-known and uncontroversial European drepanid fauna. This is unfortunate, because life histories of moths from this part of the world are rather well known in a global context (e.g. Skou, 1986; de Freina & Witt, 1987; Leraut, 2006), offering great opportunities for comparative evolutionary ecological research (Davis et al., 2016; Seifert et al., 2023). To overcome this shortcoming, the results of a molecular phylogenetic analysis of Drepanidae focusing on European taxa are presented in this paper. In the most recent checklist of European Lepidoptera, Schintlmeister (1996) listed 18 species of Drepanidae. Subsequent publications by Zolotuhin (1999), De-Gregorio et al. (2002), Leraut (2006) and Fritsch et al. (2014) increased this figure to 22, representing 14 genera and two subfamilies, Drepaninae and Thyatirinae. The current phylogenetic analysis includes 21 of the 22 species of European drepanids and all 14 genera, but also utilizes molecular data stored in public repositories originating from species occurring in other regions, in order to put the results into a broader context.

MATERIAL AND METHODS

Sample collection and DNA extraction

A total of 37 species of drepanids were included in this study. For six of them, vouchers were freshly collected from Estonia, Finland, Georgia (Caucasus) and Morocco. For a further four species, for which only barcodes were available in public repositories, 20 µL of extracted DNA were obtained from the Canadian Centre for DNA Barcoding (CCDB). For the remaining 27 species, sequence information was retrieved from the NCBI GenBank, either from whole genomes or separately stored shorter gene fragments (for details see below). The moths were identified down to species level using de Freina & Witt (1987) and Zolotuhin (1999). To insure the correctness of the identifications, DNA barcodes of all species were cross-checked using the ID engine on the Barcode of life Data Systems web page (Ratnasingham & Hebert, 2007). Sample collection areas, voucher codes and GenBank accession codes for the sequenced molecular markers from all species are listed in Table 1. The genomic DNA was isolated from the legs of the moths using the DNEasy Blood and Tissue kit (Qiagen N.V., Venlo, Netherlands) following the guidelines provided by the manufacturer.

PCR and sequencing

Eleven 'legacy' markers, which are widely used in phylogenetic studies on Lepidoptera (e.g. Õunap et al., 2011; Brehm et al., 2019; Keegan et al., 2019; Murillo-Ramos et al., 2019), were sequenced from both freshly collected samples of Drepanidae and extracts obtained from CCDB. These markers include cytochrome oxidase subunit I [COI], elongation factor 1 alpha [EF-1α], wingless [WGL], glyceraldehyde3-phosphate dehydrogenase [GAPDH], ribosomal protein S5 [RPS5], isocitrate dehydrogenase [IDH], carbamoyl phosphate synthetase [CAD], malate dehydrogenase [MDH], arginine kinase [ArgK], sarco/endoplasmic reticulum calcium ATPase [Ca-ATPase] and nexin-9-likeprotein [Nex9].

A total volume of 20 µL was used for the PCR, which included a reaction mixture consisting of 5 pmol of the primers, 10–40 ng of purified genomic DNA, 1 × BD Advantage 2 PCR buffer, 1 U of BD Advantage 2 Polymerase mix (BD Biosciences, San Jose, CA, USA) and 0.2 mm of dNTP (Thermo Scientific, Pittsburgh, PA, USA). PCR was carried out using a T1 thermocycler (Biometra, Göttingen, Germany) with the following cycling condition: a 2 min denaturing step at 94°C, 35–40 cycles of 30 s at 94°C, 30 s at different temperatures based on the primers used (see Õunap et al. (2024) for more details) and 60 s at 68°C, with a subsequent 7 min final extension at 68°C. PCR solution was purified by adding FastAP thermo-sensitive alkaline phosphatase and exonuclease I (Thermo Scientific). One unit of both enzymes was added to the PCR solution, which was incubated for 16 min at 37°C, followed by 15 min inactivation at 80°C. DNA sequencing was done using the 3730xl DNA Analyser automated sequencer (Applied Biosystems) in the Estonian Biocentre (Tartu, Estonia) (See Õunap et al. (2024) for more details). Geneious Prime 2024.0.4 was used to create the consensus sequences, which were subsequently aligned using ClustalW (Thompson et al., 1994) in BioEdit v7.0.5.2 (Hall, 1999).

Data mining and extracting the regions of interest

Whole genome data for eleven species were downloaded from the NCBI GenBank. The eleven 'legacy' markers were extracted from this data set using a proprietary process, which involved utilizing MetaEuk (Levy Karin et al., 2020), HMMER v3.3.2 (http://hmmer.org/) and a custom Python script designed for this purpose (see Õunap et al., 2024 for details). Extracted markers were com-

Table 1. List of sequences used in the construction of the phylogenetic tree. Accession codes highlighted in bold indicate sequences that were retrieved from the full genome of the respective species. For *Polyploca korbi*, NCBI GenBank accession code was not available, thus the details recorded in the BOLD Systems database are presented instead.

	COI	EF1a	WGL	GAPDH	RpS5	IDH	MDH	CAD	ArgK	Ca-ATPase	Nex9
Cimeliidae											
Axia margarita	GU828762 GU929736	GU829075 GU829358	GU829624	GU829853	GU830729	GU830147	GU830463	GU828244	×	×	×
Axia theresiae	GU929812	GU829150 GU829416	GU829697	×	GU830797	×	GU830539	GU828312	×	×	×
Drepanidae											
Cyclidiinae											
Cyclidia fractifasciata	KR872934	×	×	×	×	×	×	×	×	×	×
Cyclidia rectificata	KR872929	×	×	×	×	×	×	×	×	×	×
Cyclidia sericea	GU828830 GU929796	GU829133 GU829407	GU829683	×	GU830781	GU830210	GU830525	GU828297	×	×	×
Cyclidia substigmaria	FJ768743	FJ768760	GQ283841	×	×	×	×	GQ283525	×	×	×
Drepaninae											
Callidrepana patrana	FJ768751	FJ768767	×	×	×	×	×	GU174161	×	×	×
Cilix asiatica	PQ476154	PQ479303	PQ479346	PQ479312	PQ479339	PQ479319	PQ479327	×	PQ479284	PQ479294	PQ479336
Cilix glaucata	PQ476157	PQ479306	×	PQ479313	PQ479342	PQ479322	PQ479330	×	PQ479287	PQ479297	×
Cilix hispanica	PQ476160	PQ479308	×	PQ479315	×	×	PQ479332	×	PQ479290	PQ479299	×
Ditrigona conflexaria	FJ768750	FJ768766	×	×	×	×	×	×	×	×	×
Drepana arcuata	GCA_016069955				×	GCA_016069955					
Drepana curvatula	PQ476152	PQ479301	PQ479344	PQ479310	×	PQ479317	PQ479325	×	PQ479282	PQ479292	PQ479334
Drepana falcataria	**OX243952**	**OX243922**	**OX243923**	**OX243936**	×	**OX243925**	**OX243928**	**OX243925**	**OX243922**	**OX243925**	**OX243926**
Falcaria lacertinaria	**OX602140**	**OX602109**	**OX602110**	**OX602123**	×	**OX602111**	**OX602115**	**OX602111**	**OX602109**	**OX602111**	**OX602114**
Macrocilix maia	FJ768749	FJ768765	×	×	×	×	×	×	×	×	×
Macrocilix mysticata	FJ768744	AB265512	JQ786928	×	×	×	×	×	×	×	×
Microblepsis leucosticta	FJ768748	FJ768764	×	×	×	×	×	×	×	×	×
Sabra harpagula	PQ476156	PQ479305	PQ479348	×	PQ479341	PQ479321	PQ479329	×	PQ479286	PQ479296	PQ479338
Tridrepana fulvata	FJ768747	FJ768747	×	×	×	×	×	×	×	×	×
Watsonalla binaria	**OV838950**	**OV838918**	**OV838919**	**OV838933**	**OV838923**	**OV838922**	**OV838924**	**OV838922**	**OV838918**	**OV838922**	**OV838921**
Watsonalla cultraria	PQ476159	PQ479307	PQ479349	PQ479314	×	PQ479323	PQ479331	×	PQ479289	PQ479298	×
Watsonalla uncinula	PQ476153	PQ479302	PQ479345	PQ479311	×	PQ479318	PQ479326	×	PQ479283	PQ479293	PQ479335
Oretinae											
Hypsomadius insignis	FJ768745	FJ768761	×	×	×	×	×	×	×	×	×
Oreta vatama	FJ768746	FJ768762	×	×	×	×	×	×	×	×	×
Thyatirinae											
Achlya flavicornis	**OX392497**	**OX392469**	**OX392468**	**OX392483**	**OX392470**	**OX392472**	**OX392475**	**OX392472**	**OX392469**	**OX392472**	**OX392473**
Asphalia ruficollis	PQ476158	×	×	×	PQ479343	×	×	×	PQ479288	×	×
Cymatophorina diluta	PQ476155	PQ479304	PQ479347	×	PQ479340	PQ479320	PQ479328	×	PQ479285	PQ479295	PQ479337
Gaurena fletcheri	FJ768742	FJ768759	×	×	×	×	×	×	×	×	×
Habrosyne conscripta	FJ768740	FJ768757	×	×	×	×	×	×	×	×	×
Habrosyne pyritoides	**OU015616**	**OU015587**	**OU015586**	**OU015600**	×	**OU015591**	**OU015594**	**OU015591**	**OU015587**	**OU015591**	**OU015590**
Ochropacha duplaris	**OX592721**	**OX592692**	**OX592691**	**OX592704**	×	**OX592694**	**OX592699**	**OX592694**	**OX592692**	**OX592694**	**OX592695**
Parapsestis lichenea	FJ768741	FJ768758	×	×	×	×	×	×	×	×	×
Polyploca ridens	**OX596273**	**OX596245**	**OX596246**	**OX596258**	**OX596248**	**OX596247**	**OX596251**	**OX596247**	**OX596245**	**OX596247**	**OX596249**
Polyploca korbi	LPALE3339-23	×	×	×	×	×	×	×	×	×	×
Tethea ocularis	**OY741987**	**OY741958**	**OY741957**	**OY741970**	**OY741960**	**OY741959**	**OY741964**	**OY741959**	**OY741958**	**OY741959**	**OY741961**
Tethea or	PQ476151	PQ479300	×	PQ479309	×	PQ479316	PQ479324	×	PQ479281	PQ479291	PQ479333
Tetheella fluctuosa	**OX578245**	**OX578215**	**OX578214**	**OX578228**	**OX578216**	**OX578218**	**OX578221**	**OX578218**	**OX578215**	**OX578218**	**OX578217**
Thyatira batis	**LR990516**	**LR990487**	**LR990486**	**LR990500**	**LR990488**	**LR990489**	**LR990492**	**LR990489**	**LR990487**	LR990489	LR990490

bined with the original data set. In addition, publicly available ‘legacy’ markers of fifteen additional species of Drepanidae (see Table 1), were downloaded from the NCBI GenBank to increase the taxonomic coverage of this dataset.

Phylogenetic tree inference

For inferring the phylogeny, data for all markers were concatenated together into one large dataset. In addition to 37 species of Drepanidae, this data matrix included two species of Cimeliidae (*Axia margarita* (Hübner, 1813) and *A. theresiae* (Korb, 1900)), which were used as an outgroup. The concatenated data matrix included 1536 bp from COI, 1188 bp from EF-1alpha, 393 bp from WGL, 691 bp from GAPDH, 620 bp from RPS5, 684 bp from IDH, 733 bp from MDH, 850 bp from CAD, 388 bp from ArgK, 398 bp from Ca-ATPase and 372 bp from Nex9, totalling 7853 bp.

In the search for the best partitioning scheme, each marker was initially treated as an independent partition. The software ModelFinder (Kalyaanamoorthy et al., 2017), integrated into IQ-TREE v2.1.2 (Nguyen et al., 2015), was run on the University of Tartu HPC cluster (University of Tartu. UT Rocket. share.neic.no. https://doi.org/10.23673/PH6N-0144) to determine the best scheme and substitution models. The optimal partitioning scheme was determined by potentially consolidating partitions to alleviate over-parametrization, enhance model fit and incorporate the FreeRate model (-m TESTNEWMERGEONLY). Partitions were permitted to evolve at varying rates (-spp) and ‘-rcluster’ was set to 30 in order to speed up the analysis. ModelFinder search resulted in dividing the data into 3 partitions, with each of them in-

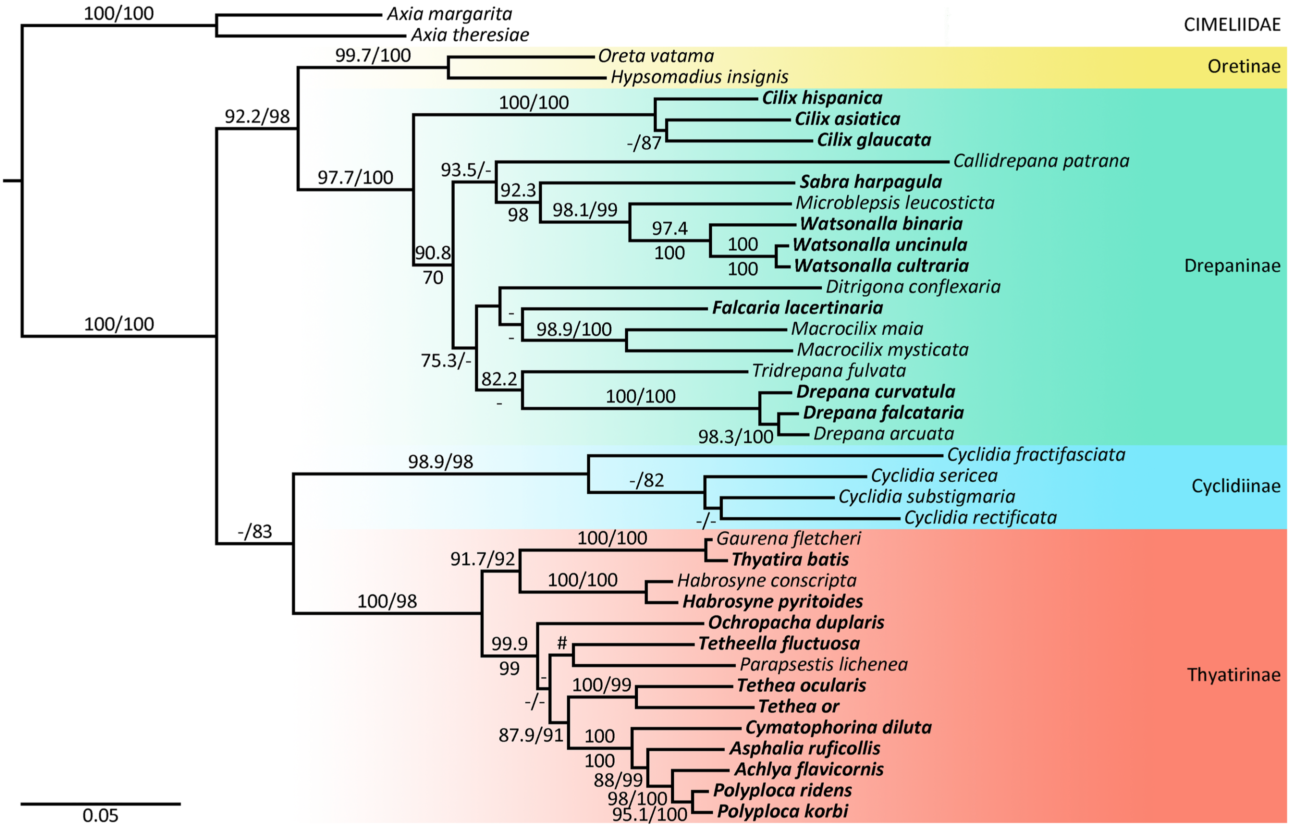

Fig. 1. Maximum likelihood phylogeny for Drepanidae. Numbers above or below branches or slashes indicate SH-Like support and UFBoot2 support, respectively. Values of the support indices inferior to 70% are not shown. A very short branch with statistical support for SH-Like = 84.4%, UfBoot2 = 94% is indicated by the symbol #. European species are highlighted in bold.

cluding 1 to 5 loci. In order to construct the phylogenetic tree, the partition scheme obtained from the previous run with IQ-TREE was used. Partitions were allowed to evolve at varying speeds (-spp), and a more thorough NNI search was activated (-allnni). In addition, NNI based directly on bootstrap alignments (-bnni) was conducted to minimize the risk of overestimating branch supports. Node supports were estimated using 1,000 ultrafast bootstrap (UFBoot2, Hoang et al., 2018) replications and 1,000 SH-Like approximate likelihood ratio test (SH-Like, Guindon et al., 2010) iterations. In order to secure that the analysis was not trapped in a local optimum, five independent runs were carried out with IQ-Tree. Final trees were visualized and compared in FigTree v1.4.4 (Rambaut, 2012), using Cimeliidae as an outgroup and to root the tree (Fig. 1).

RESULTS AND DISCUSSION

A phylogenetic tree for 37 species of Drepanidae, rooted by two species of Cimeliidae, was inferred using a set of eleven ‘legacy’ markers, providing new insights into the evolutionary relationships within this group of moths. The attempt to retrieve the ‘legacy’ markers from the downloaded whole genome dataset was exceptionally successful: for six species, all eleven markers were obtained, and for a further five species ten markers. The success in retrieving stored ‘legacy’ markers from GenBank was mixed, as seven and four markers were available for one species, three for two species and two for nine species. Sequencing of freshly collected specimens of six species was reasonably successful, with results ranging from 7 to 10 markers per species. Sequencing particular gene fragments from DNA extracts of four species obtained from CCDB was slightly less successful, as three to eight markers per species were eventually obtained. For three species present in the phylogeny, only downloaded DNA barcodes were available. Outgroup species were represented by eight (*A. margarita*) and six (*A. theresiae*) markers. GenBank accession numbers of both newly generated and downloaded sequences are provided in Table 1. While developing the phylogenetic tree, all five runs with IQ-Tree resulted in identical topologies, indicating that the recovered phylogeny is robust. The results of the runs only slightly differed in terms of branch lengths and statistical support for the nodes. A tree with the best likelihood score (Fig. 1) was selected to be the basis of further discussion.

In this phylogenetic analysis, all four subfamilies of Drepanidae formed statistically very well supported (SH-Like ≥ 97.7, UFBoot 2 ≥ 98) monophyletic groups, confirming the earlier finding of Wu et al. (2010) (Fig. 1). This analysis divided Drepanidae into two clades, both of which include two subfamilies (Fig. 1). One of these clades, which had very strong statistical support (SH-Like = 92.2%, UFBoot2 = 98%) includes Drepaninae and Oretinae, while the second, a statistically poorly supported (SH-Like = 58.1%, UFBoot2 = 83%) lineage, includes Thyatirinae and Cyclidiinae.

Of these relationships, the position of Cyclidiinae is the most intriguing. Mutanen et al. (2010), Wu et al. (2010)

and Regier et al. (2013) reported that Cyclidiinae are sister to all other drepanids. On the other hand, Regier et al. (2009) and Kawahara et al. (2019) showed that Cyclidiinae are sister to Thyatirinae instead. The results presented here (Fig. 1), although only suggestive due to poor statistical support for that particular node, are nevertheless in line with the more recent finding of Kawahara et al. (2019), which, unlike other studies, used a phylogenomic approach relying on data for more than 2,000 protein-coding genes.

The sister-group relationship between Oretinae and Drepaninae has been reported by Wu et al. (2010) and Regier et al. (2013), but it is worth noting that other molecular studies cited above did not incorporate both of these subfamilies simultaneously, thus leaving the question about the relationships between them unanswered. The results presented (Fig. 1) fully support the position of Oretinae as sister to Drepaninae.

One prominent characteristic of Drepanidae is the tendency to evolve a falcate, elongated forewing apex, which has earned them vernacular names in various languages (e.g. the English "hook-tips"). In fact, there is a whole spectrum of forewing tip shapes present in Drepanidae, from completely round (*Cilix* Leach, 1815) and sharp apex (*Ditrigona* Moore, 1888) to a small "hook" (*Watsonalla* Minet, 1985) and a very prominent "hook" (*Oreta* Walker, 1855). While hooked wingtips are absent, and sharp wingtips occur in only some species of Cyclidiinae and Thyatirinae, much more variation is reported in the other two subfamilies (e.g. de Freina & Witt, 1987; Holloway, 1998; Song et al., 2012). The current analysis, though based on a limited number of taxa, demonstrates that hooked wingtips have appeared or disappeared repeatedly in the Oretinae + Drepaninae lineage: forewing tip is rounded in *Cilix* and *Macrocilix* Butler, 1886, and sharp in *Ditrigona*, while larger or smaller "hooks" are present in all other genera. As such wing morphology is rare among Marcoheterocera (unique in the European fauna), it appears reasonable to assume that it evolved once among the ancestors of Oretinae + Drepaninae, and has been lost several times in the course of the evolution of this clade. Interestingly, the loss of the hooked forewing tips appears to be associated with the evolution of white colouration (in addition to *Cilix* and *Macrocilix* represented in current tree, consider e.g. the Nearctic *Eudeilinia* Packard, 1876 and Oriental *Teldenia* Moore, 1883), which is likely related to a shift from mimicking dry leaves to mimicking bird droppings.

Within Thyatirinae, the results also revealed a few patterns that deserve further research. First, in some genera distinct pink or orange blotches are present on the forewings. All such genera included in the derived tree (*Gaurena* Walker, 1865, *Habrosyne* Hübner, 1821, *Thyatira* Ochsenheimer, 1816) formed a clade with mixed statistical support (SH-Like = 91.7, UFBoot2 = 92) as sister to the rest of the subfamily (Fig. 1). It remains to be determined whether other, currently unsampled Thyatirinae genera that exhibit similar elements of the forewing pattern (e.g. *Euthyatira* Smith, 1891, *Horithyatira* Matsumura, 1933, *Macrothyatira* Marumo, 1916) also belong to this clade. Second, Laszlo et al. (2007) divided Thyatiridae into two subfamilies, Thyatirinae and Polyplocinae, which could now be treated as tribes due to the downranking of the "false-owlets" to a subfamily in many recent studies. The current results clearly reveal a conflict with the classification of Laszlo et al. (2007), as genera placed in Polyplocinae in their system (*Achlya* Billberg, 1820, *Asphalia* Hübner, 1821, *Cymatophorina* Spuler, 1908 and *Polyploca* Hübner, 1821) form a distinct clade that is positioned within their concept of Thyatirinae as sister to *Tethea* Ochsenheimer, 1816 (Fig. 1). Third, according to Laszlo et al. (2007) *Asphalia* and *Cymatophorina* belong to tribe Demipsestini, while *Achlya* and *Polyploca* are placed in Polyplocini. The current findings are in conflict with their classification, as *Cymatophorina* was recovered as sister to the remaining three genera (Fig. 1), thus indicating that Demipsestini sensu Laszlo et al. (2007) are paraphyletic.

Though the delimitation of the lower ranks of Polyplocinae sensu Laszlo et al. (2007) may need revision, the group itself received maximum statistical support in the current analysis (Fig. 1). Laszlo et al. (2007) pointed out that one important ecological characteristic of this group is the cold-tolerance of adults, whose flight period is in late autumn, winter or early spring. The habit of adult moths to be active very early or late in a year is known to be correlated with other ecological traits of the species (the "winter moth syndrome", Hunter, 1995). Disentangling causal relationships underlying this syndrome could advance our understanding of insect ecology in general (e.g. Snäll et al., 2007), with a comparative approach benefitting from an increasing number of phylogenetically independent cases of "winter flight". The current study adds one phylogenetically resolved case to those known for the family Geometridae (Wahlberg et al., 2010). In particular, the sister clade relationship of the early autumnal *Cymatophorina* and the proper winter moths provides an analogy to the case of *Epirrita* Hübner, 1822 (mid-autumn) vs *Operophtera* Hübner, 1825 (late autumn), suggesting a gradual transition to the winter moth phenotype. Also, the same relationship provides evidence that supports the view that evolutionary switches between early and late season flight are frequent (Yamamoto & Sota, 2007; Wahlberg et al., 2010; Truuverk et al., 2017). Resolving the phylogenetic relationships of the early- or late-flying thyatirine genera from other parts of the world (e.g. *Bycombia* Benjamin, 1938, *Ceranemota* Clarke, 1938, *Nemacerota* Hampson, 1893) would further contribute to resolving the evolution of the winter moth syndrome.

In conclusion, the present study offers a comprehensive understanding of the phylogeny of European Drepanidae in a wide context. The results indicate that Oretinae and Drepaninae form one main subdivision of Drepanidae, and Cyclidiinae and Thyatirinae belong to another. Multiple cases of losing a falcate forewing apex, a characteristic of Drepanidae, were discovered in Drepaninae. All thyatirine genera with a colourful forewing pattern form a distinct clade, whereas all early spring species also appeared to be very closely related to each other. By providing a clear understanding of the phylogeny of Drepanidae, this study not only sheds light on the evolution of this group of moths, but will also help in determining the ecological roles and adaptations of drepanids. Future research should extend the sampling globally and employ more advanced molecular techniques to delve deeper into the phylogeny of this interesting family.

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