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Applications

The Phylogenetic Placement of an Enigmatic Moth *Egybolis Vaillantina* Based on Museomics

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Abstract

Here, we present multi-locus sequencing results from the enigmatic Afrotropical monotypic genus *Egybolis* Boisduval (occurring in East- and South Africa—previously placed in the subfamily Catocalinae, Noctuidae). Model-based phylogenetic analysis places *Egybolis* within a strongly supported clade comprising four Old World Tropical genera *Cocytia* Boisduval, *Avatha* Walker, *Anereuthina* Hübner, and *Serrodes* Guenée from the family Erebidae, subfamily Erebinae. Hence, we propose to formally assign the monotypic genus *Egybolis* to the subfamily Erebinae and the tribe Cocytiini. Timing of divergence analysis reveals the late Oligocene origin around 25 million years ago (Ma) for the tribe Cocytiini, and an early Miocene (~21 Ma) for the split between *Cocytia* and *Egybolis*.

Introduction

Megadiverse groups of organisms tend to have taxa with unknown phylogenetic placement, often because they are highly autapomorphic, have a striking appearance, or, conversely, lack any shared derived morphological characters with other lineages. Molecular data is finally helping us to sort out the phylogenetic positions of such taxa (e.g., Buckley et al., 2009; Kanda et al., 2016; Sihvonen et al., 2021; Tihelka et al., 2020; Twort et al., 2021), leading to a better understanding of the evolutionary history of these lineages.

Lepidoptera are undoubtedly the largest radiation of phytophagous insects (Scoble, 1992) with over 158,000 described species (Pogue, 2009; van Nieukerken et al., 2011). The most diverse lineage of Lepidoptera is undeniably the superfamily Noctuoidea with more than 45,890 described species (Pogue, 2009; van Nieukerken et al., 2011). Noctuoid species are placed in approximately 5,630 genera (Pogue, 2009), but there are numerous undescribed species, particularly from tropical regions. Moreover, many of these tropical lineages have not been phylogenetically studied; thus, their evolutionary relationships are unknown. In particular, some of them have been previously assigned to the wrong taxonomic groups (e.g., Sihvonen et al., 2021; Zilli & Grishin, 2019).

Not long ago, researchers were using the biological material stored in museum collections only at a morphological study level. Due to the high level of degradation and fragmentation of the genetic material in historical samples, the DNA from these specimens was considered to be too degraded to be utilized in molecular studies (Shapiro & Hofreiter, 2012). Consequently, molecular approaches have been restricted, on one hand, to sequencing technologies (e.g., Sanger sequencing) (Hajibabaei et al., 2006; Hebert et al., 2013) and, on the other hand, were limited to quality materials (e.g., freshly collected samples, proper killing agent, etc.). However, recently, next-generation sequencing (NGS) technologies have made the DNA in museum specimens more accessible, either through whole-genome sequencing (WGS) (Allio et al., 2020; Call et al., 2021; Sproul & Maddison, 2017; Twort et al., 2021) or genome reduction methods (Breinholt et al., 2018; Mayer et al., 2021; Suchan et al., 2016). These advanced sequencing approaches have opened a new field with great potential for studying the evolutionary history of taxa that are difficult to collect: museomics (Call et al., 2021).

Here, using a WGS museomics approach, we investigate the phylogenetic position of the enigmatic African genus *Egybolis* Boisduval, 1847—a monotypic moth genus previously placed in the family Noctuidae. The genus was placed in the subfamily Catocalinae in Noctuidae (e.g., Pinhey, 1975; Poole, 1989; Vári & Kroon, 1986). When the family Erebidae was officially established in 2011 (Zahiri et al., 2011), it "automatically" followed the rest of Catocalinae into that family. On the Afromoths website, it is currently listed in Erebidae, Erebinae (De Prins & De Prins, 2011). Its only species—*Egybolis vaillantina* (Stoll, [1790]), the African peach moth—is found in East- and South Africa. Unlike

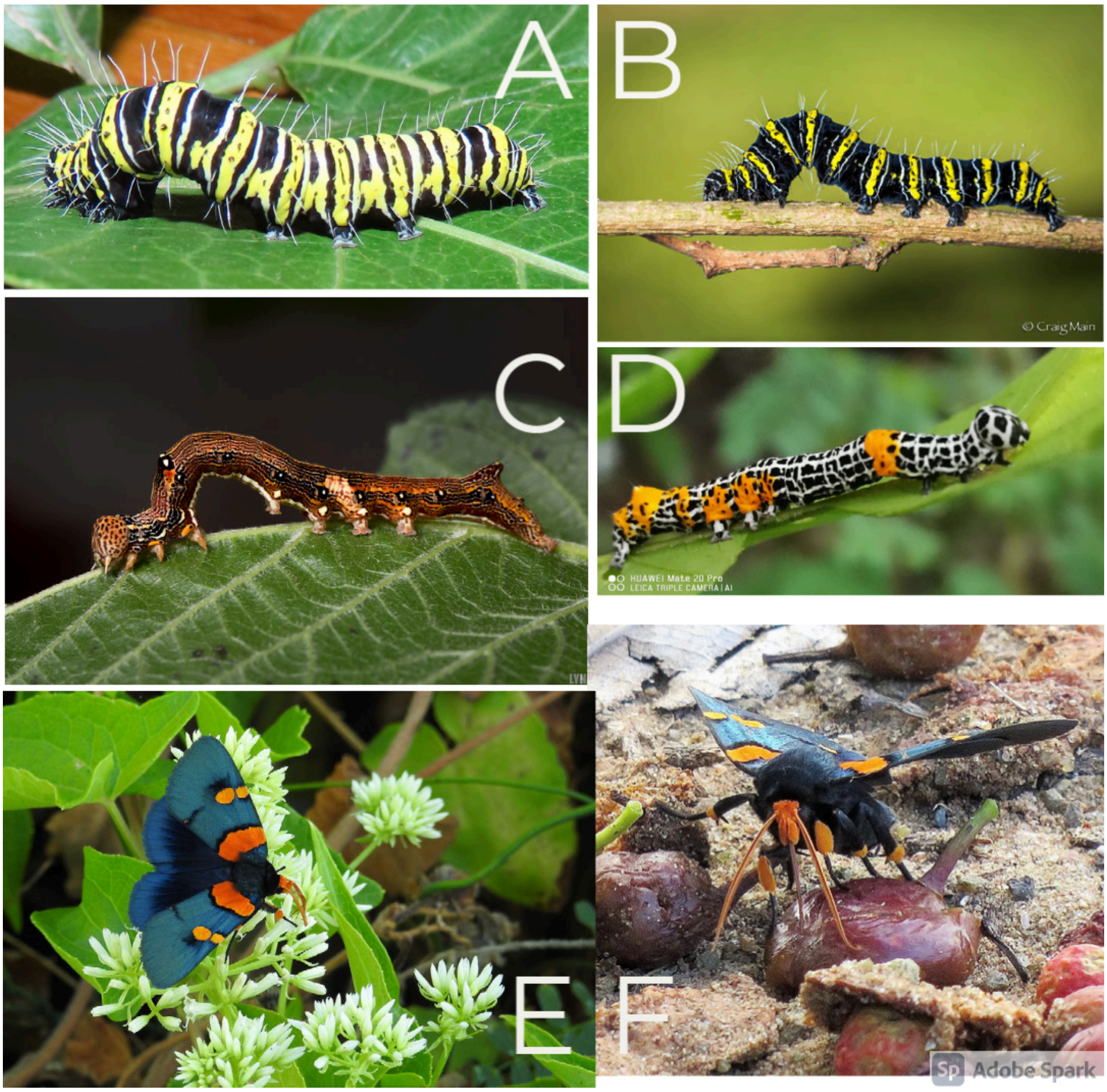

Figure 1. A, B) Caterpillars of the striking African Peach Moth (https://www.inaturalist.org/observations/66703880; https://www.inaturalist.org/observations/48096039); C) Caterpillar of *Avatha* (https://www.inaturalist.org/observations/41976344); D) Caterpillar of *Anereuthina* (https://www.inaturalist.org/observations/95109281); E, F) Adults of the striking African Peach Moth (https://www.inaturalist.org/observations/84578502; https://www.inaturalist.org/observations/67839106).

most owlet moths, the striking African Peach Moth is diurnal, and the aposematic larvae (Fig. 1A, B) feed on peach (Rosaceae) and *Sapindus* (Sapindaceae) species (Robinson et al., 2010). Adults are also aposematic with orange antennae and head, a dark blue body, and one orange band and two orange spots on each forewing (Fig. 1E, F). Although not known, both adults and larvae may be sequestering toxins from their hostplant. Wingspan is 50–60 mm.

Material and methods

Genomic DNA was extracted from a leg of an individual collected in 1993 in Tanzania, which is currently housed at the Natural History Museum, University of Oslo. The extraction was done in 2011 with the original intention to sequence PCR products from the specimen (according to protocols described in Zahiri et al. (2012)). However, all PCRs failed, and the extract has been stored at +2°C since then. Here we use the same extract for whole genome shotgun sequencing following protocols published in Twort et al. (2021). Briefly, DNA was blunt-end repaired with T4 Polynucleotide Kinase (New England Biolabs), followed by a reaction clean-up with the MinElute purification kit (Qiagen). This was followed by adapter ligation, reaction purification, and adapter fill-in. The resulting reactions were then indexed using unique dual indexes. Indexing PCR was carried out in six independent reactions to avoid amplification bias, with 15 cycles for each reaction. Indexing PCR reactions were pooled prior to magnetic bead clean-up with Sera-Mag SpeedBeads™ carboxylate-modified hydrophilic (Sigma-Aldrich). An initial bead concentration of 0.5X was used to remove long fragments that are likely to represent contamination from fresh DNA, and libraries were selected with a bead concentration of 1.8X to size select the expected library range of ~300 bp. The resulting library was quantified and quality checked with Quanti-iT™ PicoGreen™ dsDNA assay and with Bioanalyzer 2100, respectively. The final indexed library was pooled with 53 other samples prior to sequencing on an Illumina Novaseq platform (one lane, 2x150 bp, S4 flow cell) at Swedish National Genomics Infrastructure (NGI) in Stockholm.

Following Twort et al. (2021), we checked the quality of the raw reads with FASTQC v0.11.5 (Andrews, 2010). We removed reads with ambiguous bases (N's) from the dataset using Prinseq 0.20.4 (Schmieder & Edwards, 2011). We then used Trimmomatic 0.38 (Bolger et al., 2014) to remove low-quality bases from their beginning (LEADING: 3) and end (TRAILING: 3), using a sliding window approach. Quality was measured for sliding windows of four base pairs and had to be higher than 25 on average. Reads below 30 bp were also removed. We then de novo assembled the genome of *Egybolis* with SPAdes v3.13.1 (Bankevich et al., 2012) with k-mer values of 21, 33, and 55.

We extracted the standard eight gene regions used by Zahiri et al. (2012), using TBLASTN—with *Bombyx mori* amino acid sequences as the reference, as all of these sequences represent one exon within the gene of interest. A preliminary analysis using the dataset published by Zahiri et al. (2011) clearly placed *Egybolis* within Erebidae; thus, we used the dataset of Zahiri et al. (2012) for further analyses.

Model-based phylogenetic analyses were based on DNA sequences of eight protein-coding genes—cytochrome *c* oxidase subunit I (COI) from the mitochondrial genome and elongation factor-1α (EF-1α), ribosomal protein S5 (RpS5), carbamoylphosphate synthase domain protein (CAD), cytosolic malate dehydrogenase (MDH), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), isocitrate dehydrogenase (IDH), and wingless genes from the nuclear genome—from 201 representatives of the major lineages of Erebidae and 36 representatives from the five other families of Noctuoidea: Oenosandridae (one species), Notodontidae (three species), Nolidae (five species), Euteliidae (four species), and Noctuidae (23 species), giving a total of 237+1 (= *Egybolis vaillantina*) terminal taxa (dataset A, Table 1). We included an additional species from BOLD (Ratnasingham & Hebert, 2007) (with only barcode region data, 658 bp): *Anereuthina renosa* (representing type species of the genus)—a genus that appears to be closely related to the *Serrodes* Guenée group (Holloway, 2005). Raw sequencing data for *Egybolis vailantina* can be found in the NCBI database under Bioproject PRJNA913480.

We ran maximum likelihood (ML) analyses with the dataset partitioned by gene using IQ-TREE 2.0.6 (Trifinopoulos et al., 2016). The best-fitting substitution models and optimal number of partitions were selected by ModelFinder (-m MFP+MERGE) (Kalyaanamoorthy et al., 2017). Support for nodes was evaluated with 1000 ultrafast bootstrap (UFBoot2) approximations (Hoang et al., 2018) and SH-like approximate likelihood ratio test (Guindon et al., 2010) using "-B 1000 -alrt 1000" options. SH-Like ⩾80 and UFBoot2 ⩾ 95 values indicate well-supported clades. To reduce the risk of overestimating branch supports in UFBoot2 test, we used the -bnni option, which optimizes each bootstrap tree using a hill-climbing nearest neighbour interchange (NNI) search. We rooted the cladograms on *Oenosandra boisduvalii* Newman, which is the putative sister family to the remainder of the Noctuoidea (Mutanen et al., 2010; Regier et al., 2009; Zahiri et al., 2011, 2012).

Finally, divergence times were estimated using a relaxed lognormal molecular clock model as implemented in BEAST 2.6.3 (Bouckaert et al., 2019). We applied a reduced dataset with 209 terminals (dataset B, Table 1) to estimate the dates of cladogenetic events. We assume that different positions in the alignment could potentially accumulate substitutions differently. As a result, we unlinked the partitions for the substitution models, allowing each partition to evolve under a different substitution model, but we linked the partitions for the clock and tree models. The best partitioning scheme and their substitution models were selected using ModelFinder implemented in IQ-TREE (-m TEST-MERGEONLY -mset mrbayes) to choose the best set of substitution models in BEAST. The tree prior was set to a Birth Death model. Lepidoptera are characterized by a lack of fossils that can be confidently assigned to extant clades (Grimaldi & Engel, 2005; Wahlberg et al., 2013), and noctuoid moths are no exception. As a result, we relied on calibrations derived from phylogenomic study of Kawahara et al. (2019) using 2000 orthologous protein-coding genes of representatives of nearly all lepidopteran superfamilies and multiple fossil calibrations. We constrained the root of the superfamily Noctuoidea (69 Ma) with normal distributions encompassing the 95% credibility intervals estimated in Kawahara et al. (2019). Then we constrained the roots of the family Erebidae and the subfamily Arctiinae to 47.8 Ma and 30 Ma, respectively, with normal distributions encompassing the 95% credibility intervals (see Dataset S11; Kawahara et al., 2019). To make the run computationally easier, we used phylogenetic constraints, forcing the monophyly of the well supported clades in our phylogenetic results. For a list of the constraints check the Dataset S1.XML file in the supplementary material. The MCMC parameters were fixed to 100 million generations with sampling every 5,000 generations and the first 25% discarded as burn-in. We ran the analysis four independent times (different seed values) and confirmed the convergence of the runs before combining the results in LogCombiner (part of BEAST2 package). We used TreeAnnotator (part of BEAST2 package) to generate the maximum clade credibility (MCC) tree, representing the mean and 95% HPD interval for all nodal ages.

Distribution data for the target species and its closely related species (tribe Cocytiini) was acquired from the iNaturalist API using their tools and georeferenced in QGIS v.3.20.3 (QGIS Development Team, 2022) — a free and open source Geographic Information System — to visualize species distributions.

Table 1. List of taxa with voucher codes (specimen ID = specimen identity) and GenBank accession numbers used in the multi-locus and time of divergences analyses. – = Gene was not amplified for specimens. Raw sequencing data for *Egybolis vailantina* can be found in the NCBI database under Bioproject PRJNA913480.

Taxon	Sample ID	CAD	COI-LCO	COI-Jerry	EF1a-begin	EF1a-centre	EF1a-end	GAPDH	IDH	MDH	RpS5	Wingless
Anereuthina renosa	LEPMY039-13	–	OP933767	–	–	–	–	–	–	–	–	–
Mataeomera semialba	RZ107	JN401095	JN401323	JN401205	JN401438	–	–	JN401641	JN401745	JN401850	–	–
Simplicia pachycera	RZ166	HQ006971	HQ006175	HQ006879	HQ006272	OP921378	HQ006366	HQ006448	HQ006520	HQ006599	HQ006691	–
Amerila astreus	RZ404	OP921340	JN401288	JN401170	JN401403	JN401514	JN401514	JN401612	JN401715	–	JN401910	–
Tamba mnionomera	RZ415	–	JN401316	JN401199	JN401431	OP921478	JN401536	JN401634	JN401738	JN401844	JN401936	–
Toxonprucha sp	RZ307	–	JN401330	JN401212	JN401444	OP921423	JN401548	JN401648	JN401750	JN401854	JN401949	–
Anachrostis sp	RZ288	OP921336	OP933770	OP933776	–	–	–	–	–	OP921538	OP921544	–
Ugiodes cinerea	RZ326	OP921337	OP933771	OP933777	–	–	–	OP921523	–	–	OP921545	–
Tinolius eburneigutta	RZ331	OP921338	OP933772	OP933778	OP921349	–	–	OP921524	OP921531	OP921539	OP921546	–
Egybolis vaillantina	RZ687	–	OP933774	OP933781	–	–	–	–	–	–	–	–
Pseudophaloe troetschi	06-srnp-35191	GU828046	GU828534	GU828336	GU828880	–	GU829180	–	GU829925	GU830258	GU830569	GU829441
Cisseps fulvicollis	AM-94-0396	GQ283518	GU828535	GU828337	GU828881	–	GU829181	GU829722	GU829926	–	GU830570	GU829442
Asota caricae	MM00145	GU828115	GU828615	GU828413	GU828949	–	GU829240	–	GU830003	GU830325	GU830624	GU829509
Dysauxes famula	MM00154	GU828120	GU828619	GU828417	GU828954	–	GU829244	–	GU830008	GU830328	–	GU829514
Scoliopteryx libatrix	MM00407	GU828140	GU828641	GU828439	GU828975	–	GU829260	–	GU830028	GU830348	GU830643	GU829532
Lymantria monacha	MM01048	GU828152	GU828655	GU828453	GU828986	–	GU829270	–	GU830042	GU830361	GU830654	GU829542
Pechipogo strigilatus	MM01286	GU828160	GU828663	GU828461	GU828994	–	GU829277	GU829790	GU830050	GU830369	GU830660	GU829549
Hypena proboscidalis	MM01545	GU828165	GU828668	GU828466	GU828999	–	GU829282	GU829794	GU830055	GU830374	GU830664	GU829553
Hypenodes humidalis	MM01780	GU828168	GU828671	GU828469	–	–	GU829285	–	GU830058	–	GU830666	GU829556
Arctia caja	MM03713	GU828185	GU828693	GU828489	–	–	GU829305	GU829813	GU830080	GU830398	–	GU829573
Catocala sponsa	MM04358	GU828189	GU828700	GU828495	GU829023	–	GU829312	GU829816	GU830086	GU830404	GU830688	GU829576
Trisateles emortualis	MM04877	GU828195	GU828707	GU828502	GU829030	–	GU829319	GU829821	GU830093	GU830411	GU830695	GU829583
Lygephila pastinum	MM05092	GU828199	GU828711	GU828506	–	–	GU829323	–	GU830097	GU830415	GU830699	GU829587
Leucoma salicis	MM06740	GU828232	GU828748	GU929722	GU829062	–	GU829347	–	GU830132	GU830449	GU830719	GU829611
Coscinia cribraria	MM05671	HQ006949	HQ006149	HQ006856	HQ006247	–	HQ006342	–	HQ006499	KJ723677	HQ006666	HQ006758
Callistege mi	MM05469	HQ006950	HQ006150	HQ006857	HQ006248	–	HQ006343	–	HQ006500	HQ006578	HQ006667	HQ006759
Antichloris viridis	MM05380	HQ006951	HQ006151	HQ006858	HQ006249	–	HQ006344	HQ006433	HQ006501	HQ006579	HQ006668	HQ006760
Calyptra thalictri	MM00963	HQ006955	HQ006156	HQ006861	HQ006252	–	HQ006348	HQ006435	HQ006504	HQ006582	HQ006671	HQ006763
Parascotia fuliginaria	MM00340	HQ006954	HQ006154	HQ006862	HQ006253	–	HQ006347	HQ006436	HQ006505	HQ006583	HQ006672	HQ006764
Apisa canescens	MM05843	KJ723662	HQ006146	HQ006853	–	–	HQ006339	–	KJ723675	–	HQ006663	HQ006765
Oraesia emarginata	RZ102	HQ006958	HQ006159	HQ006864	HQ006256	OP921354	HQ006351	HQ006439	HQ006508	HQ006586	HQ006675	HQ006768
Saroba pustulifera	RZ104	OP921334	HQ006160	HQ006865	HQ006257	OP921356	HQ006352	OP921517	HQ006509	–	HQ006676	HQ006769
Erebus ephesperis	RZ11	HQ006959	HQ006161	HQ006866	HQ006258	OP921359	HQ006353	HQ006440	HQ006510	HQ006587	HQ006677	HQ006770

Taxon	Sample ID	CAD	COI-LCO	COI-Jerry	EF1a-begin	EF1a-centre	EF1a-end	GAPDH	IDH	MDH	RpS5	Wingless
Eulepidotis rectimargo	RZ12	HQ006960	HQ006162	HQ006960	HQ006259	OP921364	HQ006354	–	HQ006511	HQ006588	HQ006678	HQ006771
Catephia alchymista	RZ127	HQ006961	HQ006164	HQ006961	HQ006261	OP921365	HQ006355	HQ006441	–	HQ006590	HQ006680	HQ006773
Phytometra viridaria	RZ129	HQ006962	HQ006165	HQ006962	HQ006262	OP921366	HQ006356	HQ006442	HQ006512	HQ006591	HQ006681	HQ006774
Gonitis involuta	RZ13	HQ006963	HQ006166	HQ006963	HQ006263	OP921367	HQ006357	OP921518	OP921529	HQ006592	HQ006682	HQ006775
Orgyia antiqua	RZ130	HQ006964	HQ006167	HQ006964	HQ006264	OP921368	HQ006358	HQ006443	HQ006513	HQ006593	HQ006683	HQ006776
Acantholipes regularis	RZ135	–	HQ006168	HQ006872	HQ006265	OP921369	HQ006359	–	–	–	HQ006684	HQ006777
Callimorpha dominula	RZ136	HQ006965	HQ006169	HQ006873	HQ006266	HQ006360	HQ006360	HQ006444	HQ006514	HQ006594	HQ006685	HQ006778
Araeopteron sp	RZ137	HQ006966	HQ006170	HQ006874	HQ006267	OP921370	HQ006361	–	HQ006515	–	HQ006686	HQ006779
Micronoctua sp	RZ138	HQ006967	HQ006171	HQ006875	HQ006268	OP921371	HQ006362	HQ006445	HQ006516	HQ006595	HQ006687	HQ006780
Hypopyra capensis	RZ149	HQ006968	HQ006172	HQ006876	HQ006269	OP921373	HQ006363	OP921519	HQ006517	HQ006596	HQ006688	HQ006781
Miniodes phaeosoma	RZ153	HQ006969	HQ006173	HQ006877	HQ006270	OP921374	HQ006364	HQ006446	HQ006518	HQ006597	HQ006689	HQ006782
Eudocima fullonia	RZ16	HQ006970	HQ006174	HQ006878	HQ006271	OP921377	HQ006365	HQ006447	HQ006519	HQ006598	HQ006690	HQ006783
Hypsoropha hormos	RZ17	HQ006972	HQ006176	HQ006880	HQ006273	OP921380	HQ006367	HQ006449	HQ006521	HQ006600	HQ006692	HQ006784
Marcipa sp	RZ177	HQ006973	HQ006177	HQ006881	–	OP921381	HQ006368	HQ006450	HQ006522	HQ006601	–	HQ006785
Masca abactalis	RZ18	HQ006974	HQ006178	HQ006882	HQ006274	OP921382	HQ006369	HQ006451	HQ006523	–	HQ006693	HQ006786
Achaea serva	RZ19	HQ006975	HQ006179	HQ006883	HQ006275	OP921385	HQ006370	HQ006452	HQ006524	HQ006602	HQ006694	HQ006787
Mocis latipes	RZ20	HQ006976	HQ006180	HQ006884	HQ006276	OP921387	HQ006371	HQ006453	HQ006525	HQ006603	HQ006695	HQ006788
Ophiusa coronata	RZ21	HQ006977	HQ006181	HQ006885	HQ006277	OP921389	HQ006372	HQ006454	HQ006526	HQ006604	HQ006696	HQ006789
Azeta ceramina	RZ22	HQ006978	HQ006182	HQ006886	HQ006278	OP921390	HQ006373	OP921520	HQ006527	HQ006605	HQ006697	HQ006790
Oxycilla ondo	RZ24	HQ006980	HQ006184	HQ006888	HQ006280	OP921392	HQ006375	HQ006456	HQ006529	HQ006607	–	HQ006792
Ulotrichopus macula	RZ241	–	HQ006185	HQ006889	HQ006281	–	–	HQ006457	HQ006530	HQ006608	HQ006699	HQ006793
Heteropalpia acrosticta	RZ243	HQ006981	HQ006186	HQ006890	HQ006282	–	HQ006376	–	–	–	HQ006700	HQ006794
Ophiusa tirhaca	RZ246	HQ006982	HQ006187	HQ006891	HQ006283	OP921393	HQ006377	HQ006458	–	HQ006609	HQ006701	HQ006795
Clytie devia	RZ247	HQ006983	HQ006188	HQ006892	HQ006284	OP921394	HQ006378	HQ006459	–	HQ006610	–	HQ006796
Acantholipes circumdata	RZ248	HQ006984	HQ006189	HQ006893	HQ006285	OP921395	HQ006379	–	HQ006531	–	HQ006702	HQ006797
Schrankia costaestrigalis	RZ27	HQ006987	HQ006192	HQ006896	HQ006288	OP921404	HQ006382	HQ006461	–	HQ006613	HQ006705	HQ006800
Brunia antica	RZ28	OP921335	HQ006193	HQ006897	HQ006289	HQ006383	HQ006383	HQ006462	HQ006534	HQ006614	HQ006706	HQ006801
Erygia apicalis	RZ29	HQ006988	HQ006194	HQ006898	HQ006290	OP921415	HQ006384	–	HQ006535	HQ006615	HQ006707	HQ006802
Sphingomorpha chlorea	RZ291	–	HQ006195	HQ006899	HQ006291	–	HQ006385	–	–	HQ006616	HQ006708	HQ006803
Oxidercia toxea	RZ295	HQ006989	HQ006196	HQ006900	HQ006292	–	–	–	–	HQ006617	HQ006709	HQ006804
Laspeyria flexula	RZ3	HQ006990	HQ006197	HQ006901	HQ006293	OP921421	HQ006386	HQ006463	HQ006536	HQ006618	HQ006710	HQ006805
Creatonotos transiens	RZ30	HQ006991	HQ006198	HQ006902	HQ006294	HQ006387	HQ006387	OP921522	HQ006537	HQ006619	HQ006711	HQ006806
Pantydia diemeni	RZ309	HQ006992	HQ006199	HQ006903	HQ006295	OP921424	HQ006388	HQ006464	HQ006538	HQ006620	HQ006712	HQ006807
Sypnoides fumosa	RZ313	HQ006994	HQ006201	HQ006905	HQ006297	OP921426	HQ006390	HQ006466	HQ006539	HQ006622	HQ006714	HQ006809
Serrodes campana	RZ318	HQ006995	HQ006202	HQ006906	HQ006298	OP921429	HQ006391	HQ006467	HQ006540	HQ006623	HQ006715	HQ006810

Taxon	Sample ID	CAD	COI-LCO	COI-Jerry	EF1a-begin	EF1a-centre	EF1a-end	GAPDH	IDH	MDH	RpS5	Wingless
Pandesma robusta	RZ321	HQ006997	HQ006204	HQ006908	HQ006300	OP921432	HQ006393	–	HQ006542	HQ006625	HQ006717	HQ006812
Ercheia cyllaria	RZ33	HQ006998	HQ006205	HQ006909	HQ006301	OP921433	HQ006394	–	HQ006543	HQ006626	HQ006718	HQ006813
Anoba anguliplaga	RZ332	OP921339	HQ006206	HQ006910	HQ006302	OP921434	HQ006395	HQ006469	HQ006544	HQ006627	–	HQ006814
Plusiodonta nitissima	RZ333	–	HQ006207	HQ006911	HQ006303	–	–	HQ006470	–	HQ006628	HQ006719	HQ006815
Gonodonta fernandezi	RZ335	–	HQ006208	HQ006912	HQ006304	–	–	HQ006471	HQ006545	HQ006629	HQ006720	HQ006816
Nygmia plana	RZ34	HQ006999	HQ006209	HQ006913	HQ006305	OP921438	HQ006396	HQ006472	HQ006546	HQ006630	HQ006721	HQ006817
Corgatha nitens	RZ36	HQ007001	HQ006211	HQ006915	HQ006307	OP921446	HQ006398	HQ006474	HQ006547	HQ006632	HQ006723	HQ006819
Prolophota trigonifera	RZ37	HQ007002	HQ006212	HQ006916	HQ006308	OP921450	HQ006399	HQ006475	–	HQ006633	HQ006724	HQ006820
Anisoneura salebrosa	RZ38	HQ007003	HQ006213	HQ006917	HQ006309	OP921453	HQ006400	HQ006476	HQ006548	OP921541	HQ006725	HQ006821
Ericeia subcinerea	RZ39	HQ007004	HQ006214	HQ006918	HQ006310	OP921460	HQ006401	HQ006477	HQ006549	HQ006635	HQ006726	HQ006822
Colobochyla salicalis	RZ4	HQ007005	HQ006215	HQ006919	HQ006311	OP921466	HQ006402	HQ006478	OP921532	HQ006636	HQ006727	HQ006823
Pangrapta bicornuta	RZ40	HQ007006	HQ006216	HQ006920	HQ006312	OP921467	HQ006403	HQ006479	HQ006550	HQ006637	HQ006728	HQ006824
Metaemene atrigutta	RZ41	HQ007007	HQ006218	HQ006922	HQ006314	OP921473	HQ006405	HQ006481	HQ006552	HQ006639	HQ006730	HQ006826
Asota heliconia	RZ44	HQ007009	HQ006220	HQ006924	HQ006316	OP921481	HQ006407	HQ006483	HQ006554	HQ006641	HQ006732	HQ006828
Ugia insuspecta	RZ45	HQ007010	HQ006221	HQ006925	–	OP921483	HQ006408	HQ006484	HQ006555	HQ006642	HQ006733	HQ006829
Artena dotata	RZ46	HQ007011	HQ006222	HQ006926	HQ006317	OP921485	HQ006409	–	HQ006556	HQ006643	HQ006734	HQ006830
Sympis rufibasis	RZ48	HQ007012	HQ006223	OP933780	HQ006318	OP921490	HQ006410	HQ006485	HQ006557	HQ006644	HQ006735	HQ006831
Paracolax tristalis	RZ5	HQ007013	HQ006224	HQ006927	HQ006319	OP921491	HQ006411	–	–	–	HQ006736	HQ006832
Thysania zenobia	RZ53	HQ007014	HQ006225	HQ006928	HQ006320	OP921493	HQ006412	HQ006486	HQ006558	HQ006645	HQ006737	HQ006833
Rusicada metaxantha	RZ55	HQ007016	HQ006227	HQ006930	HQ006322	OP921495	HQ006414	–	HQ006560	HQ006647	HQ006739	HQ006835
Phyllodes eyndhovi	RZ56	OP921342	HQ006228	HQ006931	HQ006323	OP921496	HQ006415	OP921527	HQ006561	HQ006648	HQ006740	HQ006836
Lygephila maxima	RZ57	OP921343	HQ006229	HQ006932	HQ006324	OP921497	HQ006416	HQ006487	HQ006562	HQ006649	HQ006741	HQ006837
Melipotis jucunda	RZ58	HQ007017	HQ006230	HQ006933	HQ006325	OP921498	HQ006417	–	HQ006563	HQ006650	HQ006742	HQ006838
Panopoda rufimargo	RZ59	HQ007018	HQ006231	HQ006934	HQ006326	OP921499	HQ006418	HQ006488	HQ006564	HQ006651	HQ006743	HQ006839
Herminia tarsicrinalis	RZ6	–	HQ006232	HQ006935	HQ006327	OP921500	HQ006419	HQ006489	–	–	–	HQ006840
Audea bipunctata	RZ60	HQ007019	HQ006233	HQ006936	HQ006328	OP921501	HQ006420	–	HQ006565	HQ006652	HQ006744	HQ006841
Pangrapta decoralis	RZ66	HQ007022	HQ006236	HQ006939	HQ006331	OP921502	HQ006423	–	HQ006568	–	HQ006747	HQ006844
Eublemma purpurina	RZ7	–	HQ006237	HQ006940	HQ006332	OP921504	HQ006424	HQ006491	HQ006569	HQ006655	HQ006748	HQ006845
Amata phegea	RZ8	OP921345	HQ006238	HQ006941	–	HQ006425	HQ006425	HQ006492	OP921535	HQ006656	HQ006749	HQ006846
Euclidia glyphica	RZ82	HQ007023	HQ006239	HQ006942	HQ006333	OP921505	HQ006426	–	HQ006570	HQ006657	HQ006750	HQ006847
Dysschema leucophaea	RZ88	–	HQ006240	–	HQ006334	HQ006427	HQ006427	HQ006493	HQ006571	HQ006658	HQ006751	HQ006848
Arctornis sp	RZ89	HQ007024	HQ006241	HQ006943	HQ006335	OP921506	HQ006428	HQ006494	HQ006572	HQ006659	HQ006752	HQ006849
Scolecocampa liburna	RZ9	HQ007025	HQ006242	HQ006944	HQ006336	OP921507	HQ006429	HQ006495	HQ006573	HQ006660	HQ006753	HQ006850
Pericyma cruegeri	RZ99	HQ007027	HQ006244	HQ006946	HQ006338	OP921514	HQ006431	HQ006497	HQ006575	HQ006662	HQ006755	HQ006852
Ossonoba torpida	RZ411	JN401050	JN401252	JN401134	JN401369	OP921475	JN401480	JN401582	–	–	JN401893	JN400939

Taxon	Sample ID	CAD	COI-LCO	COI-Jerry	EF1a-begin	EF1a-centre	EF1a-end	GAPDH	IDH	MDH	RpS5	Wingless
Rhesala imparata	RZ265	JN401053	JN401255	JN401137	JN401372	OP921400	JN401483	JN401585	JN401685	JN401791	–	JN400940
Bocula bifaria	RZ413	JN401055	JN401258	JN401140	JN401375	OP921477	JN401485	JN401587	JN401686	–	–	JN400942
Alesua etialis	RZ94	–	JN401260	JN401142	JN401377	OP921511	JN401487	JN401589	JN401688	JN401794	–	JN400943
Marcipa sp	RZ200	–	JN401261	JN401143	JN401378	OP921388	JN401488	JN401590	JN401689	JN401795	–	JN400944
Plecoptera major	RZ183	–	JN401262	JN401144	–	OP921384	JN401489	JN401591	JN401690	JN401796	–	JN400945
Crithote prominens	RZ109	JN401057	JN401263	JN401145	JN401379	OP921358	JN401490	JN401592	JN401691	JN401797	–	JN400946
Rema costimacula	RZ103	JN401058	JN401264	JN401146	JN401380	OP921355	JN401491	JN401593	JN401692	JN401798	–	JN400947
Baniana strigata	RZ92	JN401059	JN401265	JN401147	JN401381	OP921509	JN401492	JN401594	JN401693	JN401799	–	JN400948
Deinopa signiplena	RZ311	JN401060	JN401266	JN401148	JN401382	OP921425	JN401493	JN401595	JN401694	JN401800	–	JN400949
Hypena laceratalis	RZ368	JN401062	JN401268	JN401150	JN401384	OP921448	JN401495	–	JN401696	–	JN401896	JN400950
Cultripalpa sp	RZ394	–	JN401269	JN401151	JN401385	OP921464	JN401496	JN401597	JN401697	JN401802	JN401897	JN400951
Chrysograpta igneola	RZ408	–	JN401270	JN401152	JN401386	OP921471	JN401497	JN401598	–	JN401803	JN401898	JN400952
Hyposemansis singha	RZ279	JN401063	JN401271	JN401153	JN401387	OP921410	JN401498	JN401599	JN401698	JN401804	JN401899	JN400953
Gracilodes caffra	RZ292	JN401064	JN401272	JN401154	JN401388	OP921417	JN401499	JN401600	JN401699	JN401805	JN401900	JN400954
Episparis costistriga	RZ319	–	JN401273	JN401155	JN401389	OP921430	JN401500	JN401601	JN401700	–	JN401901	JN400955
Schistorhynx argentistriga	RZ119	JN401065	JN401274	JN401156	JN401390	OP921363	JN401501	JN401602	JN401701	JN401806	JN401902	JN400956
Idia aemula	RZ271	JN401066	JN401275	JN401157	JN401391	OP921406	JN401502	OP921521	JN401702	JN401807	–	JN400957
Lysimelia neleusalis	RZ260	JN401067	JN401276	JN401158	–	OP921398	JN401503	JN401603	JN401703	JN401808	–	JN400958
Nodaria verticalis	RZ180	–	JN401277	JN401159	JN401392	OP921383	JN401504	JN401604	JN401704	–	–	JN400959
Neochera inops	RZ346	JN401068	JN401278	JN401160	JN401393	–	–	JN401605	JN401705	JN401809	–	JN400960
Euplocia membliaria	RZ345	–	JN401279	JN401161	JN401394	OP921442	JN401505	JN401606	JN401706	JN401810	–	JN400961
Peridrome orbicularis	RZ280	–	JN401280	JN401162	JN401395	OP921411	JN401506	JN401607	JN401707	JN401811	JN401903	JN400962
Mecodina praecipua	RZ268	JN401069	JN401281	JN401163	JN401396	OP921402	JN401507	JN401608	JN401708	JN401812	–	JN400963
Psimada quadripennis	RZ47	JN401070	JN401282	JN401164	JN401397	OP921488	JN401508	–	JN401709	JN401813	JN401904	JN400964
Garudinia simulana	RZ399	JN401071	JN401283	JN401165	JN401398	JN401509	JN401509	JN401609	JN401710	JN401814	JN401905	JN400965
Eugoa bipunctata	RZ400	JN401072	JN401284	JN401166	JN401399	JN401510	JN401510	–	JN401711	JN401815	JN401906	JN400966
Cyana sp	RZ398	JN401073	JN401285	JN401167	JN401400	JN401511	JN401511	JN401610	JN401712	JN401816	JN401876	JN400967
Barsine sp	RZ397	JN401074	JN401286	JN401168	JN401401	JN401512	JN401512	–	JN401713	JN401817	JN401878	JN400968
Nyctemera baulus	RZ387	–	JN401287	JN401169	JN401402	JN401513	JN401513	JN401611	JN401714	JN401818	JN401909	JN400969
Epitausa dilina	RZ93	OP921346	JN401289	JN401171	JN401404	OP921510	JN401515	JN401613	JN401716	JN401819	JN401911	JN400970
Hemiceratoides sittaca	RZ155	–	JN401290	JN401172	JN401405	OP921375	JN401516	JN401614	JN401717	JN401820	JN401912	JN400971
Calyptra hokkaida	RZ336	JN401075	JN401292	JN401174	JN401407	OP921435	JN401518	JN401616	JN401718	JN401823	JN401914	JN400972
Sanys irrosca	RZ343	JN401079	JN401298	JN401180	JN401412	–	–	JN401621	JN401722	JN401829	JN401919	JN400973
Anticarsia gemmatilis	RZ267	–	JN401299	JN401181	JN401413	OP921401	JN401522	JN401622	JN401723	JN401830	JN401920	JN400974
Antiblemma fuscireticulata	RZ334	–	JN401297	JN401179	JN401411	–	–	JN401620	–	JN401828	JN401918	JN400975

Taxon	Sample ID	CAD	COI-LCO	COI-Jerry	EF1a-begin	EF1a-centre	EF1a-end	GAPDH	IDH	MDH	RpS5	Wingless
Rivula ochrea	RZ159	JN401054	JN401257	JN401139	JN401374	OP921376	JN401484	JN401586	OP921530	JN401793	–	JN400979
Hypocala andremona	RZ340	JN401078	JN401295	JN401177	JN401410	OP921439	JN401521	JN401619	JN401721	JN401826	JN401917	JN400980
Anomis flava	RZ100	JN401052	JN401254	JN401136	JN401371	OP921352	JN401482	JN401584	JN401684	–	–	JN400981
Rusicada fulvida	RZ101	JN401051	JN401253	JN401135	JN401370	OP921353	JN401481	JN401583	JN401683	JN401790	JN401894	JN400983
Hypocala deflorata	RZ105	JN401077	JN401294	JN401176	JN401409	OP921357	JN401520	JN401618	JN401720	JN401825	JN401916	JN400985
Oglasa ansorgei	RZ167	JN401056	JN401259	JN401141	JN401376	OP921379	JN401486	JN401588	JN401687	–	–	JN400986
Oraesia excavata	RZ337	JN401076	JN401293	JN401175	JN401408	OP921436	JN401519	JN401617	JN401719	JN401824	JN401915	JN400987
Eublemma anachoresis	RZ98	–	JN401319	JN401202	JN401434	OP921513	JN401539	JN401637	JN401741	JN401847	JN401939	JN400989
Eudocima salaminia	RZ338	JQ784511	JN401291	JN401173	JN401406	OP921437	JN401517	JN401615	JN401740	JN401821	JN401913	JN400990
Platyjionia mediorufa	RZ111	JN401084	JN401306	JN401188	JN401420	OP921360	JN401528	JN401628	JN401729	–	JN401926	JN400991
Biuncus sp	RZ475	JN401088	–	JN401193	JN401425	OP921489	JN401532	JN401630	JN401732	JN401838	JN401931	JN400992
Hypena baltimoralis	RZ367	JN401061	JN401267	JN401149	JN401383	OP921447	JN401494	JN401596	JN401695	JN401801	JN401895	JN400993
Biuncus sp	RZ476	JN401089	JN401311	JN401194	JN401426	–	–	JN401631	JN401733	JN401839	JN401908	JN400994
Anticarsia irrorata	RZ370	JN401080	JN401300	JN401182	JN401414	OP921451	JN401523	JN401623	JN401724	JN401831	JN401921	JN400995
Zurobata rorata	RZ385	JN401091	JN401313	JN401196	JN401428	OP921457	JN401534	OP921525	JN401735	JN401841	JN401933	JN400996
Hypospila bolinoides	RZ116	–	JN401325	JN401207	JN401440	OP921362	JN401544	JN401643	JN401743	JN401851	JN401944	JN400997
Daddala lucilla	RZ320	JN401098	JN401327	JN401209	JN401441	OP921431	JN401545	JN401645	JN401747	JN401852	JN401946	JN400998
Hypotacha brandbergensis	RZ275	JN401105	JN401337	JN401219	JN401451	OP921407	JN401554	JN401654	JN401757	–	JN401956	JN400999
Calyptis idonea	RZ473	JN401107	JN401339	JN401221	JN401453	–	–	JN401656	JN401759	JN401861	JN401958	JN401000
Chalciope mygdon	RZ391	JN401881	JN401348	JN401230	JN401462	OP921461	JN401562	JN401665	JN401768	JN401865	JN401967	JN401001
Heteranassa sp	RZ350	–	JN401328	JN401210	JN401442	OP921444	JN401546	JN401646	JN401748	JN401860	JN401947	JN401002
Avatha uloptera	RZ317	–	JN401345	JN401227	JN401459	OP921428	JN401559	JN401662	JN401765	JN401867	JN401964	JN401003
Hemeroplanis finitima	RZ298	–	JN401301	JN401183	JN401415	OP921420	JN401524	JN401624	JN401725	JN401832	JN401922	JN401004
Autophila chamaephanes	RZ276	JN401081	JN401302	JN401184	JN401416	OP921408	JN401525	JN401625	JN401726	JN401833	JN401923	JN401005
Poeta denotalis	RZ445	–	JN401304	JN401186	JN401418	OP921482	JN401526	JN401626	JN401727	–	JN401925	JN401006
Tamsia hieroglyphica	RZ389	JN401083	JN401305	JN401187	JN401419	OP921459	JN401527	JN401627	JN401728	JN401835	JN401907	JN401007
Luceria striata	RZ42	OP921341	JN401308	JN401190	JN401422	OP921480	JN401530	OP921526	OP921533	–	JN401928	JN401008
Luceria oculalis	RZ369	JN401086	JN401309	JN401191	JN401423	OP921449	JN401531	–	JN401731	–	JN401929	JN401009
Condate sp	RZ393	JN401090	JN401312	JN401195	JN401427	OP921463	JN401533	JN401632	JN401734	JN401840	JN401932	JN401010
Homodes crocea	RZ412	JN401092	JN401314	JN401197	JN401429	OP921476	JN401535	–	JN401736	JN401842	JN401934	JN401011
Enispodes purpurea	RZ390	JN401093	JN401315	JN401198	JN401430	–	–	JN401633	JN401737	JN401843	JN401935	JN401012
Araeopteron sp	RZ410	JN401094	JN401318	JN401201	JN401433	OP921474	JN401538	JN401636	–	JN401846	JN401938	JN401013
Eublemma albifascia	RZ220	–	JN401320	JN401203	JN401435	OP921391	JN401540	JN401638	JN401742	–	JN401940	JN401014
Metalectra edilis	RZ372	–	JN401321	JN401204	JN401436	OP921452	JN401541	JN401639	JN401744	JN401848	JN401941	JN401015
Hypenagonia sp	RZ409	–	JN401322	–	JN401437	OP921472	JN401542	JN401640	–	JN401849	JN401942	JN401016

Taxon	Sample ID	CAD	COI-LCO	COI-Jerry	EF1a-begin	EF1a-centre	EF1a-end	GAPDH	IDH	MDH	RpS5	Wingless
Euaontia semirufa	RZ285	JN401096	JN401324	JN401206	JN401439	OP921414	JN401543	JN401642	JN401746	–	JN401943	JN401017
Zale bethunei	RZ270	–	JN401329	JN401211	JN401443	OP921405	JN401547	JN401647	JN401749	JN401853	JN401948	JN401018
Pseudbarydia crespula	RZ91	JN401099	JN401331	JN401213	JN401445	OP921508	JN401549	JN401649	JN401751	JN401855	JN401950	JN401019
Bulia deducta	RZ314	JN401100	JN401332	JN401214	JN401446	OP921427	JN401550	JN401650	JN401752	JN401856	JN401951	JN401020
Forsebia perlaeta	RZ284	JN401101	JN401333	JN401215	JN401447	OP921413	JN401551	JN401651	JN401753	JN401857	JN401952	JN401021
Melipotis punctifinis	RZ342	JN401102	JN401334	JN401216	JN401448	OP921441	JN401552	JN401652	JN401754	JN401858	JN401953	JN401022
Phoberia atomaris	RZ286	JN401103	JN401335	JN401217	JN401449	–	–	–	JN401755	JN401859	JN401954	JN401023
Audea humeralis	RZ290	JN401104	JN401336	JN401218	JN401450	OP921416	JN401553	JN401653	JN401756	–	JN401955	JN401024
Spirama retorta	RZ359	JN401106	JN401338	JN401220	JN401452	–	–	JN401655	JN401758	–	JN401957	JN401025
Ommatophora luminosa	RZ407	JN401108	JN401340	JN401222	JN401454	OP921470	JN401555	JN401657	JN401760	JN401862	JN401959	JN401026
Platyja umminea	RZ261	–	JN401341	JN401223	JN401455	OP921399	JN401556	JN401658	JN401761	JN401863	JN401960	JN401027
Praxis porphyretica	RZ308	JN401109	JN401342	JN401224	JN401456	–	–	JN401659	JN401762	JN401864	JN401961	JN401028
Ischyja manlia	RZ269	JN401110	JN401343	JN401225	JN401457	OP921403	JN401557	JN401660	JN401763	JN401822	JN401962	JN401029
Oxyodes scrobiculata	RZ113	JN401111	JN401344	JN401226	JN401458	OP921361	JN401558	JN401661	JN401764	JN401866	JN401963	JN401030
Cocytia durvillii	RZ401	JN401112	JN401346	JN401228	JN401460	OP921468	JN401560	JN401663	JN401766	JN401869	JN401965	JN401031
Bastilla praetermissa	RZ306	JN401113	JN401347	JN401229	JN401461	OP921422	JN401561	JN401664	JN401767	JN401868	JN401966	JN401032
Allotria elonympha	RZ294	JN401115	JN401349	JN401231	JN401463	OP921418	JN401563	JN401666	JN401769	JN401870	JN401968	JN401033
Thyas metaphaea	RZ190	JN401116	JN401350	JN401232	JN401464	OP921386	JN401564	JN401667	JN401770	JN401871	JN401969	JN401034
Rivula sericealis	MM01404	GU828161	GU828664	GU828462	GU828995	–	GU829278	GU829791	GU830051	GU830370	–	JQ787019
Hulodes caranea	RZ126	–	OP933768	–	OP921347	–	–	–	–	OP921536	OP921543	OP921548
Nychioptera noctuidalis	RZ283	–	OP933769	OP933775	OP921348	–	–	–	–	OP921537	–	OP921549
Gabara stygialis	RZ297	JN401085	JN401307	JN401189	JN401421	OP921419	JN401529	JN401629	JN401730	JN401836	JN401927	OP921550
Tautobriga glaucopis	RZ354	–	OP933773	OP933779	–	–	–	–	–	OP921540	–	OP921551
Parolulis absimilis	RZ392	–	JN401317	JN401200	JN401432	OP921462	JN401537	JN401635	JN401739	JN401845	JN401937	OP921552
Eutelia adulatrix	MM00160	GU828122	GU828621	GU828419	GU828956	–	GU829246	GU829764	GU830010	GU830330	GU830629	GU829516
Lophoptera hemithyris	MM07614	GU828274	GU828802	GU929772	GU829107	–	GU829385	GU829879	GU830183	GU830501	GU830759	GU829661
Targalla subocellata	RZ35	HQ007000	HQ006210	HQ006914	HQ006306	OP921443	HQ006397	HQ006473	MW352507	HQ006631	HQ006722	HQ006818
Aegilia sp	RZ287	JN401036	JN401234	JN401118	JN401352	–	–	JN401566	–	JN401772	JN401873	JN400927
Archiearis parthenias	NW107-1	EU141303	DQ018928	DQ018928	DQ018899	DQ018899	DQ018899	EU141485	EU141539	EU141604	EU141381	–
Eucocytia meeki	RZ87	JN401047	JN401247	JN401129	–	–	–	–	–	JN401785	JN401888	–
Aedia leucomelas	RZ277	–	JN401250	JN401132	JN401367	OP921409	JN401478	JN401580	JN401681	JN401788	JN401891	–
Raphia cf. abrupta	CWM-94-0372	GU828059	GU828548	GU828350	GU828893	–	GU829193	GU829728	GU829939	GU830270	GU830579	GU829455
Condica vecors	CWM-95-0471	GU828061	GU828550	GU828352	GU828895	–	GU829194	–	GU829941	–	GU830581	GU829457
Diaphone sp	MF-05-0053	GU828076	GU828571	GU828372	GU828913	–	GU829206	GU829738	GU829960	GU830285	GU830591	GU829475
Autographa gamma	MM00328	GU828135	GU828636	GU828434	GU828970	–	GU829256	–	GU830023	GU830344	GU830640	GU829528

Taxon	Sample ID	CAD	COI-LCO	COI-Jerry	EF1a-begin	EF1a-centre	EF1a-end	GAPDH	IDH	MDH	RpS5	Wingless
Amphipyra perflua	MM01162	GU828157	GU828660	GU828458	GU828991	–	GU829275	GU829787	GU830047	GU830366	GU830657	GU829546
Panthea coenobita	MM04583	GU828191	GU828702	GU828497	GU829025	–	GU829314	–	GU830088	GU830406	GU830690	GU829578
Noctua fimbriata	MM04752	GU828194	GU828705	GU828500	GU829028	–	GU829317	GU829820	GU830091	GU830409	GU830693	GU829581
Periscepta polysticta	MM07669	GU828289	GU828820	GU929788	GU829125	–	GU829400	GU829892	GU830201	GU830519	GU830773	GU829674
Xanthodes albago	MM09894	GU828308	GU828844	GU929808	GU829145	–	GU829412	–	GU830224	GU830535	GU830792	GU829693
Dyops chromatophila	RZ10	HQ006957	HQ006158	–	HQ006255	OP921351	HQ006350	HQ006438	HQ006507	HQ006585	HQ006674	HQ006767
Ecpatia longinquua	RZ25	HQ006985	HQ006190	HQ006894	HQ006286	OP921396	HQ006380	–	HQ006532	HQ006611	HQ006703	HQ006798
Arcte modesta	RZ54	HQ007015	HQ006226	HQ006929	HQ006321	OP921494	HQ006413	–	HQ006559	HQ006646	HQ006738	HQ006834
Pseudoarcte sp	RZ147	JN401037	JN401235	JN401119	JN401353	OP921372	JN401465	JN401567	JN401669	JN401773	JN401874	JN400928
Belciana biformis	RZ384	JN401039	JN401237	JN401121	JN401355	OP921456	JN401467	JN401569	JN401671	JN401775	JN401877	JN400929
Belciana kala	RZ416	JN401040	JN401238	–	JN401356	OP921479	JN401468	JN401570	JN401672	JN401776	JN401879	JN400930
Diopa corone	RZ472	JN401041	JN401239	JN401122	JN401357	KT879739	KT879739	JN401571	–	JN401777	JN401880	JN400931
Dyrzela plagiata	RZ395	JN401042	JN401240	JN401123	JN401358	OP921465	JN401469	JN401572	JN401673	JN401778	JN401881	JN400932
Encruphion leena	RZ351	JN401044	JN401243	JN401126	JN401361	OP921445	JN401472	JN401575	JN401676	JN401781	JN401884	JN400933
Parangitia temperata	RZ463	JN401045	JN401244	JN401127	JN401362	OP921486	JN401473	–	–	JN401782	JN401885	JN400934
Parangitia mosaica	RZ464	JN401046	JN401245	–	JN401363	OP921487	JN401474	–	JN401677	JN401783	JN401886	JN400935
Antitrisuloides catocalina	RZ388	JN401048	JN401248	JN401130	JN401365	OP921458	JN401476	JN401578	JN401679	JN401786	JN401889	JN400936
Thiacidas sp	RZ459	–	JN401249	JN401131	JN401366	OP921484	JN401477	JN401579	JN401680	JN401787	JN401888	JN400937
Hemicephalis alesa	RZ341	JN401049	JN401251	JN401133	JN401368	OP921440	JN401479	JN401581	JN401682	JN401789	JN401892	JN400938
Ramadasa pavo	RZ382	–	JN401241	JN401124	JN401359	OP921455	JN401470	JN401573	JN401674	JN401779	JN401882	JN400978
Diloba caeruleocephala	MM09267	KT713813	JN401246	JN401128	JN401364	–	JN401475	–	JN401678	JN401784	JN401887	JN400982
Amyna axis	RZ50	–	JN401242	JN401125	JN401360	OP921492	JN401471	JN401574	JN401675	JN401780	JN401883	JN400984
Sosxetra grata	RZ281	JN401038	JN401236	JN401120	JN401354	OP921412	JN401466	JN401568	JN401670	JN401774	JN401875	JN400988
Nola aerugula	MM01776	GU828167	GU828670	GU828468	GU829001	–	GU829284	–	GU830057	GU830376	GU830665	GU829555
Earias clorana	MM06650	GU828231	GU828747	GU929721	GU829061	–	GU829346	GU829845	GU830131	GU830448	GU830718	GU829610
Negeta contrariata	RZ26	HQ006986	HQ006191	HQ006895	HQ006287	OP921397	HQ006381	HQ006460	HQ006533	HQ006612	HQ006704	HQ006799
Eligma narcissus	RZ97	HQ007026	HQ006243	HQ006945	HQ006337	OP921512	HQ006430	HQ006496	HQ006574	HQ006661	HQ006754	HQ006851
Risoba obstructa	RZ381	JN401035	JN401233	JN401117	JN401351	OP921454	OP921515	JN401565	JN401668	JN401771	JN401872	JN400926
Phalera bucephala	MM00122	GU828108	GU828607	GU828405	GU828941	–	GU829235	–	GU829995	GU830318	GU830617	GU829502
Stauropus fagi	MM00981	GU828148	GU828651	GU828449	GU828983	–	GU829266	GU829780	GU830038	GU830357	GU830650	GU829539
Notodonta dromedarius	MM00998	GU828150	GU828653	GU828451	GU828984	–	GU829268	GU829781	GU830040	GU830359	GU830652	GU829540
Oenosandra boisduvalii	MM07590	GU828266	GU828791	GU929762	GU829098	–	GU829377	GU829871	GU830173	GU830492	GU830751	GU829651
Discophlebia sp	RZ403	GQ283529	HQ006217	HQ006921	HQ006313	OP921469	HQ006404	HQ006480	HQ006551	HQ006638	HQ006729	HQ006825

Results

We were able to recover sequences of seven of the eight gene regions, only wingless was not recovered from the genome assembly of *Egybolis*. The optimal topology found by dataset A in our analysis and those of the Erebidae dataset of Zahiri et al. (2012) are very similar with slight differences in a few terminal taxa placements. Our results fully resolve the phylogenetic position of the enigmatic *Egybolis* moth with confidence and suggested a close relationship (SH-Like = 100 / aBayes = 1 / UFBoot2 = 100) within the tribe Cocytiini Boisduval, 1829 (Fig. 2). *Egybolis* is placed within the Erebinae in a strongly-supported sister relationship with the genera of the *Serrodes* group of Holloway (2005) and the striking New Guinea and Moluccan genus *Cocytia* in Cocytiini (Zahiri et al., 2012) (Fig. 2). The three genera (i.e., *Serrodes*, *Avatha*, and *Anereuthina*) included by Holloway (2005) shared general similarity of facies, particularly the presence and disposition of blocks of black on the forewing. In *Egybolis,* there are bands of black that define the yellow band and anterior spots.

The results of this study corroborate those of Zahiri et al. (2012) by confirming a strongly supported sister relationship between Cocytiini and a clade containing the tribes Poaphilini + Ophiusini (Fig. 2). This clade is well supported (SH-Like = 99.9/aBayes = 1/UFBoot2 = 100) and is divided into two major assemblages—tribes Ophiusini and Poaphilini, each with strong support for reciprocal monophyly (SH-Like = 100/aBayes = 1/UFBoot2 = 100 (Fig. 2).

The chronogram (Fig. 3) obtained from the Bayesian dating analyses allows us to infer a median age of 24.96 Ma (late Oligocene) for the tribe Cocytiini, with 95% highest posterior densities (HPD) of 20.49–29.34 Ma . Within Cocytiini, *Serrodes* is the first lineage to branch off, followed by *Avatha* (about 23.39 Ma), and finally with the bifurcation of *Egybolis* from *Cocytia* happening in the late Miocene some 21.12 Ma (Fig. 3). Our analysis of divergence time suggests that the ancestors of Erebinae began diversifying in the mid-Eocene some 46.33 Ma (Fig. 3).

Discussion

We find that closely related members of Cocytiini moths are widely separated on the various austral landmasses (Fig. 4). The enigmatic Afrotropical *Egybolis* moth has a close evolutionary relationship with other members of the tribe Cocytiini (Erebidae, Erebinae) that are principally distributed in humid climates in tropical regions of the Old World. *Avatha* is an Indo-Malayan/Australasian genus, *Serrodes* is an Old-Tropic genus (Indo-Malayan/Australasian/African), *Anereuthina* is Indo-Malayan, and *Cocytia* is a New Guinea and Moluccan genus (Fig. 4).

Cocytia typifies the tribe Cocytiini and was illustrated in Holloway (2001). The status of *Cocytia* was also studied by Speidel et al. (1997), with illustrations, and further details of its morphology presented by Heppner (2010). Speidel et al. (1997) noted that the proboscis has a smooth, rather than nodulose, apex, and all the styloconic sensilla are dorsal. This condition is shared with many genera that they examined in Erebinae as constituted in the study of Zahiri et al. (2012). The structures of the male genitalia could also be interpreted as being a modification of the condition in *Avatha* (see Holloway, 2005). The larvae of the *Serrodes* group of Holloway are of the ophiusine type, and a pupal bloom has been noted in a species of *Avatha* Walker, but not in *Serrodes* or *Anereuthina*. The prolegs all appear well developed, except those on A3 which are reduced; the plantae are expanded to give each a T-shape. The condition of reduced prolegs on A3 is exactly the same condition that occurs in the larvae of *Egybolis* (Fig. 1). Host plants in *Serrodes* and *Avatha* are predominantly from the Sapindaceae, but *Anereuthina* larvae are palm-feeders. The larvae of *Egybolis* feed on peach and *Sapindus* species. The strong association from DNA sequence evidence and morphology of the members of Cocytiini led Zahiri et al. (2012) to suggest that the larva of *Cocytia* may also feed on Sapindaceae, like those of *Serrodes* and *Avatha* as well as *Egybolis* in this study. *Avatha* includes Indo-Australian species (Fig. 3) of the old but inappropriate concept of *Athyrma* Hübner (type species *adjutrix* Cramer, Surinam), a genus essentially restricted to South America and not related to the species discussed here. *Serrodes* species are Afro-Indo-Australian, and *Anereuthina* includes Indo-Malayan species (Fig. 3). *Egybolis* and *Cocytia* are both monotypic genera with restricted geographical distributions— in southeastern Africa and New Guinea, respectively— compared to their sister taxa (Fig. 3).

The time of diversification of the tribe Cocytiini in the Miocene is similar to other groups of Lepidoptera that have connections between Africa and Southeast Asia (e.g., Aduse-Poku et al., 2009; Chazot et al., 2021; Kawahara et al., 2019; Kodandaramaiah & Wahlberg, 2007; Toussaint et al., 2019). The sister relationship of *Egybolis* and *Cocytia*, and their disjunct distributions in Africa and New Guinea, respectively, suggest that their common ancestor may have been widespread from Africa to New Guinea. Indeed, there may be extant species that are found currently in Southeast Asia that are more related to one or the other that we simply have not yet investigated. Given the surprising phylogenetic position of *Egybolis*, this is quite a likely scenario.

Conclusion

In conclusion, using a museomics approach, we have been able to ascertain the phylogenetic position of an enigmatic moth species, and thus transfer *Egybolis vaillantina* from Noctuidae to Erebidae: Erebinae: Cocytiini. Museomics approaches show great potential for resolving such mysteries, which megadiverse groups of organisms tend to be laden with. Given that the whole genome of *Egybolis* was sequenced in this study, the data can be reused in the future in genome-level studies, including phylogenomic analyses of Erebidae (e.g., Ghanavi et al., 2022; Homziak et al., 2019).

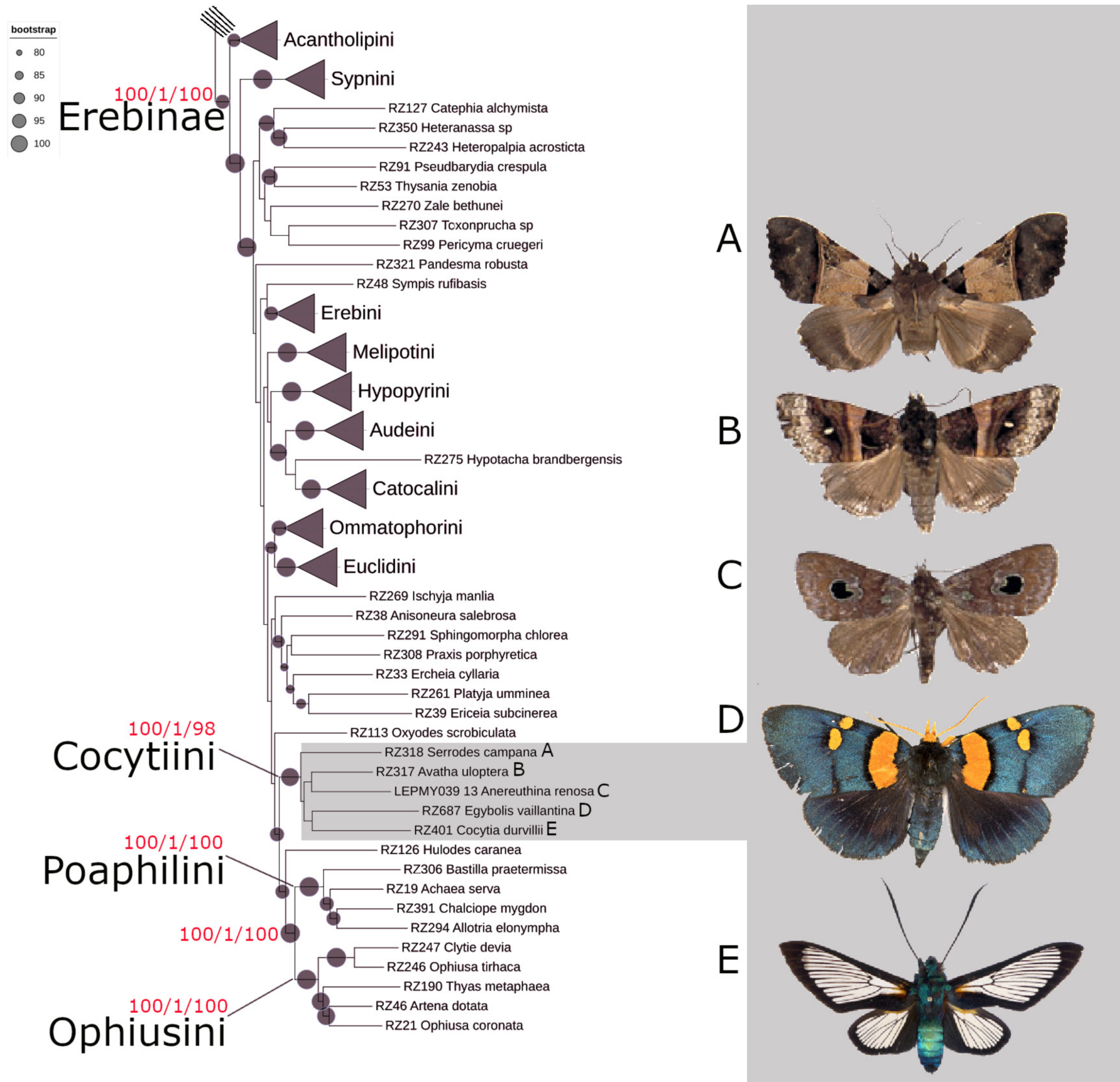

Figure 2. The maximum likelihood tree from analysis of the eight-gene regions of Erebidae dataset of Zahiri et al. (2012) and additional terminals. The subfamily Erebinae is only shown here (see suppl. Fig 1 for a full tree). Ultrafast bootstrap values (please see legend) are displayed as grey circles on the tree branches. Red numbers above the selected nodes (Erebinae, Cocytiini, Poaphilini, Ophiusini, Poaphilini+Ophiusini) are SH-aLRT support (%)/aBayes support/ultrafast bootstrap support (%). Names of moths shown in the figure from top to bottom: A) *Serrodes campana,* B) *Avatha uloptera,* C) *Anereuthina renosa,* D) *Egybolis vaillantina,* and E) *Cocytia durvillii.*

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Data Availability

Raw sequencing data for *Egybolis vailantina* can be found in the NCBI database under Bioproject PRJNA913480. GenBank accession numbers for the rest of the DNA sequences can be found in Table 1. XML input file for BEAST generated in BEAUti is available in Dataset S1.

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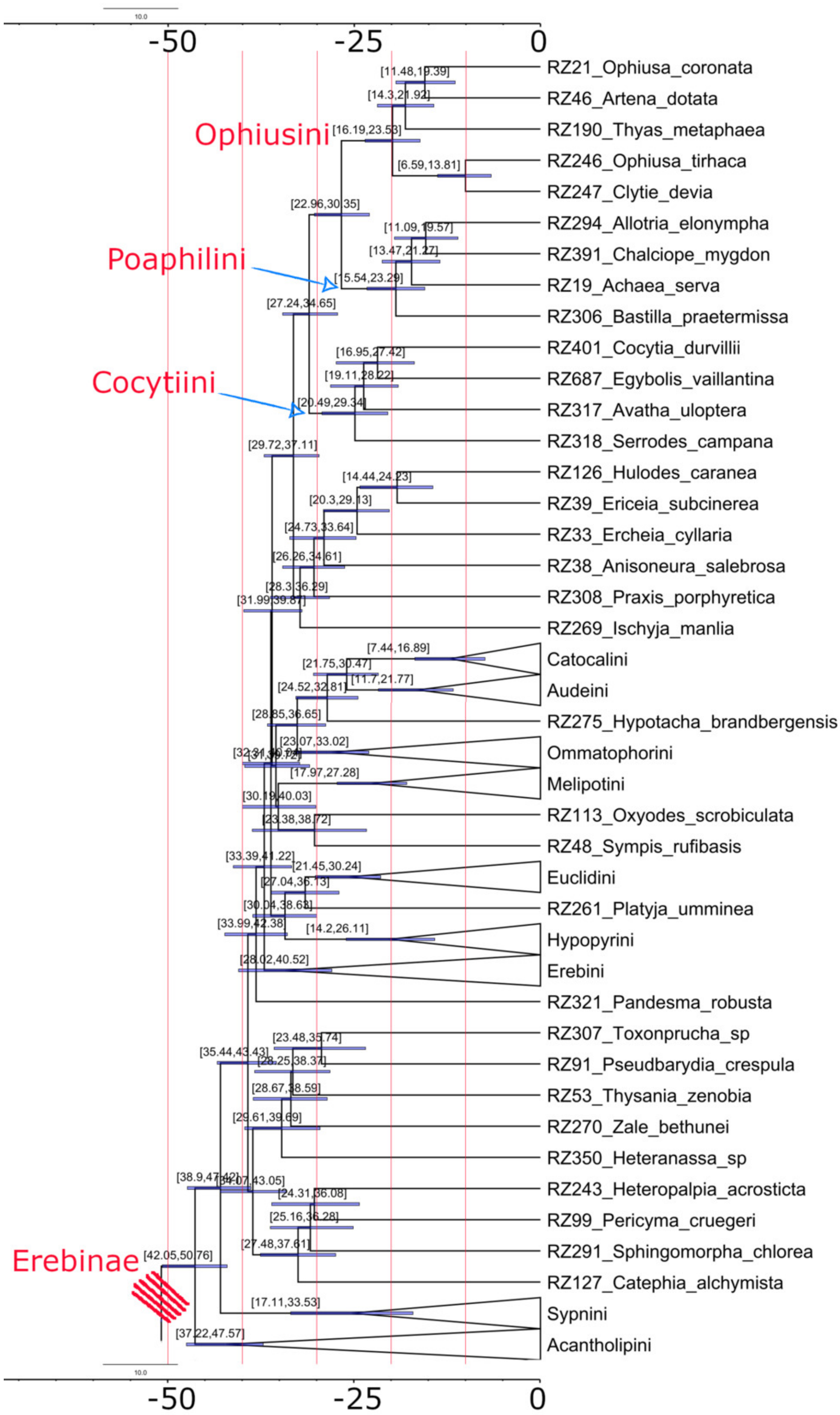

Figure 3. Maximum credibility tree with median ages (Ma) from the Bayesian uncorrelated uniform analysis under BEAST for the tribe Cocytiini. A ten Myr-timescale is placed at the top of the chronogram. Horizontal blue bands represent the 95% HPD heights (in Myr) for the major nodes of the chronogram. Node numbers in brackets above the nodes are the

95% HPD heights (in Myr).

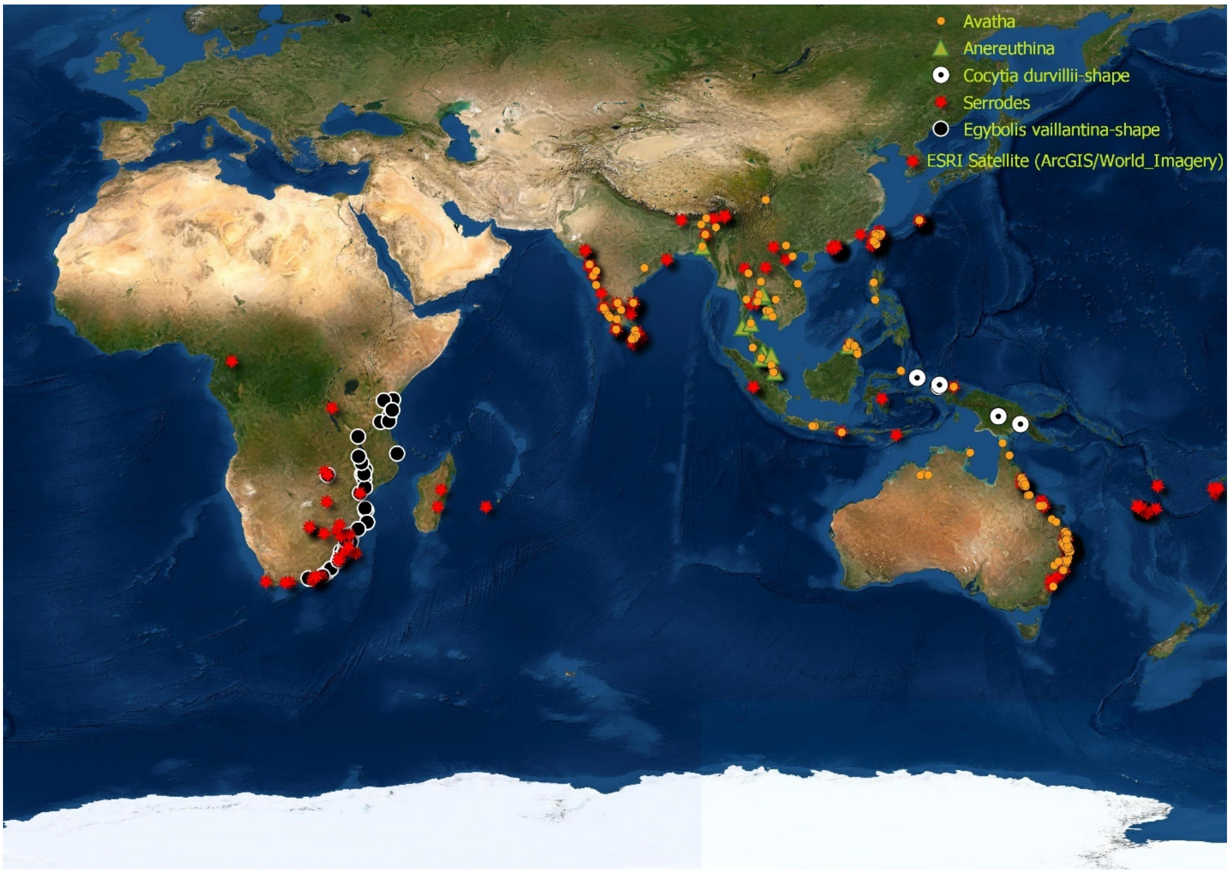

Figure 4. Distribution map of members of the tribe Cocytiini (Erebidae, Erebinae).

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Supplementary Materials

Supplementary Fig. 1. The Maximum likelihood tree from analysis of the eight-gene regions of Erebidae dataset of Zahiri et al. [y@207201] and additional terminals. Ultrafast bootstrap values (please see legend) are displayed as grey circles on the tree branches.

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Dataset S1. XML input file for BEAST generated in BEAUti (part of BEAST2 package).

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