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Palaeo island-affinities revisited – Biogeography and systematics of the Indo-Pacific genus *Cethosia* Fabricius (Lepidoptera: Nymphalidae)

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ABSTRACT

The Indo-Pacific is a very complex region encompassing several micro-continents with unique tectonic and geomorphologic histories. Unsurprisingly, the biogeographic history of Indo-Pacific biota is generally poorly known, especially that of organisms found in the heart of the region, the biodiversity hotspot known as Wallacea. Here, we explore the biogeographic history of the Indo-Pacific butterfly genus *Cethosia* using all known species and many distinctive subspecies. *Cethosia* butterflies span the Indo-Pacific tropics, including several lineages with localized endemism that are critically important when reconstructing biogeographic history of the Indo-Pacific and, in particular, of Wallacea. A phylogenetic hypothesis is proposed, based on sequences of the mitochondrial genes cytochrome oxidase subunit I (COI) and NADH dehydrogenase 5 (ND5), and the nuclear wingless gene. Both Maximum Parsimony and Bayesian analyses showed that the genus is monophyletic and consistently recovered seven, generally very well supported, clades, namely the *cydippe*, *leschenault*, *biblis*, *nietneri*, *hypsea*, *penthesilea* and *cyane* clades. Species group relationships are largely concordant with general morphology (i.e., wing pattern and colouration) and, based on the phylogeny, we propose a revised systematic classification at the species level. The evolution of the genus appears associated with the inferred geological history of the region, in particular with respect to the expanding Wallacea theory, whereby ancient connected terranes were fragmented during the mid Miocene to early Pliocene, approximately 14–3 Mya. Recent diversification events in *Cethosia* were likely promoted by climatic fluctuations during the Pliocene and, to a lesser extent, the Pleistocene. Our results support the view that, while dispersal has been important for *Cethosia* throughout much of the region, the high levels of island endemism and the essentially allopatric radiations recovered in *Cethosia* in Wallacea are better explained by vicariant processes linked to the history of formation of micro-continent and associated palaeo islands.

1. Introduction

The Indo-Pacific, while a biological hotspot, is still poorly known biogeographically. In the heart of the region, Wallacea, bounded by Wallace's (1863) Line and Weber's Line, (Dickerson, 1928) is renowned for its high degree of endemism (e.g., Duffels, 1990; de Jong, 1990; Michaux, 1994; Myers et al., 2000; Inger and Voris, 2001). Wallacea has a very unique geological history influenced by the amalgamation of 'palaeo islands' that likely represent fragments that were sliced from the northern margin of New Guinea (Australian Plate) along the Yapen-Sorong Fault Zone (or similar palaeo-faults) at different times during the Tertiary (Hall, 2002; Hinschberger et al., 2005). From a biogeographic perspective, Wallacea has commonly been regarded as a transitional zone between the Oriental and Australian regions (e.g., Vane-Wright and de Jong, 2003; Braby and Pierce, 2007), but few clues exist as to the temporal relationship of Wallacean taxa with respect to those occurring in other parts of the Indo-Pacific.

Butterflies are an important group of organisms to address historical biogeographical questions in the Indo-Pacific. They tend to show highly localized endemism in the region and high species diversity (Kitching et al., 2001; Braby and Pierce, 2007). One butterfly family in particular, Nymphalidae, has been the focus of several recent phylogenetic studies (e.g., Simonsen et al., 2006; Wahlberg, 2006; Brown et al., 2007; Kodandaramaiah and Wahlberg, 2007, 2009; Wahlberg and Freitas, 2007; Wahlberg and Saccheri, 2007; Peña and Wahlberg, 2008; Leneveu et al., 2009; Wahlberg et al., 2009; Kodandaramaiah et al., 2010a,b; Müller et al., 2010). Nonetheless, most of these studies were based on taxa that occur outside of the Indo-Pacific region.

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Here, we focus on the phylogenetic and biogeographic history of the nymphalid genus *Cethosia*. These butterflies, commonly known as Lacewings, are popular with naturalists, artists, researchers and collectors worldwide, as both adults and early stages are striking. The systematic position of *Cethosia* is still controversial (Harvey, 1991; Brower, 2000; Penz and Peggie, 2003; Freitas and Brown, 2004; Silva-Brandão et al., 2008). The genus is distributed widely in the Indo-Pacific, occurring from India and Sri Lanka, through Indo-China, Peninsula Malaysia and the Philippines to New Guinea (as far east as the Bismarck Archipelago) and northern Australia. Seventeen species have been described, although the number of recognised species varies between authors (e.g., d'Abrera, 1977, 1985). Nearly all inhabit tropical, lowland rainforest and/or vine thicket, although one species, the recently described Papuan *C. vasilia* Müller, is restricted to higher altitudes. The larvae, where known, are gregarious, aposematic and invariably feed on members of the family Passifloraceae. Many Indo-Pacific *Cethosia* species have limited ranges, being restricted to certain small islands, or groups of islands. For these reasons, the genus represents an ideal subject for detailed biogeographical analysis in the highly dynamic and complex Indo-Pacific region. The current work is the first comprehensive molecular analysis of *Cethosia* and indeed, together with the study of Indo-Pacific *Charaxes* by Müller et al. (2010), it is the first study to include multiple gene sequence data for all known species in a Lepidopteran genus in the region. This work adds to an ongoing comparative study (e.g., Müller et al., 2010) that shows that Wallacea is not simply a transitional biogeographic zone, but is a very unique region with a distinct geological history and resulting biogeographic pattern.

2. Materials and methods

2.1. Taxon sampling

Forty-eight exemplars (Table 1) were sampled for the combined analysis, comprising all known species, and many of the more dis-

Table 1
Cethosia species groups and composition.

Tribe	Taxon name	Author	Voucher Code	Locality	COI	ND5	Wingless
Outgroup							
Acraeini	*Acraea andromacha*	(Fabricius, 1775)	NW115-8	AUSTRALIA	EU275522	–	EU275417
	Altinote stratonice	(Latreille, [1813])	NW90-14	ECUADOR: Sucumbios	AY218233	HM998352	DQ018892
Argynnini	*Argynnis paphia*	(Linnaeus, 1758)	NW76-12	SWEDEN: Stockholm	AY090200	HM998353	AY090133
Heliconiini	*Heliconius hecale*	(Fabricius, 1775)	NW70-6	UK: Stratford Butterfly Farm	AY090202	–	AY090135
	Heliconius heurippa	Hewitson, 1854	STRI-B-40	COLOMBIA: Cundinamarca	AY748059	–	–
	Heliconius heurippa	Hewitson, 1854	–	COLOMBIA: Cundinamarca	C-4-6	–	AF169895
Vagrantini	*Vagrans egista*	(Cramer, 1780)	NW71-8	AUSTRALIA: Cairns, Queensland	AY090203	–	AY090136
Ingroup							
Cethosiini	*Cethosia biblis biblis*	(Drury, 1773)	CJM-126-001	INDIA: Khasi Hills	HM998316	–	HM998382
	Cethosia biblis picta	C. & R. Felder, 1867	CJM-160-001	INDONESIA: Mamassa, south Sulawesi	HM998317	HM998354	HM998380
	Cethosia cyane	(Drury, 1770)	CJM-137-003	THAILAND: Bangkok	HM998319	–	–
	Cethosia cyane	(Drury, 1770)	NW100-12	BANGLADESH: Sylhet Div.	DQ922870	–	DQ922838
	Cethosia cyane	(Drury, 1770)	CJM-159-001	INDIA: Khasi Hills	HM998318	HM998355	HM998378
	Cethosia cydippe antoni	Kawai, 1996	CJM-105-001	INDONESIA: Tanimbar Is.	HM998320	HM998356	HM998390
	Cethosia cydippe chrysippe	(Fabricius, 1775)	CJM-168-006	AUSTRALIA: Cairns, Queensland	HM998321	–	–
	Cethosia cydippe cydippe	(Linnaeus, 1763)	CJM-149-002	INDONESIA: Ambon Is.	HM998322	–	–
	Cethosia cydippe damasippe	C. & R. Felder, 1867	CJM-129-004	PAPUA NEW GUINEA: Baiyer River, Western Highlands Province	–	HM998357	HM998388
	Cethosia cydippe obiana	Fruhstorfer, 1903	CJM-128-001	INDONESIA: Obi Is.	HM998323	–	HM998394
	Cethosia gabinia	Weymer, 1883	CJM-152-001	INDONESIA: Nias Is.	HM998324	HM998358	HM998384
	Cethosia hypsea aeole	Moore, 1857	CJM-162-006	INDONESIA: Sumatra	HM998325	–	–
	Cethosia hypsea batuensis	Stichel, 1907	CJM-164-001	INDONESIA: Tanahmasa Is.	HM998326	–	–
	Cethosia hypsea fruhstorferi	Stichel, 1907	CJM-163-001	INDONESIA: Bali is.	HM998327	HM998359	HM998386
	Cethosia hypsea hypsea	Doubleday, 1847	CJM-125-003	INDONESIA: Kelapis, Kalimantan	HM998328	HM998360	HM998389
	Cethosia hypsea munjava	Fruhstorfer, 1912	CJM-165-007	INDONESIA: West Java	HM998329	–	–
	Cethosia lamarcki	Godart, 1819	CJM-138-001	INDONESIA: Wetar	HM998330	HM998361	HM998383
	Cethosia leschenault	Godart, 1823	CJM-117-001	INDONESIA: Wetar	HM998331	HM998362	HM998395
	Cethosia luzonica boholica	Semper, 1888	CJM-150-004	PHILIPPINES: Bohol	HM998332	–	–
	Cethosia luzonica luzonica	C. & R. Felder, 1863	CJM-122-003	PHILIPPINES: Luzon	HM998333	HM998363	HM998376

(*continued on next page*)

Table 1 (*continued*)

Tribe	Taxon name	Author	Voucher Code	Locality	COI	ND5	Wingless
	Cethosia luzonica magindanica	Semper, 1888	CJM-144-006	PHILIPPINES: Mindanao	HM998334	HM998364	HM998377
	Cethosia methypsea carolinae	Forbes, 1885	CJM-112-005	INDONESIA: Sumatra	HM998335	HM998365	HM998379
	Cethosia mindanensis mindanensis	C. & R. Felder, 1863	CJM-146-001	PHILIPPINES: Tawi Tawi Is.	HM998336	HM998366	–
	Cethosia mindanensis mindanensis	C. & R. Felder, 1863	CJM-147-002	PHILIPPINES: Tawi Tawi Is.	–	HM998367	–
	Cethosia moesta	C. & R. Felder, 1867	CJM-115-001	INDONESIA: Batjan Is.	HM998337	–	–
	Cethosia myrina myrina	C. & R. Felder, 1867	CJM-127-001	INDONESIA: Salawatang, south Sulawesi	HM998338	HM998368	HM998396
	Cethosia myrina myrina	C. & R. Felder, 1867	NW106-8	INDONESIA: Sulawesi	EU275514	–	EU275410
	Cethosia myrina ribbei	Honrath, 1886	CJM-131-001	INDONESIA: Peleng Is.	HM998339	HM998369	–
	Cethosia nietneri nietneri	C. & R. Felder, 1867	CJM-161-001	SRI LANKA	HM998340	HM998370	HM998387
	Cethosia obscura antippe	Grose-Smith, 1889	CJM-103-001	PAPUA NEW GUINEA: Whiteman Range, West New Britain	HM998341	–	HM998392
	Cethosia obscura hormisda	Fruhstorfer, 1915	CJM-142-003	PAPUA NEW GUINEA: Tascul, New Hannover	HM998342	–	–
	Cethosia obscura obscura	Guérin-Meneville, 1838	CJM-157-001	PAPUA NEW GUINEA: Cape Suesat, western New Ireland	HM998343	–	HM998393
	Cethosia obscura obscura	Guérin-Meneville, 1838	CJM-157-007	PAPUA NEW GUINEA: Tabar Is., New Ireland Prov.	HM998344	–	–
	Cethosia obscura obscura	Guérin, 1838	CJM-124-005	PAPUA NEW GUINEA: Taron, southern New Ireland	–	HM998371	–
	Cethosia penthesilea exsanguis	Fruhstorfer, 1896	CJM-135-001	INDONESIA: Lombok Is.	HM998345	HM998372	HM998385
	Cethosia penthesilea paksha	Fruhstorfer, 1905	CJM-114-001	INDONESIA: Kapan, West Timor	HM998346	–	–
	Cethosia penthesilea paksha	Fruhstorfer, 1905	NW118-13	INDONESIA: West Java	EU275515	–	EU275411
	Cethosia tambora floresiana	Fruhstorfer, 1902	CJM-133-003	INDONESIA: Flores Is.	HM998347	HM998373	–
	Cethosia tambora narmada	Fruhstorfer, 1896	CJM-102-001	INDONESIA: Lombok Is.	HM998348	–	HM998381
	Cethosia tambora tambora	Doherty, 1891	CJM-153-005	INDONESIA: Sumbawa Is.	HM998349	–	–
	Cethosia vasilia	Müller, 1999	CJM-155-001	PAPUA NEW GUINEA: Schleinitz Mts., central New Ireland	HM998350	HM998374	x
	Cethosia vasilia	Müller, 1999	CJM-156-004	PAPUA NEW GUINEA: Hans Meyer Range, southern New Ireland	HM998351	HM998375	HM998391

tinctive subspecies, of *Cethosia*. Six Heliconiine species, namely *Argynnis paphia* (Linnaeus, 1758), *Vagrans egista* (Cramer, 1780), *Heliconius heurippa* Hewitson, 1854, *H. hecale* (Fabricius, 1775), *Altinote stratonice* (Latreille, 1813) and *Acraea andromacha* (Fabricius, 1775) represent outgroup taxa (Table 1). Selection from the subfamily Heliconiinae, to which *Cethosia* belongs, potentially reduces the attraction of long branches during phylogenetic analysis. Outgroup taxa were selected based on inferred phylogenetic relationships proposed by Ackery et al. (1999) and Penz and Peggie (2003). The trees were rooted with *Argynnis paphia*.

2.2. Molecular markers

Sequence data from mitochondrial COI and ND5 and nuclear wingless gene were used to assess phylogenetic relationships of *Cethosia*. Each of these gene fragments have been widely used in phylogenetic studies of insects and, since they show variable rates of substitution, their combination is ideal for resolving divergence at different levels. In Lepidoptera, COI has been used for resolving relatively recent divergence events, particularly at the genus and species level (Caterino et al., 2000; Sperling, 2003). ND5 is a protein-encoding gene that has great application in the resolution of lower level relationships, such as those between populations within a species or between closely related species within a genus (e.g., Simon et al., 1994; Su et al., 1998; Yagi et al., 1999; Szalanski et al., 2006; Albre et al., 2008; Dodo et al., 2008). Wingless is a protein-encoding gene in the nuclear genome partly responsible for wing pattern formation. It belongs to the wnt gene family, whose paralogs are easily distinguishable, and shows a relatively rapid rate of substitution. In Lepidoptera, it has been used to resolve relationships at both higher and lower systematic levels (Brower and Egan, 1997; Brower and DeSalle, 1998; Brower, 2000; Campbell et al., 2000; Wahlberg et al., 2003).

2.3. Molecular techniques

DNA was normally extracted from two legs, but sometimes thoracic tissue, of dried butterflies. Voucher specimens are located at the following depositories: Chris Müller reference collection, Sydney; Australian National Insect Collection (ANIC), Canberra, Australia; School of Biological Sciences Collection, Macquarie University, Sydney, Australia.

A Chelex (Bio-Rad, Hercules, CA) (Walsh et al., 1991) method, modified from that of Walsh et al. (1991) was used to extract DNA. Tissue samples were ground manually in a 1.5 mL microcentrifuge tube and 500 μL of 5% Chelex resin, and 5 μL of 20 g/L Proteinase K (ProK) (Sigma P2308) enzyme digester were added before vortexing for a few seconds. Samples were incubated at 53 °C overnight and then at 93 °C for 30 min. Standard PCR reactions were conducted in a PTC-100 MJ Research thermocycler, with a total volume of 25 μL: 2 μL of gDNA template at various dilutions, with 11.625 μL of distilled H_2O, 2.5 μL of buffer (100 mM), 2.5 μL Magnesium Chloride, 5 μL of each dNTP (2.5 mM), 0.5 μL of each primer and 0.375 μL of Taq polymerase (5 U μL^{-1}).

For COI, a 654 bp fragment was amplified using Folmer et al. (1994) LCO (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′). For older tissue samples that could not be amplified using the LCO/HCO pair, internal primers Ron (5′-GGAGCYCCWGATATAGCTTTCCC-3′) and Nancy (5’-CCTGGTAAAATTAAAATATAAACTTC-3′), as provided by Caterino and Sperling (1999), were used. The primers LepWG1 (5′-GARTGYAARTGYCAYGGYATGTCTGG-3′) and LepWG2 (5′-ACTICGCARCACCARTGGAATGTRCA-3′) (Brower and DeSalle, 1998) were used to amplify the 433 bp wingless gene fragment. For the amplification of the 397 bp of ND5, the primers A1 (5′-AATATDAGGTATAAATCATAT-3′) and C2 (5′-ATCYTTWGAATAAAAYCCAGC-3′) (Torres et al., 2001) were employed.

The cycling protocol for COI was 2 min at 94 °C, five cycles of 1 min at 94 °C, 1.5 min at 45 °C and 1.5 min at 72 °C, followed by 35 cycles of 1 min at 93 °C, 1.5 min at 50 °C and 1.5 min at 72 °C, with a final extension of 2 min at 72 °C. For ND5, the protocol used was 5 min at 95 °C, 30 cycles of 1 min at 94 °C, 1 min at 45 °C and 2 min at 72 °C, and a final extension of 10 min at 72 °C, while for wingless, the thermal cycling protocol was 5 min at 95 °C, followed by 35 cycles of 0.5 min at 94 °C, 0.5 min at 47 °C and 1.5 min at 72 °C, with a final extension of 10 min at 72 °C. Negative controls were included in all PCR’s. The PCR products were separated by electrophoresis and purified using an UltraClean 15 DNA Purification Kit (MO BIO Laboratories Inc.).

All DNA sequencing was performed on an Applied Biosystems 3130xl using the manufacture’s protocol. Resultant chromatograms were edited manually and then aligned in SEQUENCHER 4.1.2. Nucleotide sequences were visually inspected for miscalls, reading frame errors, and termination codons. Ambiguities were treated as missing data. All sequences were aligned against other published Lepidoptera sequences (e.g., Brower and DeSalle, 1998; Campbell et al., 2000). For COI, the consensus sequences were aligned against the published reference sequence for *Drosophila yakuba* Burla (Clary and Wolstenholme, 1985) and/or other Lepidoptera sequences on GenBank. GenBank accession numbers for all sequences are in Table 1.

2.4. Phylogenetic analysis

We used MEGA version 4.1 (Tamura et al., 2006) to assess sequence properties. During MP analysis, phylogenetic trees were reconstructed using unweighted MP as implemented in PAUP* 4.0.b10 (Swofford, 2002). We conducted heuristic searches with the tree-bisection-reconnection (TBR) branch-swapping algorithm, stepwise addition with up to 1000 random starts to check for islands of trees, and the multiple trees option in effect. When more than one equally parsimonious tree was recovered, strict consensus trees were calculated. To determine levels of stability at each node, bootstrap analysis (Felsenstein, 1988), based on a full heuristic search of 1000 pseudo-replicates using TBR branch swapping and simple stepwise addition, was carried out for each analysis. We also evaluated clade robustness with Bremer support (Bremer, 1994) using the program TreeRot 3 (Sorenson and Franzosa, 2007), where partitioned support was calculated to assess the contribution of each data partition to the total Bremer support values in the combined analysis.

We performed Bayesian analyses, partitioned by gene fragment (COI, ND5, wingless), using MrBayes 3.0b4 (Ronquist and Huelsenbeck, 2003), after analysing the dataset using MODELTEST 3.06 (Posada and Crandall, 1998). All partitions were assigned with the GTR+G model. Several independent Bayesian runs at temperatures ranging from 0.2 to 0.4 were performed on the data using metropolis-coupled Markov chain Monte Carlo simulations, for 5 million generations each, and tree sampling every 100 generations. Bayesian topology and posterior probabilities for each node were computed by majority rule consensus after deleting the first one million generations as ‘burn-in’, once likelihood values had stabilized.

2.5. Age of divergence estimations

We calibrated our *Cethosia* tree with reference to a recent study extrapolated from fossil data which estimated the age of the split between Acraeini and *Cethosia* at 44 Mya (SD 3.5 Mya) (Wahlberg et al., 2009). The sister group relation of *Cethosia* and Acraeini is now quite certain (see Wahlberg et al., 2009). Other potential geological and/or geographical events were deemed unreliable as calibration points since, in the Indo-Pacific, these are generally widespread or are too localised.

The program BEAST (Drummond and Rambaut, 2007) was used to estimate divergence times. The relaxed molecular clock method was used for the molecular dating, allowing branch lengths to vary according to an uncorrelated lognormal distribution. The tree prior was set to the Yule process, and the age at the node of the common ancestor of *Cethosia* and *Acraea* was set to 44 million years (with a standard deviation of 3.5 million years). All other priors were left to the defaults in BEAST. We ran a Bayesian analysis twice for 5 million generations. We used the program Tracer (Drummond and Rambaut, 2007) to determine whether our parameter estimates and tree topology were at equilibrium. The first one million generations were discarded as burn-in. Posterior credibility and error estimates (posterior probability for the nodes, standard error and Bayesian credibility interval for the age estimates) were computed for each internal node estimate.

2.6. Biogeographical analysis

The historical biogeography of *Cethosia* was investigated using dispersal–vicariance analysis, implemented in the program DIVA (Ronquist, 1997). We used the default settings of the program, where vicariance events cost zero but dispersal and extinction events cost one per unit area. The optimal (and most parsimonious) ancestral reconstruction of the DIVA model is the one with the lowest cost. We used the Bayesian topology (as derived from BEAST) for this analysis.

Geographical distribution was coded as 14 states for *Cethosia*, enabling radiation and diversification patterns to be assessed at fine resolution within the Indo-Pacific region, where many of the subject taxa have restricted ranges. These states do not necessarily define political boundaries but harbour at least one endemic taxon. These zones (states) are defined as follows (see Fig. 1): Palearctic (A); South America (B); India/Sri Lanka (C); Australia (D); Indo-China (E), comprising non-peninsula Thailand, Laos, Cambodia, Vietnam, southern China and Myanmar; Sundaland (F), encompassing peninsula Thailand, Peninsula Malaysia, Sumatra, Borneo and Java; Philippines (G), excluding Palawan; Sulawesi (H), Sulawesi and satellite islands, including Sula Islands, Sangihe, Buton and Muna Islands; Lesser Sunda Islands (I), comprised of all islands between Lombok and Alor, excluding Timor and Wetar; Timor/Wetar (J); Tanimbar Islands (K); New Guinea mainland (L); Bismarck Archipelago (M) and Maluku (N), except Wetar Is.

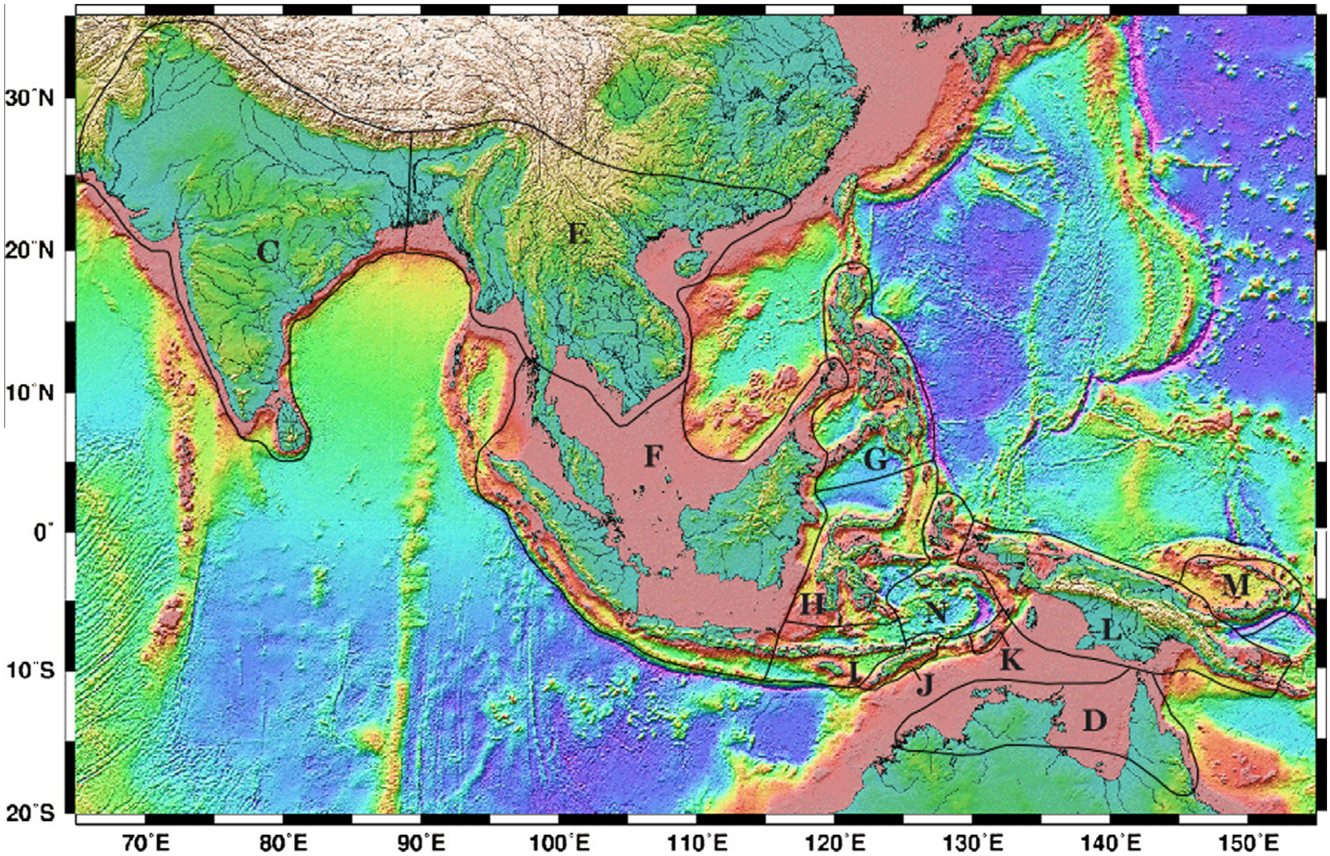

Fig. 1. Map showing assigned biogeographic zones used in the dispersal–vicariance analysis of *Cethosia*, defined as follows: Palearctic (A) (not shown); South America (B) (not shown); India/Sri Lanka (C); Australia (D); Indo-China (E), comprising non-peninsula Thailand, Laos, Cambodia, Vietnam, southern China and Myanmar; Sundaland (F), encompassing peninsula Thailand, Peninsula Malaysia, Sumatra, Borneo and Java; Philippines (G), excluding Palawan; Sulawesi (H), Sulawesi and satellite islands, including Sula Islands, Sangihe, Buton and Muna Islands; Lesser Sunda Islands (I), comprised of all islands between Lombok and Alor, excluding Timor and Wetar; Timor/Wetar (J); Tanimbar Islands (K); New Guinea mainland (L); Bismarck Archipelago (M) and Maluku (N), except Wetar Is.

3. Results

3.1. Dataset properties

The final dataset comprised 40 taxa, including six outgroups. For certain taxa, we were unable to amplify all of the three gene fragments (Table 1). The complete combined sequence data contained 1448 nucleotides, or characters, of which 480 sites (33.15%) were variable. Approximately 302 (20.86%) of the variable sites were parsimony informative. At the individual gene level, COI had the highest proportion of parsimony informative sites, with 25.08%. Base frequencies were near equal in the wingless, but were strongly A–T biased in the mitochondrial genes ($A = 28.4$, $T = 39.3$, $G = 14.4$, $C = 17.8$).

Decay indices, as yielded from partitioned Bremer support of the combined dataset implied generally high congruence levels between the three genes for most nodes. Some conflict was recorded in the wingless gene, but least with COI. This is in contrast with other Lepidopteran studies focusing at and above the level of species group, where wingless contributed more to the phylogenetic signal than COI (e.g., Silva-Brandão et al., 2005; Braby et al., 2006). The lack of divergence in the wingless gene at and below the species level, as well as the absence of wingless sequences for some taxa in our dataset for *Cethosia* may be partly responsible for such conflict. Silva-Brandão et al. (2008), in their study of the nymphalid tribe Acraeini, showed some level of incongruence with the wingless gene, possibly because that group comprises several closely related species with minimal divergence for that gene.

3.2. Phylogenetic patterns

A strict consensus tree, taken from 71 equally most parsimonious trees from the combined MP analysis of the three genes, is shown in Fig. 2. The Bayesian topology (MrBayes Fig. 3; BEAST Fig. 4) was similar to that generated by MP, with nodes for both analyses being generally well resolved. *Cethosia* was recovered as a well-supported monophyletic group under both MP and BI (bootstrap 100% MP, 1.00 BI).

Seven distinctive monophyletic clades were recovered by both MP and BI, namely the *cydippe*, *leschenault*, *biblis*, *hypsea*, *penthesilea*, *cyane* and *nietneri* clades. All but the latter clade showed strong statistical support. In both analyses, the *cydippe* and *leschenault* clade were sister, and the remaining clades showed similar topologies and pairings as well. One exception was the position of the *leschenault* clade, which was resolved as sister to the *cydippe* clade under BI, yet sister to all *Cethosia* less the *cydippe* clade under MP. Also, the phylogenetic position of the *nietneri* clade is uncertain, as it is sister to *biblis* and *hypsea* + (*penthesilea* + *cyane*) under MP, and sister to *biblis* and *cyane* + (*penthesilea* + *hypsea*) in BI analyses, with little support in either.

Certain clades (*biblis*, *cydippe* and *leschenault*) were characterised by relatively long branches between taxa under Bayesian analysis. Conversely, those of remaining clades had much shorter branch lengths, especially those in the *hypsea* clade. There was moderate-strong support for nodes below, and most nodes above, the basal splitting of parts of the *hypsea* clade, as well as the node giving rise to *C. methypsea* and *C. obscura* exemplars. Some

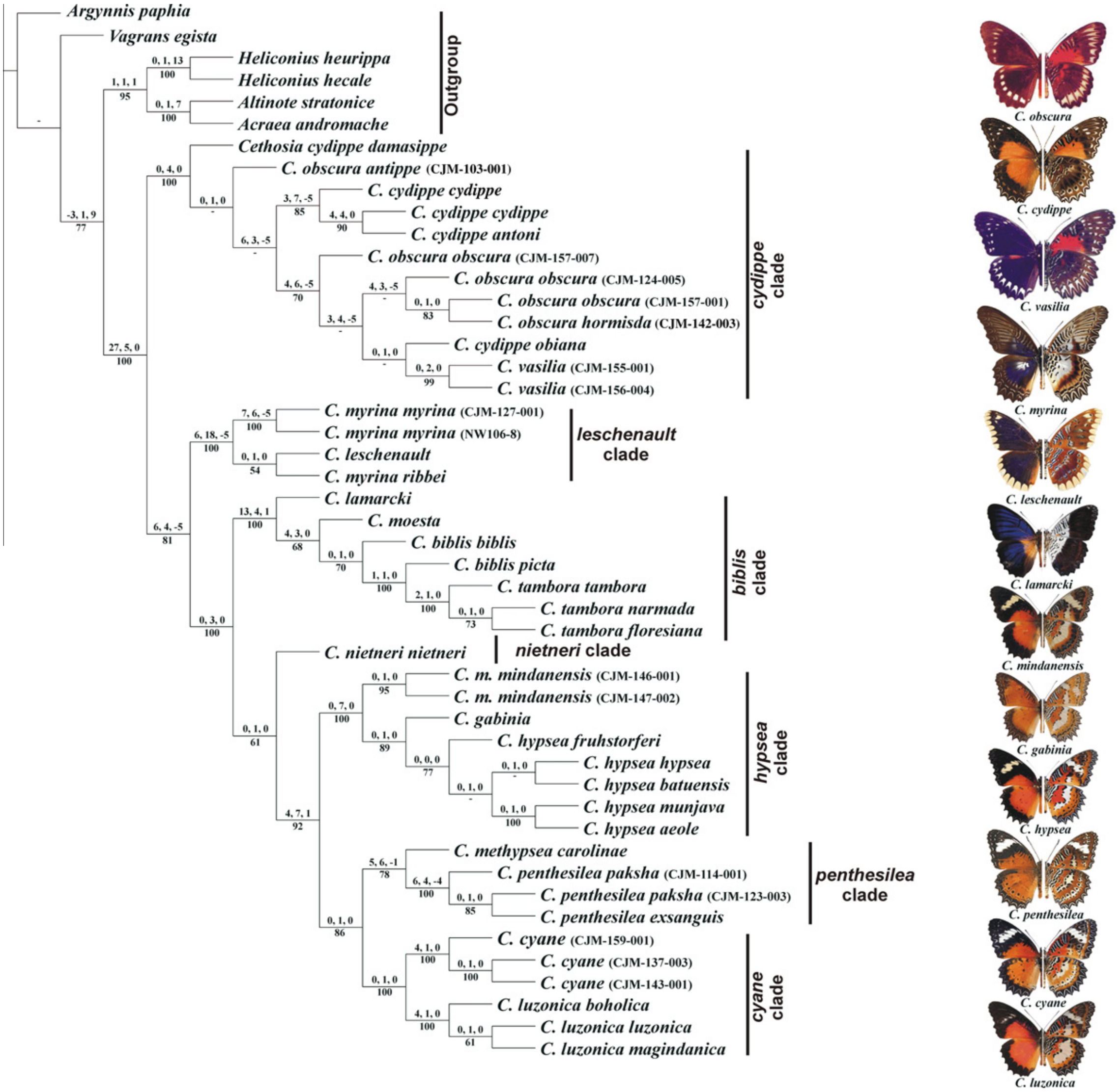

Fig. 2. 50% majority rule phylogram from the Bayesian analysis of the combined COI, ND5 and wingless dataset. Numbers to the left of nodes are the posterior probabilities of those nodes.

examples of species paraphyly are recorded, namely in *C. cydippe* and *C. obscura* and (under MP) within the *leschenault* clade. Additionally, the position of *C. biblis* relative to other members of the *biblis* clade was variable in the different trees.

Uncorrected pairwise divergence between recognised *Cethosia* species ranged from ca. 0.3% to nearly 5.0%, for COI. The lowest degrees of divergence were between taxa in the *hypsea* clade (see under Systematic Implications), while the highest rates were recorded for COI sequences of the para/sympatric *C. obscura* and *C. vasilia*, as well the Wallacean *C. leschenault* and *C. myrina*. With the exception of the fore-mentioned sister pair *C. obscura* and *C. vasilia*, all other sister pairs of species are allopatric in distribution.

3.3. Divergence ages

The chronogram in Fig. 4 illustrates our estimation of divergence times within *Cethosia*, which revealed that basal splitting in *Cethosia* commenced in the mid to late Oligocene, approximately 25–21 Mya. The earliest lineages, which formed at that time, were the *biblis*, *leschenault* and *cydippe* clades. The mid Miocene to Pliocene, ca. 11–2 Mya, saw the peak of diversification in *Cethosia*, when many of the extant species (or their ancestors) evolved. Further diversification has taken place at subspecific level during the Pleistocene.

3.4. Biogeographical analysis

The geographic origin of the ancestor of *Cethosia* and *Acraea* was undetermined, based on the dispersal-vicariance (DIVA) model using the Bayesian tree derived in BEAST. According to our DIVA analysis, the majority of diversification within *Cethosia* beyond the species group level was driven by vicariance, as demonstrated by the essentially allopatric radiations. Splitting between Wallacean taxa in the *leschenault* and *biblis* clades, namely those occurring in Sulawesi, Maluku, the Lesser Sunda Islands and Timor/Wetar occurred between 11 and 3 Mya. The node separating the sister pair *C. leschenault* and *C. myrina*, of Sulawesi and Timor, respectively, was dated at 11 Mya. Other notable events include the splitting the Indo-Chinese *C. cyane* and Philippine *C. luzonica* at approximately 19.5 Mya.

Exceptions to the vicariant mode of speciation include the inferred dispersal of the ancestor of (*C. mindanensis* + (*C. hypsea hypsea* + *C. h. fruhstorferi*)) into the Philippines from a Sundanian ancestor. However, the separation of *C. mindanensis* from *C. hypsea*

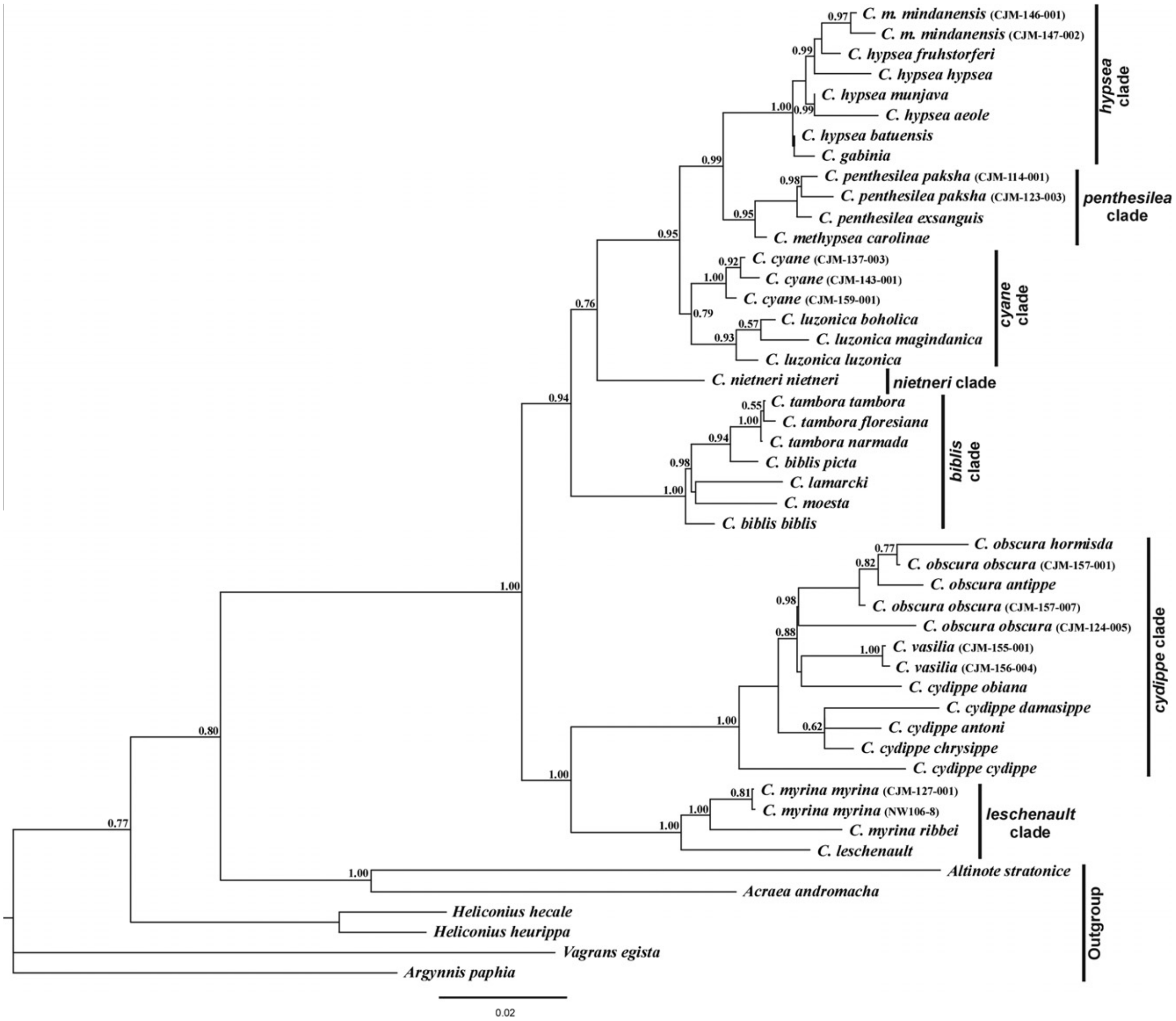

Fig. 3. Strict consensus tree of 71 equally parsimonious trees ((length 1396 steps, consistency index (CI) = 0.45, retention index (RI) = 0.52)) for the combined COI, ND5 and wingless dataset. Numbers above branches are Partitioned Bremer support indices for COI, ND5 and wingless respectively and those below are bootstrap values >50% for the node to the right. The tree is rooted with *Argynnis paphia*.

was inferred as a vicariant event. The DIVA model also implies that *C. penthesilea* separated from *C. methypsea* by dispersal from Sundaland to the Lesser Sundas, although we are unable to explain the within-Sundaland boundary between the latter pair of species. The Moluccan subspecies of *C. cydippe* have evidently dispersed and differentiated from their mainland New Guinea ancestors.

4. Discussion

4.1. Systematic implications

Seven distinctive clades were recovered by both MP and BI, namely the *leschenault, biblis, cydippe, hypsea, penthesilea, cyane* and *nietneri* clades. All but the latter clade showed strong statistical support, both under MP and BI. Consistent topologies across the analyses and strong statistical support is in part likely supported by our comprehensive sampling, which reduces phylogenetic error (Zwickl and Hillis, 2002). As well as genetic affinity, the majority of these groupings is in accordance with morphological similarity, especially wing pattern and colour. In particular, high genetic and phenotypic divergence is evident in the essentially Wallacean, *leschenault* and *biblis* clades, while the remainder are essentially of similar morphotype (orange–brown, or black, uppersides, with similarly patterned undersides).

Küppers (2006) revised the systematics of *Cethosia*, based on morphology, although he omitted at least one species (*C. vasilia*) and was unable to decipher the date of publication for the well known taxon, *C. cydippe antoni* Kawai (Tennent and Rawlins, 2008). Küppers (2006) arranged *Cethosia* into three species groups, namely the *biblis*, *hypsea* and *cydippe* groups. Küppers correctly grouped *C. biblis*, *C. tambora* and *C. lamarcki* into the former clade, based on similar underside patterns and male genitalic structure. However, he erroneously reported *C. tambora* as ranging to Timor, which is undoubtedly false, as the only member of the *biblis* group occurring in Timor is *C. lamarcki*. Küppers also likely erroneously states that the distribution of *C. tambora* encompasses East Java, which is occupied by *C. biblis*. We consider the above highly unlikely, as all members of the *biblis* clade are allopatric in distribution. Indeed, our molecular analysis suggests that all “species” within the *biblis* clade may be best regarded as conspecific, since both *C. biblis biblis* and *C. biblis picta* are paraphyletic. Küppers (2006) con-

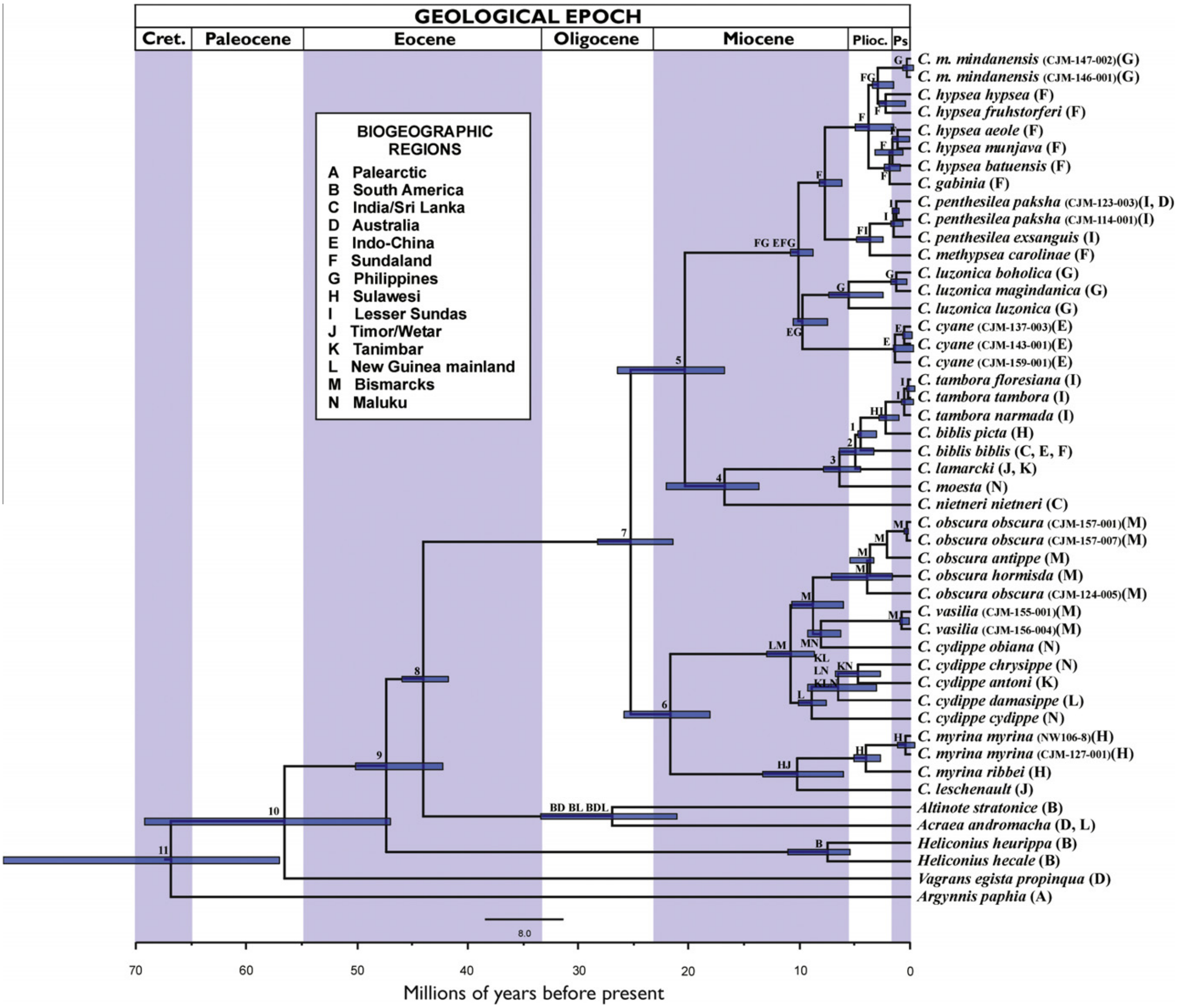

Fig. 4. Chronogram based on the Bayesian topology with associated posterior credibility limits. Results of the dispersal-vicariance analysis, with unrestricted ancestral areas, are shown for each node. Certain nodes revealed too many ancestral distributions to fit on the figure and are listed in Appendix B.

sidered the *hypsea* group to comprise eight species, which encompasses our *cyane, penthesilea, hypsea, leschenault* and *nietneri* clades. In our analyses, the latter monotypic clade was relatively poorly supported and showed affinities with both the *biblis* and (*hypsea* + (*penthesilea* + *cyane*)) clades. The *cyane*, *penthesilea* and *hypsea* clades recovered in our analyses always shared a common ancestor but the *leschenault* clade was invariably sister to *cydippe* and/or the *biblis* clade. Küppers suggested that the external facies and genitalia placed *C. penthesilea* between *C. cyane* and *C. methypsea* but our phylogeny implies a sister relationship with the latter. Hence Küppers' statement that "the outer appearance as well as the genital structures [of] *C. methypsea* shows more affinities to *C. hypsea* than to *C. penthesilea*" is not corroborated in our molecular analysis. Küppers considered that *C. luzonica* is closely related to *C. mindanensis* and *C. hypsea*. He stated that it had undergone a degree of separation from the remainder of his *hypsea* group of taxa, with the greatest differentiation in the male genitalia. Conversely, our phylogeny implies a sister relationship of *C. luzonica* with *C. cyane*. Küppers stated that the genitalic structures of *C. leschenault* showed the greatest divergence from the typical *Cethosia* structure, which reflects the significant molecular divergence of this species with its sister taxon, *C. myrina*. Although Küppers' morphological analysis corroborates fairly closely with our molecular results, we propose a new classification based on the strong support of our groupings (see Table 2).

4.2. Historical biogeography and diversification

The zoogeographical distributions of each *Cethosia* taxon were coded on our phylogeny to produce a taxon-area cladogram (Fig. 4). Broadly, the distribution of *Cethosia* taxa can be grouped by clade, with the *hypsea* clade being endemic to Sundaland and the Philippines, the *methypsea* clade ranging in Sundaland and the Lesser Sunda Islands, while the *cydippe* clade is restricted to the Australasian Region. Both the *biblis* and *myrina* clades are essentially confined to Wallacea, the latter strictly so. The speciation events between Wallacean taxa are notably older than those between species in the Oriental region and New Guinea, and long branches imply extended isolation of Wallacean *Cethosia*. Interestingly, such a pattern has been documented for other groups of butterflies co-occurring in Wallacea (Müller et al., 2010, Müller and Beheregary unpublished). The recognition of comparatively older

Table 2
Exemplar taxa used in this study, with collection localities and GenBank accession numbers.

Küpper's classification		Current classification	
Species group/clade	Taxon	Species group/clade	Taxon
hypsea	*C. gabinia*	*hypsea*	*C. hypsea*
	C. hypsea	*cyane*	*C. cyane*
	C. mindanensis		*C. luzonica*
	C. cyane	*penthesilea*	*C. methypsea*
	C. luzonica		*C. penthesilea*
	C. penthesilea	*leschenault*	*C. leschenault*
	C. methypsea		*C. myrina*
	C. leschenault	*nietneri*	*C. nietneri*
	C. myrina	*biblis*	*C. biblis*
	C. nietneri		*C. lamarcki*
biblis	*C. biblis*		*C. tambora*
	C. tambora	*cydippe*	*C. cydippe*
	C. lamarcki		*C. obscura*
cydippe	*C. cydippe*		*C. vasilia*
	C. obscura		

Wallacean lineages in the genus *Cethosia* is analogous to that discussed in detail for the genus *Charaxes* by Müller et al. (2010). The temporal importance of Wallacea for the evolution of butterflies has remained enigmatic and all studies appear to be focused on Sulawesi taxa. Vane-Wright and de Jong (2003) showed in their reproduced phylogenies of various Indo-Pacific genera that certain endemic Sulawesi taxa represented ancient lineages. In his reconstructed phylogenies of Indo-Pacific cicadas, de Boer (1995) implied that genera and tribes endemic to Sulawesi were essentially of early and ancient lineages.

4.2.1. Diversification

4.2.1.1. Geological vicariance. Two primarily vicariant events likely attributable to geological processes are recognised, namely the allopatric diversification of the two predominantly Wallacean clades (*leschenault*, *biblis*) from separate common ancestors. In particular, the sister relationship of *C. leschenault* and *C. myrina* is of paramount significance and is analogous to the vicariant splitting of Sulawesi/Timor taxa in *Charaxes* (Müller et al., 2010).

Wallacea has a very unique geological history and the islands that comprise it, for the most part, likely represent fragments that were sliced from the northern margin of New Guinea (Australian Plate and Bird's Head Microplate) along the Yapen-Sorong Fault Zone (or similar palaeo-faults) at different times during the Tertiary (Hall, 2002; Hinschberger et al., 2005). Some micro-continents were amalgamated to form the "Banda" micro-continent (Hinschberger pers. comm. 2008). West Sulawesi, on the other hand, separated from east Borneo in the Eocene, resulting in opening of the Makassar Strait. During the Late Eocene and Oligocene the Makassar Straits, and much of East Borneo and West Sulawesi was a wide, and locally deep, marine region, although with some elevated areas to the west and east, forming a substantial barrier between Sundaland and Sulawesi (Hall, 2009). Such a barrier is evident in the distribution of the *Cethosia* clades, with the *hypsea* clade not crossing this line to the east, nor the *myrina* clade to the west.

Subduction at the Banda Trench was initiated during the early Miocene and subsequently lead to back-arc spreading and the inception of the Banda Basins (Banda Sea) (Réhault et al., 1994; Honthaas et al., 1998; Hinschberger et al., 2000, 2005), as well as the formation of Wallacea, as we now know it. The "Banda" micro-continent was likely dismembered as a result of such successive basin opening towards the south–east. Such fragmentation likely resulted in the allopatric speciation within the two Wallacean *Cethosia* clades from an ancestor that occurred in this micro-continent.

The initial genesis of Wallacea coincides temporally closely with the earliest splitting in the two fore-mentioned clades, *leschenault* and *biblis*, with basal nodes at ca. 11 and 6.5 Mya, respectively. The split between the Sulawesi endemic *C. myrina* and Timorese *C. leschenault* is markedly similar in age to that between the endemic *Charaxes mars* (Sulawesi) and *C. marki* (Timor), which was estimated at 8.5 Mya (Müller et al., 2010).

Buru and Seram islands were probably part of a single micro-continent, as shown by stratigraphic similarities (Pigram and Panggabean, 1984; Linthout and Helmers, 1994; Linthout et al., 1997; Milsom, 2000). Seram was almost a part of the Australian Plate for the last 1 Mya (approximate age of the accretion of the Banda back-arc area to the Australian Plate). Hence, dispersal of the *biblis* clade into the Moluccas recently was likely made possible at this time, as was the colonisation by *C. cydippe* from the New Guinea mainland.

Timor "Fore-Arc"

The well-supported sister relationship of *C. leschenault* and *C. myrina* has implications for a Sulawesi-Timor palaeo-island connection, which was also postulated for the sister relationship between *Charaxes mars* and *C. marki* (Müller et al., 2010). A similar connection between members of the *Parantica tityoides* group was unable to be explained by Ackery and Vane-Wright (1984), who suggested that the presence of a Timorese endemic, *P. timorica* (Grose-Smith), likely meant extinction in much of the Lesser Sunda Islands, following divergence from a Sundanian ancestor. However, the palaeo connection of Sulawesi and Timor does not require extinct taxa in the Lesser Sundas, nor a recent connection with Sundaland. The inferred geodynamics, as well as palaeontological records, imply a connection between the two islands just prior to the estimated split of the fore-mentioned sister pairs of species, during the middle Miocene.

Timor comprises mostly Australian Plate margin but the upper nappes are 'Asian' (R. Hall pers. comm. 2008; F. Hinschberger pers. comm., 2008). The Timorese fossil mammal, *Anthracothema verhoeveni* Von Koenigswald belongs to a genus otherwise known from mainland Asia (Von Koenigswald, 1967), suggesting that the Asian component of the fused Timor composite may have been aerial and harboured terrestrial taxa.

New Guinea mainland–Bismarck connection

The *cydippe* clade of three species, *C. cydippe*, *C. obscura* and *C. vasilia* is confined to Maluku, mainland New Guinea, the Bismarck Archipelago and various satellite islands. According to our DIVA analysis, the separation of mainland NG *cydippe* and Bismarckian (*C. obscura* + *C. vasilia*) was a vicariant event, dated at approximately 11 Mya. The mainland NG and Bismarck connection is of utmost importance in understanding the biogeography of taxa spanning, or separated by, this interface, as the latter archipelago boasts very high endemism at high ranking species level, particularly in Lepidoptera (Müller, 1999a,b; Müller, 2001, 2002, 2003; Müller and Tennent, 1999; Tennent, 2000a,b).

All major islands of the Bismarck Archipelago are comprised of both Paleogene to mid Miocene arc-type volcanics and Miocene limestone and sediments (Davies, 1990, 2009), which in all respects is mirrored by the composition of the Finisterre block (terrane) along the north coast of Papua New Guinea. The Finisterre terrane is similar to most other terranes in the eastern part of New Guinea in that it has an oceanic island arc affinity (Pigram and Davies, 1987) and has been accreted to the northern margin of the New Guinea craton. Probably the Finisterre collision was in the early Miocene, which would explain the cessation of Finisterre volcanic activity at about 20 Mya (Davies et al., 1997). The

Bismarck volcanic arc, which includes the main island of New Britain, is believed by many authors to have been connected to the Finisterre block (Davies pers. comm., 2009). At approximately 12 Mya, the Ontong Java Plateau collided with the Bismarck Sea Plate, blocking the westward subduction of the Pacific Plate, resulting in the subsequent development of the New Britain Trench. Hence, this was a time when the initial arc may have fragmented, potentially compromising the Bismarck and Finisterre (mainland NG) connection. Hence, the inferred vicariant event, separating the mainland *C. cydippe* and the Bismarckian *C. obscura* and *C. vasilia* during the middle Miocene is highly plausible, with respect to the inferred geological evolution and accretion of the NG island arcs.

Philippine–Indo-Chinese connection

Other notable events include the splitting of the Indo-Chinese *C. cyane* and Philippine *C. luzonica* during the late Miocene at approximately 9.5 Mya. Since this was inferred as a vicariant event, this has implications as to the history of connections between the two regions. A connection between Taiwan and Luzon through an arc–trench system composed of the Luzon Arc, North Luzon Trough, Hengchun Ridge and the Manila Trench has been proposed by various authors (e.g., Huang et al., 2000; Teng et al., 2000; Lallemand et al., 2001). It has been generally accepted that the formation of Taiwan resulted from the collision of the Luzon Arc with the Eurasian continental margin or with the former Ryukyu subduction zone (Hsu and Sibuet, 1995). The Luzon Arc formed during the early to middle Miocene (Hsu and Sibuet, 1995) and likely connected Taiwan with Luzon from the late Miocene to late Pliocene (approximately 10–2 Mya). Remnants of such an arc are present as the Fuga and Babuyan Islands, between North Luzon and Taiwan. Since Taiwan was connected to southern China at various stages during the Pliocene and Pleistocene, based on bathymetric studies (Yu and Song, 1993), it is plausible that the ancestor of *C. cyane* and *C. luzonica* occupied this arc connecting Luzon and Taiwan and became extinct in Taiwan. A similar palaeo connection is likely to have resulted in the distributions of the closely related sister swallowtail species *Papilio xuthus* Linnaeus (Taiwan and Indo-China) and *P. benguetanus* Joicey and Talbot (northern Luzon).

4.2.1.2. Climatic inferences in the biogeographic history of Cethosia. Various relatively recent speciation events within *Cethosia*, spanning the Pliocene and Pleistocene, are interpreted as a response to climate dynamics as they are within areas of comparative tectonic stability. In Sundaland, the Pliocene marked a period of peak diversification within the *hypsea* and *penthesilea* clades, the former of which is essentially peculiar to the Greater Sunda Islands (Borneo, Sumatra, Java). The most prominent response to climate change in the past has likely been sea level fluctuation, which would have seen the fore-mentioned islands, as well as Peninsula Malaysia, connected and isolated repeatedly, leading to diversification through genetic drift during periods of higher sea level.

While the influence of Pleistocene sea level fluctuations as a biogeographic driver has been well documented (e.g., Hutchison, 1989, 1992; Monk et al., 1997; Roy and Whitehouse, 2003), much earlier sea level changes also appear significant in the Indo-Pacific region. In fact, as with our *Cethosia* dataset, other recent examples suggest that the Pliocene period was temporally more important than the Pleistocene (e.g., Klicka and Zink, 1997; Ward, 1999; Gorog et al., 2004). Indeed, the Pliocene was a time of peak diversification among African (Aduse-Poku et al., 2009) and Indo-Pacific (Müller et al., 2010) Charaxes butterflies.

Pleistocene sea level drops appear to have shaped the genus at subspecific level in *Cethosia* in Indonesia. For example, the various subspecies of *C. tambora* occurring in the Lesser Sunda Islands likely diverged from an ancestor that spanned the entire island group, when they were connected during a period of low sea level. However, between Flores and Wetar, the islands of the Lesser Sundas were flooded at various periods during the Pliocene, maintaining isolation between *C. tambora* and the related *C. lamarcki* on Wetar. A similar pattern was noted for *Charaxes ocellatus* and *C. orilus* occupying the same areas, respectively (Müller et al., 2010).

4.2.1.3. Dispersal events. Several examples of dispersal in *Cethosia*, most of which represent more recent divergences, were inferred from our DIVA analysis. We infer dispersal of the ancestor of (*C. mindanensis* + (*C. hypsea hypsea* + *C. h. fruhstorferi*)) into the Philippines from a Sundanian ancestor. However, the separation of *C. mindanensis* from *C. hypsea* was inferred as a vicariant event. *C. penthesilea* likely separated from *C. methypsea* by dispersal from Sundaland to the Lesser Sundas, although we are unable to explain the within-Sundaland boundary between this pair of species, the former of which is distributed within both Sundaland (Java) and the Lesser Sunda Islands. The Moluccan subspecies of *C. cydippe* have evidently dispersed and differentiated from their mainland New Guinea ancestors. This would no doubt have been facilitated by the relatively recent (<1 Mya) docking of Seram onto the Australian Plate margin (New Guinea mainland), as discussed above, and also the New Guinea – Halmahera connection that persisted during the late Pliocene and Pleistocene.

Interestingly, no examples of dispersal within Wallacea were indicated by our DIVA analysis. Indeed, the absence of the Sulawesi endemic, *C. myrina*, in the proximal Sula Islands, indicates the relatively poor dispersive power of these insects. The Sula Islands have likely never had any terrestrial connection with Sulawesi, despite the collision of the Sula Block with East Sulawesi (Ali and Hall, 1995). The patterns of diversification reported in this study strongly show that vicariance predominates over dispersal. The Wallacean *Cethosia* lineages are relatively old, yet are still unobscured today due to sea barriers that have existed for several millions of years, even though Wallace's Line is so narrow (between Borneo-Sulawesi it is only approximately 50 km, and even less between Bali and Lombok).

4.2.1.4. Ecological influences. Only few evidenced examples of terrestrial ecological speciation (i.e., sympatric) are recorded in the literature (Berlocher and Feder, 2002). The parapatric pair of divergent sister species, *Cethosia obscura* and *C. vasilia* (*cydippe* clade) on New Ireland, is intriguing and their separation several millions of years ago (according to our dispersal-vicariance analysis) is inferred to have been prompted by ecological isolation. While *C. obscura* is generally widespread in the lowlands throughout much of the Bismarck Archipelago, *C. vasilia* is restricted to the central cordillera of New Ireland, from 900 to 2400 m (Müller, 1999b). The two taxa overlap partially below 1000 m, although *C. obscura* is only rarely encountered above 500 m (Müller, unpublished).

Where known, larvae of *C. cydippe* races feed on *Adenia* species (Passifloraceae), robust passion vines that grow high into the rainforest canopy. Larvae of *C. obscura* likewise feed upon the foliage of *Adenia heterophylla* (Blume) Koord., while *C. vasilia* is specialised on a small, ground creeper, *Passiflora* near *hollrungii* K. Schum. (Müller, unpublished). Like the adults, the early stages of the two species are morphologically distinct and differences in adult behaviour were recorded by Müller (1999b). The distribution of the two Bismarckian *Cethosia* appears to be governed by their food plant ranges and it is likely that they experienced an ecological shift some millions of years ago.

4.2.1.5. Chronology. Our chronogram showing reconstructed separation between the various clades and taxa agrees closely with the interpreted fragmentation and/or isolation of ancestral areas. This is particularly so in central Indonesia (Wallacea) where numerous highly divergent taxa have undergone allopatric speciation between 11 and 3 Mya. This coincides closely with the opening of the Flores and Banda Basins and ultimately, the inception of Wallacea. Also, vicariance induced by extreme climate dynamics during the Pliocene and Pleistocene has played a major role in shaping the phylogenetic patterns within *Cethosia*.

Of the relatively few biogeographic studies within Wallacea, only a small proportion have postulated topology of divergence. Essentially the same chronological pattern was shown for Indo-Pacific *Charaxes*, as in *Cethosia* in the *myrina* and *biblis* clades, for diversification within and across Wallacea (Müller and Beheregaray, unpublished), with diversification between sister species dated at between 13 and 3 Mya. The grasshopper genus *Chitaura* has apparently been diversifying within Sulawesi for 7–14 Mya and reached the Moluccas from North Sulawesi some 2.5–5 Mya (Butlin et al., 1998), although these authors only used an uncalibrated ‘molecular clock’.

5. Conclusions

Seven distinct lineages were recognised within *Cethosia*, which show a strong correlation between morphology and genotype. Biogeographical analysis of the genus has provided insight into the history of the region and the processes that have shaped the evolution of its species groups.

Cethosia began diversifying in the mid to late Oligocene, approximately 25–21 Mya, with the inception of the *biblis*, *leschenault* and *cydippe* clades. The peak of diversification in *Cethosia* ranged from mid Miocene to Pliocene, ca. 11–2 Mya. Further diversification has taken place at subspecific level during the Pleistocene. The majority of diversification within *Cethosia* beyond the species group level was driven by vicariance, through geological and climatic processes, as demonstrated by the essentially allopatric radiations. Conversely, as suggested by the high levels of local endemism in the genus, inter-island dispersal has been a rare, random event. Sea barriers have separated the aerial fragments for many millions of years until the present.

Ancient land connections between Sulawesi and Timor are postulated, as is a palaeo connection for mainland New Guinea and at least part of the Bismarck Archipelago. A probable land bridge may have existed between Taiwan and Luzon.

Patterns and theories elucidated from this study of *Cethosia* agree with our analysis of Indo-Pacific *Charaxes* and should be used as models for future Indo-Pacific biogeographic studies.

Acknowledgments

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Appendix A

Taxonomy of *C. hypsea*.

Cethosia hypsea mindanensis Felder and Felder, 1863, stat. rev.

Cethosia mindanensis C. and R. Felder, 1863, Wiener Entomologische Monatschrift, 7, 106.

Cethosia hypsea festiva Fruhstorfer, 1909, stat. rev.

Cethosia mindanensis festiva Fruhstorfer, 1909, Stettin. Entomologische Zeitung, LXX, 229.

Remarks. *C. h. mindanensis* and *C. h. festiva* resemble other subspecies of *C. hypsea*, although their markings are generally more diffuse. The pairwise divergence for COI between *C. hypsea fruhstorferi* and *C. h. festiva* was measured at 0.311%. Küppers (2006) also considered the taxon *mindanensis* to show a close relationship to *C. hypsea*.

Note that the above two subspecies were formerly considered as races of *C. mindanensis*.

Cethosia hypsea gabinia Weymer, 1883, stat. rev.

Cethosia gabinia Weymer, 1883, Entomologische Nachrichten, 9, 191.

Remarks. *C. h. gabinia* is superficially very distinct, owing to the relatively unmarked orange upperside of the male and pied colouration of the female. However, the markings, despite being vestigial, are congruous with other *C. hypsea* taxa. The calculated pairwise divergence for COI between *C. hypsea gabina* and *C. h. batuensis* was 0.308%. Küppers (2006) correctly interpreted that *gabinia* and *hypsea* had a common ancestor.

Appendix B

Unrestricted ancestral areas for nodes, as a result of the dispersal-vicariance analysis.

Node 1: CI EI CEI FI CFI EFI CEFI CHI EHI CEHI FHI CFHI EFHI CEFHI

Node 2: IK CIK EIK CEIK FIK CFIK EFIK CEFIK HIK CHIK EHIK CEHIK FHIK CFHIK EFHIK CEFHIK IJK CIJK EIJK CEIJK FIJK CFIJK EFIJK CEFIJK HIJK CHIJK EHIJK CEHIJK FHIJK CFHIJK EFHIJK CEFHIJK

Node 3: IKN CIKN EIKN CEIKN FIKN CFIKN EFIKN CEFIKN HIKN CHIKN EHIKN CEHIKN FHIKN CFHIKN EFHIKN CEFHIKN IJKN CIJKN EIJKN CEIJKN FIJKN CFIJKN EFIJKN CEFIJKN HIJKN CHIJKN EHIJKN CEHIJKN FHIJKN CFHIJKN EFHIJKN CEFHIJKN

Node 4: CIKN CEIKN CFIKN CEFIKN CHIKN CEHIKN CFHIKN CEFHIKN CIJKN CEIJKN CFIJKN CEFIJKN CHIJKN CEHIJKN CFHIJKN CEFHIJKN

Node 5: CEFGIKN CEFGHIKN CEFGIJKN CEFGHIJKN

Node 6: HM JM HJM HLM JLM HJLM

Node 7: CEFGHIJKMN CEFGHIJKLMN

Node 8: CEFGHIJKLMN BCEFGHIJKLMN CDEFGHIJKLMN BCDEFGHIJKLMN

Node 9: BCEFGHIJKLMN BCDEFGHIJKLMN

Node 10: BCDEFGHIJKLMN

Node 11: ABCDEFGHIJKLMN

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