



'After Africa': the evolutionary history and systematics of the genus *Charaxes* Ochsenheimer (Lepidoptera: Nymphalidae) in the Indo-Pacific region

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The predominantly Afrotropical genus *Charaxes* is represented by 31 known species outside of Africa (excluding subgenus *Polyura* Billberg). We explored the biogeographic history of the genus using every known non-African species, with several African species as outgroup taxa. A phylogenetic hypothesis is proposed, based on molecular characters of the mitochondrial genes *cytochrome oxidase subunit I* (*COI*) and *NADH dehydrogenase 5* (*ND5*), and the nuclear *wingless* gene. Phylogenetic analyses based on maximum parsimony and Bayesian inference of the combined dataset implies that the Indo-Pacific *Charaxes* form a monophyletic assemblage, with the exception of *Charaxes solon* Fabricius. Eight major lineages are recognized in the Indo-Pacific, here designated the *solon* (+African), *elwesi*, *harmodius*, *amycus*, *mars*, *eurialus*, *latona*, *nitebis*, and *bernardus* clades. Species group relationships are concordant with morphology and, based on the phylogeny, we present the first systematic appraisal and classification of all non-African species. A biogeographical analysis reveals that, after the genus originated in Africa, the evolutionary history of *Charaxes* in the Indo-Pacific, in particular Wallacea, may be correlated with the inferred geological and climatic history of the region. We propose that Wallacea was the area of origin of all *Charaxes* (excluding *C. solon*) occurring to the east of Wallace's [1863] Line. The earliest Indo-Pacific lineages appear to have diverged subsequent to the initial fragmentation of a palaeo-continent approximately 13 million years ago. Further diversification in Indo-Pacific *Charaxes* appears primarily related to climatic changes during the Pliocene and possibly as recently as the Pleistocene. Although both dispersal and vicariance have played important roles in the evolution of the genus within the region, the latter has been particularly responsible for diversification of *Charaxes* in Wallacea. © 2010 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2010, **100**, 457–481.

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INTRODUCTION

Butterflies are among the most well studied invertebrates, with most species probably already described. In particular, the family Nymphalidae has been the subject of numerous phylogenetic and biogeographical studies in recent years (Simonsen *et al.*, 2006; Wahlberg, 2006; Brown *et al.*, 2007; Wahlberg & Freitas,

2007; Wahlberg & Saccheri, 2007; Peña & Wahlberg, 2008; Kodandaramaiah & Wahlberg, 2009; Leneveu, Chichvarkhin & Wahlberg, 2009). Most of these have been at the genus level. Those pertaining to species level phylogenies are essentially for groups occurring outside of the Indo-Pacific [e.g. the genera *Pararge* (Hübner) (Weingartner, Wahlberg & Nylin, 2006) and *Junonia* (Hübner) (Kodandaramaiah & Wahlberg, 2007)]. In the Indo-Pacific, published species-level molecular phylogenies are few for butterflies [e.g.

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Arhopala Boisduval (Megens *et al.*, 2004) and *Ornithoptera* Boisduval (Morinaka *et al.*, 2000)]. Indeed, no studies incorporating multiple DNA regions for all known species in a genus have been published.

Despite being poorly known generally, the biogeography of taxa occurring in the Oriental and Australian (Indo-Pacific) zoogeographical regions has been of great interest to evolutionary biologists ever since Wallace (1863) first formalized the faunal affinities of these areas. Wallace (1863) noted a major faunal discontinuity between the islands of Borneo and Sulawesi in the north and Bali and Lombok [Lombok] in the south. This discontinuity separated areas which he termed the 'Indo-Malayan Region', in the west and the 'Austro-Malayan Region' to the east of what has become popularly known as Wallace's Line. The area between Wallace's [1863] Line and Weber's Line was named Wallacea by Dickerson (1928). Dickerson also included the Philippines as part of Wallacea but more recent references (Vane-Wright, 1990; Hall, 2009) to the region tend to exclude the Philippines, which we adopt here. Studies documenting how the historical geology of the Oriental and Australian regions may have affected the evolutionary history of a variety of taxa (Austin, 1999; Brown & Guttman, 2002; Cannon & Manos, 2003; De Bruyn, Wilson & Mather, 2004) generally have focussed on either region, with negligible studies recognizing the significance of Wallacea itself (Beheregaray, 2008). Indeed, Wallacea has commonly been considered as a transitional zone between the Oriental and Australian regions (Braby & Pierce, 2007).

Comprehensive, fine-scale species-level phylogenies can provide a more accurate account of more recent historic biogeography than genus or species-group level phylogenies. In the present study, we use all known non-African *Charaxes* species to explore the history of diversification in the Oriental and Australian region, especially the 'transitional' Wallacea. Several of these *Charaxes* species are poorly known and/or represent recently described taxa. Indeed, the highly distinctive *C. marki* Lane & Müller is known only from the holotype (Lane & Müller, 2006). This work forms part of a larger study that demonstrates Wallacea is not only a transitional zone, but also comprises a very unique area, with distinct geological and biogeographic histories.

THE GENUS *CHARAXES*

Charaxes butterflies, commonly known as Rajahs, Pashas, and Emperors, are popular with naturalists, artists, researchers and collectors worldwide. Both adults and early stages are striking, with the former being exceptionally robust and characterized by very powerful flight. The genus is most richly represented

in Africa, where estimates of the number of recognized species currently vary. Henning (1989) suggested 152 species, whereas Williams (2008) recognized 169. Both authors, *sensu* Van Someren (1975), recognize 19 putative species groups for the African *Charaxes*. One predominantly African species, *Charaxes jasius* (Linnaeus), also occurs throughout much of the Mediterranean. Although African *Charaxes* are diverse, many species, especially the 'black' *Charaxes* of the *etheocles* group, are cryptic and have been accorded specific status only in recent years. A recent molecular study of African *Charaxes* found that three species groups were not monophyletic entities and that the genera *Euxanthe* and *Polyura* are nested within *Charaxes* (Aduse-Poku, Vingerhoedt & Wahlberg, 2009). They also found that the two species of Asian *Charaxes* sampled in their study did not form a monophyletic entity, with *Charaxes solon* being related to *Charaxes jahlunga*, and *Charaxes bernardus* to the *candiope* group.

Although the African taxa have been the subject of intensive research over the decades, the remaining 31 known species that occur within the Oriental and Australian region have received much less attention and are the primary focus of the present study. Outside of Africa and the Mediterranean, *Charaxes* is distributed from Sri Lanka and India through South-East Asia to mainland New Guinea, the Bismarck Archipelago and far northern Queensland. Only one species, *Charaxes latona* Butler, occurs east of Maluku, eastern Indonesia.

Nearly all species inhabit tropical, dense, lowland rainforest, although a few Indonesian taxa (e.g. *Charaxes mars* Staudinger, *Charaxes orilus* Butler, and *Charaxes marki*) prefer higher altitudes. The latter two species, together with *Charaxes ocellatus* Fruhstorfer and *Charaxes elwesi* Joicey & Talbot, are restricted to monsoonal vine thicket in the Lesser Sunda Islands, Indonesia. Adult males are most commonly observed on the semi-open tops of steep, jungle-clad hills or along creeks where they patrol in search of urine, dung or carrion on which to feed. Females are generally seen flying directly through forest, sometimes at great height, often in search of oviposition sites. Larvae of *Charaxes* are known to feed on a number of plant families and certain species are polyphagous. However, the life histories of comparatively few Indo-Pacific *Charaxes* have been recorded.

Many Indo-Pacific *Charaxes* species have limited ranges, being restricted to certain small islands or archipelagos. Others, particularly those occurring to the west of Wallace's Line in the Oriental Region, are more widespread. For these reasons, the genus constitutes an ideal subject for detailed biogeographical analysis. Because the systematics of Indo-Pacific

Charaxes has not been previously reviewed in any detail, we present a new classification based essentially on molecular systematics.

MATERIAL AND METHODS

TAXON SAMPLING

For the combined analysis, 65 exemplars (see Appendix, Table A1) were sampled, representing all known species of *Charaxes* occurring outside of Africa. Additionally, a number of divergent *Charaxes* taxa were included in the analysis, from several of the 19 putative species groups endemic to Africa proposed by van Someren (1969–75) and Henning (1989). Six species, also from the butterfly family Nymphalidae, were selected as outgroup taxa, namely *Calinaga buddha* Moore, *Anaea troglodyta* (Fabricius), *Archaeoprepona demophon* (Linnaeus), *Prothoe franck* (Godart), *Polyura moori* (Distant), and *Euxanthe eurinome* (Cramer) (see Appendix, Table A1). We selected outgroup taxa based on inferred phylogenetic relationships proposed by Wahlberg & Wheat (2008). The trees were rooted with *C. buddha* (Calinaginae). In total, the final data set for the combined analysis thus comprised 58 taxa (52 *Charaxes*, of which 41 were non-African). This data set is particularly significant because it is entirely comprehensive at the species level with respect to non-African *Charaxes* (excluding subgenus *Polyura*).

MOLECULAR MARKERS

Three genes [*cytochrome oxidase subunit I* (*COI*) and *NADH dehydrogenase 5* (*ND5*), and the nuclear *wingless* gene] were used to infer phylogenetic relationships between the species and species-groups of *Charaxes*. All of these gene fragments have been widely used in insect phylogenetic research and show variable rates of substitution. Hence their combination is ideal for resolving divergence at different levels. *COI* has shown great utility for resolving shallow (recent) divergence events in Lepidoptera (Caterino, Cho & Sperling, 2000; Sperling, 2003). *ND5* is a rapidly evolving protein-encoding gene that has the potential to resolve relatively recent, lower level relationships, such as those between populations within a species or between closely related species within a genus (Simon *et al.*, 1994; Su *et al.*, 1998; Yagi, Sasaki & Takebe, 1999; Szalanski *et al.*, 2006; Albre, Gers & Legal, 2008; Dodo *et al.*, 2008). *Wingless* is a protein-encoding gene in the nuclear genome involved in wing pattern formation and shows a relatively rapid rate of substitution. In Lepidoptera, it has been used successfully for resolving relationships at both higher and lower systematic levels (Brower &

Egan, 1997; Brower & DeSalle, 1998; Brower, 2000; Campbell, Brower & Pierce, 2000; Wahlberg, Weingartner & Nylin, 2003).

MOLECULAR TECHNIQUES

Two legs were removed for subsequent extraction of DNA. Voucher specimens are deposited at the following centres: Chris Müller reference collection, Sydney; Australian National Insect Collection, Canberra, Australia; School of Biological Sciences Collection, Macquarie University, Sydney, Australia.

A modified Chelex (Bio-Rad) (Walsh, Metzger & Higuchi, 1991) extraction method yielded the most consistent results. Legs and/or small amounts of body tissue were ground manually in a 1.5-mL microcentrifuge tube and 500 μ L of 5% Chelex resin, and 5 μ L of 20 g L⁻¹ 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 polymerase chain reaction (PCR) reactions were conducted in a PTC-100 MJ Research thermocycler, with a total volume of 25 μ L: 2 μ L of gDNA template at various dilutions, with 11.625 μ L of distilled H₂O, 2.5 μ L of buffer (100 mM), 2.5 μ L magnesium chloride, 5 μ L of each dNTP (2.5 mM), 0.5 μ L of each primer and 0.375 μ L of Taq polymerase (5 units μ L⁻¹).

For *COI*, a 654 bp fragment was amplified using Folmer *et al.* (1994) LCO (5'-GGTCAACAAATCATA AAGATATTGG-3') and HCO (5'-TAAACTTCAGGGT GACCAAAAATCA-3'). For older and/or degraded tissue samples that could not be amplified using the LCO/HCO pair, internal primers Ron (5'-GGAGCY CCWGATATAGCTTTCCC-3') and Nancy (5'-CCTGG TAAAATTAATAAATAAACTTC-3'), as obtained by Caterino & Sperling (1999), were trialed to amplify two fragments that were concatenated into a 654-bp *COI* sequence. To amplify the 433-bp *wingless* gene fragment, the primers LepWG1 (5'-GARTGYAAR TGYCAYGGYATGTCTGG-3') and LepWG2 (5'-ACT ICGCARCACCARTGGAATGTR CA-3') (Brower & DeSalle, 1998) were employed. The primers A1 (5'-AATATDAGGTATAAATCATAT-3') and C2 (5'-ATCY TTWGAATAAAAYCCAGC-3') (Torres *et al.*, 2001) were used in the amplification of 397 bp of *ND5*.

The cycling protocol used for *COI* comprised: 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 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, whereas, 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 mins at 72 °C, with a final extension of 10 min at 72 °C. Negative controls were included in all PCRs. The PCR products were separated by electrophoresis and purified using UltraClean 15 DNA Purification Kit (MO BIO Laboratories Inc.).

All DNA sequencing was performed on an ABI 3130 using the manufacturer's protocol. Chromatograms generated from each reaction were edited manually and then aligned in SEQUENCHER, version 4.1. All sequences were aligned against other published Lepidoptera sequences (Brower & DeSalle, 1998; Campbell *et al.*, 2000). For *COI*, the consensus sequences were aligned against the published reference sequence for *Drosophila yakuba* Burla (Clary & Wolstenholme, 1985) and/or various Lepidoptera sequences on GenBank. GenBank accession numbers for all sequences are provided in the Appendix (Table A1).

PHYLOGENETIC ANALYSIS

We assessed individual sequence properties using MEGA, version 4.1 (Tamura *et al.*, 2006). For maximum parsimony (MP) analysis, phylogenetic trees were reconstructed using unweighted and weighted MP as the optimality criterion, as in PAUP* 4.0.b10 (Swofford, 2002). Tree estimation involved 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 'MulTrees' option in effect. Strict consensus trees were computed whenever there was more than one equally parsimonious tree. Bootstrap analyses (Felsenstein, 1985, 1988), based on a full heuristic search of 1000 pseudo-replicates using TBR branch swapping and simple stepwise addition, were carried out for each analysis to determine the level of support of each node. Clade robustness was also evaluated using Bremer support (Bremer, 1994) using TREEROT 3 (Sorenson & Franzosa, 2007). 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, version 3.0b4 (Ronquist & Huelsenbeck, 2003), after analysing the dataset using MODELTEST, version 3.06 (Posada & Crandall, 1998). All partitions were assigned with the GTR+G model. Three independent Bayesian runs at temperature settings in the range 0.2–0.4 were performed on the data using metropolis-coupled Markov chain Monte Carlo simulations, from one to 5 million generations each, and tree sampling every 100 generations. Bayesian topology and branch posterior probabilities were computed by majority

rule consensus after deleting the first 1000 000 generations (10 000 trees) as 'burn-in', after confirming that likelihood values had stabilized prior to the 100 000th generation.

AGE OF DIVERGENCE ESTIMATIONS

Because there are no known fossils of *Charaxes*, we calibrated our tree by consulting a recent study based on fossil record which estimated the age of the split between *Charaxes* and *Euxanthe* at 22 ± 1.5 Mya (Peña & Wahlberg, 2008). There appear to be no other reliable calibration events because potential markers for the region studied consisted of either widespread, gradual and recurring events (e.g. geo-dynamics, sea-level fluctuations in response to climatic changes) or singular events that were too localized to be useful (e.g. volcanic eruption, impact event). Indeed, tectonic events in the Indo-Pacific during the Tertiary are still poorly known and conjectural and inferred sea levels are highly speculative beyond approximately 100 000 years ago (R. Hall, pers. comm.).

The estimation of divergence times was undertaken using BEAST (Drummond & Rambaut, 2007). The relaxed molecular clock technique 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 *Charaxes* and *Euxanthe* was set to 22 ± 1.5 Myr. All other priors were left as defaults in BEAST. We ran a Bayesian analysis twice for 5000 000 generations. We determined whether our parameter estimates and tree topology were at equilibrium using the program TRACER (Drummond & Rambaut, 2007). The first 1000 000 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.

BIOGEOGRAPHICAL ANALYSIS

We reconstructed the historical biogeography of Indo-Pacific *Charaxes* using a dispersal–vicariance optimization model implemented in DIVA (Ronquist, 1997). The model, unlike the classic vicariance single pattern model, acknowledges the need for some level of dispersal in explaining the occurrence of widespread ancestors. DIVA therefore assigns a cost of one for assumed dispersal and extinction events and a zero for vicariance and speciation events not requiring large-scale geographic distance (within-area speciation). The optimal ancestral reconstruction of the DIVA model is the one with the lowest cost and is thus the most parsimonious. DIVA requires that the

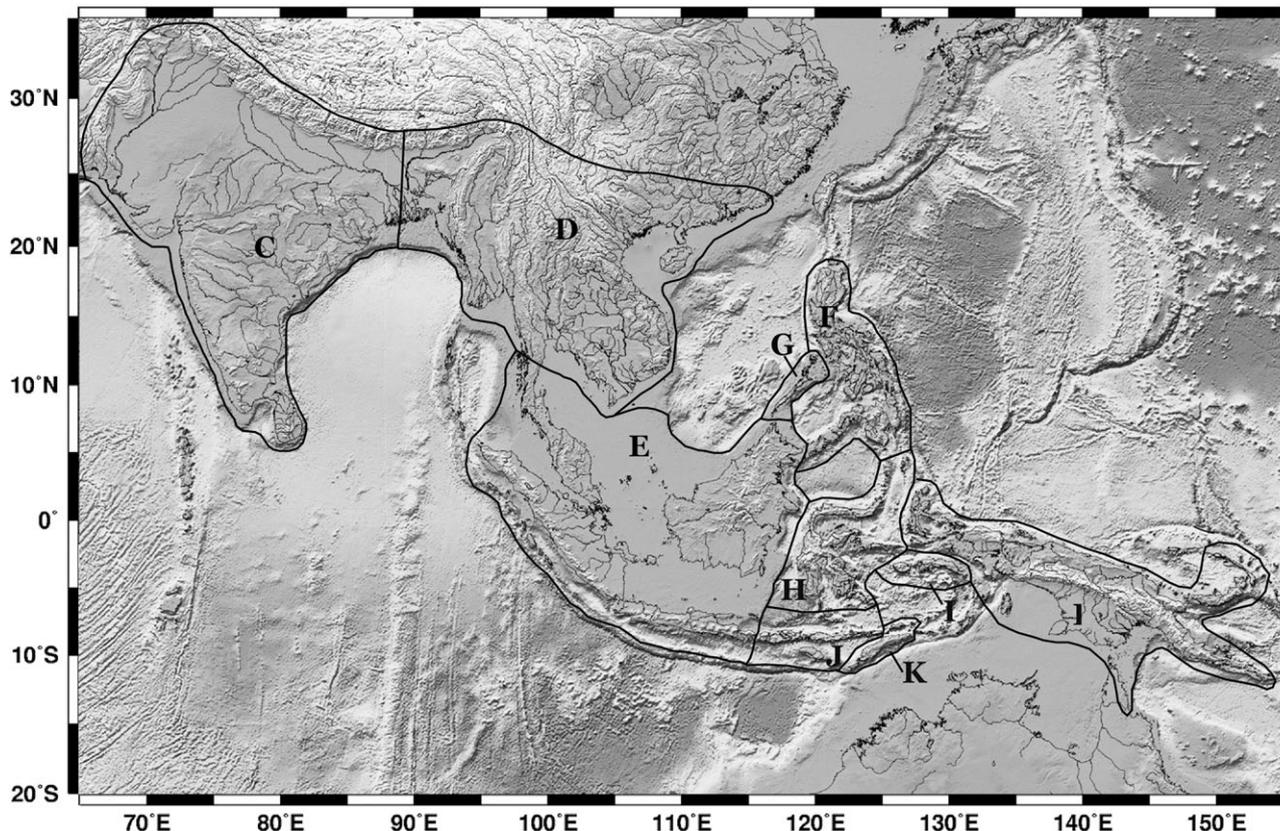


Figure 1. Map showing assigned biogeographic zones used in the dispersal-vicariance analysis of Indo-Pacific *Charaxes*, defined as: Africa (A); South America (B); India and Sri Lanka (C); Indo-China (D), comprising nonpeninsula Thailand, Laos, Cambodia, Vietnam, southern China, and Myanmar; Sundaland (E), encompassing peninsula Thailand, Peninsula Malaysia, Sumatra, Borneo, and Java; Philippines (F), excluding Palawan; Palawan (G); Sulawesi (H); Sulawesi and satellite islands, including Sula Islands, Sangihe, Buton, and Muna Islands; Central Maluku (I); including the main islands of Buru, Seram, and Ambon; Lesser Sunda Islands (J), comprised of all islands between Lombok and Alor, excluding Timor and Wetar; Timor/Wetar (K) and the New Guinea mainland (L). Note that zones (A) and (B) are not shown.

phylogenetic relationship among species is fully resolved. We thus used the Bayesian topology (as derived using BEAST) for this analysis.

Geographical distribution was coded as 12 states, enabling radiation and diversification patterns to be assessed at fine resolution within the Indo-Pacific region, where many of the subject taxa are endemic to particular islands or archipelagos. These states do not necessarily define political boundaries but are unique in their overall biological composition. These zones (states) (Fig. 1) are defined as: Africa (A); South America (B); India and Sri Lanka (C); Indo-China (D), comprising nonpeninsula Thailand, Laos, Cambodia, Vietnam, southern China, and Myanmar; Sundaland (E), encompassing peninsula Thailand, Peninsula Malaysia, Sumatra, Borneo, and Java; Philippines (F), excluding Palawan; Palawan (G); Sulawesi (H); Sulawesi and satellite islands, including Sula Islands, Sangihe, Buton, and Muna Islands; Central Maluku

(I); including the main islands of Buru, Seram, and Ambon; Lesser Sunda Islands (J), comprising all islands between Lombok and Alor, excluding Timor and Wetar; Timor/Wetar (K) and the New Guinea mainland and far north Queensland, Australia (L).

RESULTS

DATASET PROPERTIES

The final dataset consisted of 58 taxa, including six outgroups. For certain taxa, we were unable to amplify all of the three gene fragments (see Appendix, Table A1). The complete combined sequence data contained 1456 nucleotides, of which 611 (42.0%) were variable and approximately 383 (26.3%) were parsimony informative. At the individual gene level, *COI* had the highest proportion of parsimony informative sites, with 30.1%, whereas *ND5* had 21.7% and *wingless* 24.2%.

Partitioned Bremer support of the combined dataset under MP revealed a high level of congruence between the three genes for most nodes. The only source of conflict was with the *wingless* gene, and the least with *COI*. This is in contrast with previous Lepidopteran studies focusing at and above the level of species-group, where *COI* has been shown to contribute less than nuclear gene fragments (Silva-brandão *et al.*, 2005; Braby, Vila & Pierce, 2006). The causes of this are likely two-fold: 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. Partial lack of congruence with the *wingless* gene was shown by Silva-brandão *et al.* (2008) in their study of the nymphalid tribe Acraeini, which included several closely related species of *Altinote* Potts and *Actinote* Hübner.

PHYLOGENETIC PATTERNS

Results for the combined MP analysis of the three genes are shown in Figure 2, which represents a strict consensus tree from 43 equally most parsimonious trees derived using unweighted parsimony. The Bayesian analysis (MrBayes: Fig. 3; BEAST: Fig. 4) produced a topology essentially congruent with that generated by MP. There was considerable phylogenetic structure, with the majority of nodes being well resolved. This was likely a result of comprehensive sampling, which reduces phylogenetic error (Zwickl & Hillis, 2002). Inclusive of *Euxanthe* and *Polyura*, *Charaxes* is monophyletic with respect to other selected outgroup taxa. *Charaxes solon* falls within the African *Charaxes* clades in the Bayesian analysis and for MP is sister to all remaining Indo-Pacific *Charaxes* [*C. solon* + (*C. marki* + remainder of Indo-Pacific *Charaxes*)].

All Indo-Pacific *Charaxes*, with the exception of *C. solon*, form a well-supported monophyletic group [bootstrap 73% MP, 1.00 Bayesian inference (BI)]. Within the Indo-Pacific *Charaxes*, eight distinctive clades (*elwesi*, *mars*, *nitebis*, *harmodius*, *amycus*, *eurialus*, *latona*, and *bernardus*) were resolved, with invariably high statistical support yielded by both BI and MP analysis. The *bernardus* clade is sister to the remainder of Indo-Pacific *Charaxes*, a pairing which is well supported for both MP and BI. For the latter, the monotypic *nitebis* clade grouped with the *bernardus* clade. Although most Indo-Pacific clades were characterized by relatively long branches between taxa and moderate-strong statistical node support, the *bernardus* clade revealed a number of species with very little genetic divergence. The lack of resolution among the basal lineages of the *bernardus* clade may comprise a hard polytomy (rapid radiation) rather than a soft polytomy in which a lack of data or

multiple substitutions (homoplasy) is obscuring the phylogenetic signal.

AGE OF DIVERGENCE

Our estimation of divergence times and their credibility intervals, within *Charaxes* is shown in the chronogram in Figure 4. The analysis revealed that *Polyura* and *Charaxes* split in the late Oligocene, approximately 25 Mya. *Charaxes* diversified in Africa soon after, with the Oriental *C. solon* splitting from African *Charaxes eupale* + (*Charaxes etesipe* + *Charaxes nichetes*) in the mid Miocene, around 17 mya. At approximately the same time, the other Indo-Pacific *Charaxes* split from African *Charaxes* (*Charaxes bipunctatus* + (*Charaxes protoclea* + (*Charaxes cynthia* + *Charaxes boueti*))). The large *bernardus* clade separated from the remainder of Indo-Pacific *Charaxes* during the mid Miocene, at approximately 12 Mya. The peak of radiation in Indo-Pacific *Charaxes* appears to have been during the mid Miocene to late Pliocene, between 3–13 Mya.

BIOGEOGRAPHICAL ANALYSIS

Based on the DIVA model, using the Bayesian tree derived in BEAST (Fig. 4), both dispersal and vicariance have played importance roles in the diversification of *Charaxes* in the region. Indeed, vicariance appears to have been largely responsible for cladogenesis in Wallacea.

The origins of Indo-Pacific *Charaxes* are conjectural but that of the *bernardus* clade is likely to have been Asian (west of Wallace's Line), whereas that of the remaining Indo-Pacific *Charaxes* is possibly Sulawesi (or at least the ancestral distribution likely included Sulawesi). According to the DIVA model, the majority of subsequent diversification for the latter group was driven by vicariance, as demonstrated by the essentially allopatric radiations. An exception is the inferred dispersal of *C. ocellatus* from its ancestor with *C. orilus*. For the *bernardus* clade, Palawan is suggested as an origin for several taxa occurring in Indo-China, Sundaland, and the remainder of the Philippines.

Splitting between Wallacean taxa, namely those occurring in Sulawesi, central Maluku, the Lesser Sunda Islands, and Timor/Wetar occurred 3.5–13 Mya. Other notable events include the splitting of the Wallacean *mars* and Sundanian *harmodius* clade of taxa with Philippine endemics (*amycus* clade) at approximately 12 Mya, the splitting of the other Wallacean clades (*nitebis* + (*elwesi* + (*eurialus* + *affinis*))) and Sundanian *bernardus* clade at around the same time and the divergence of the Wallacean *mars* and Sundanian *harmodius* clade at approximately 11 Mya. Within Wallacea, the vicariant split between the Sulawesi *mars* and Timorese *marki* was dated at

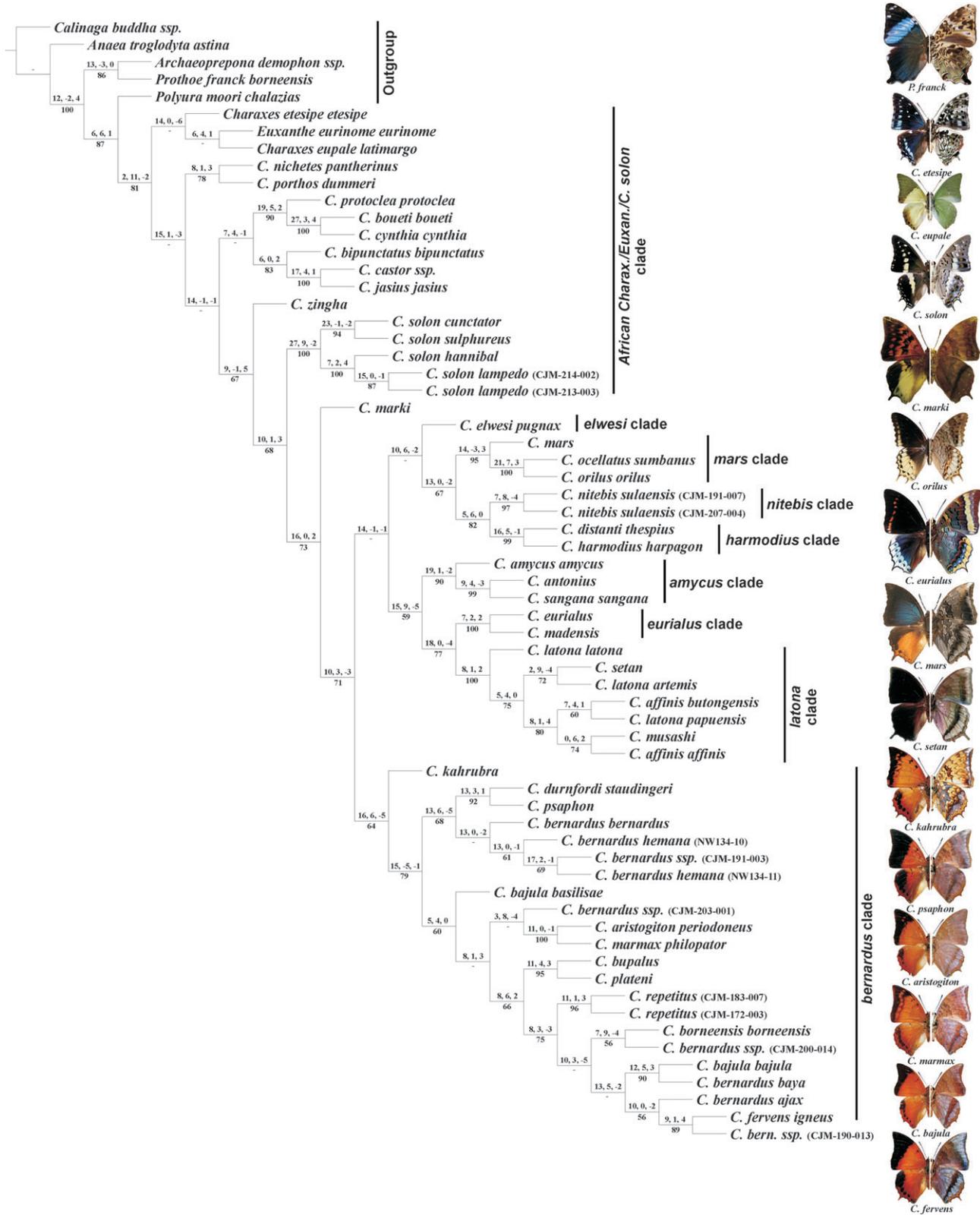


Figure 2. Strict consensus tree of 43 equally parsimonious trees (length 1980 steps, consistency index (CI) = 42, retention index (RI) = 55) for the combined *COI*, *ND5* and *wingless* dataset. Numbers above branches are Bremer support indices for *COI*, *ND5* and *wingless*, respectively, and those below are bootstrap values > 50% for the node to the right. *Calinaga buddha* is outgroup.

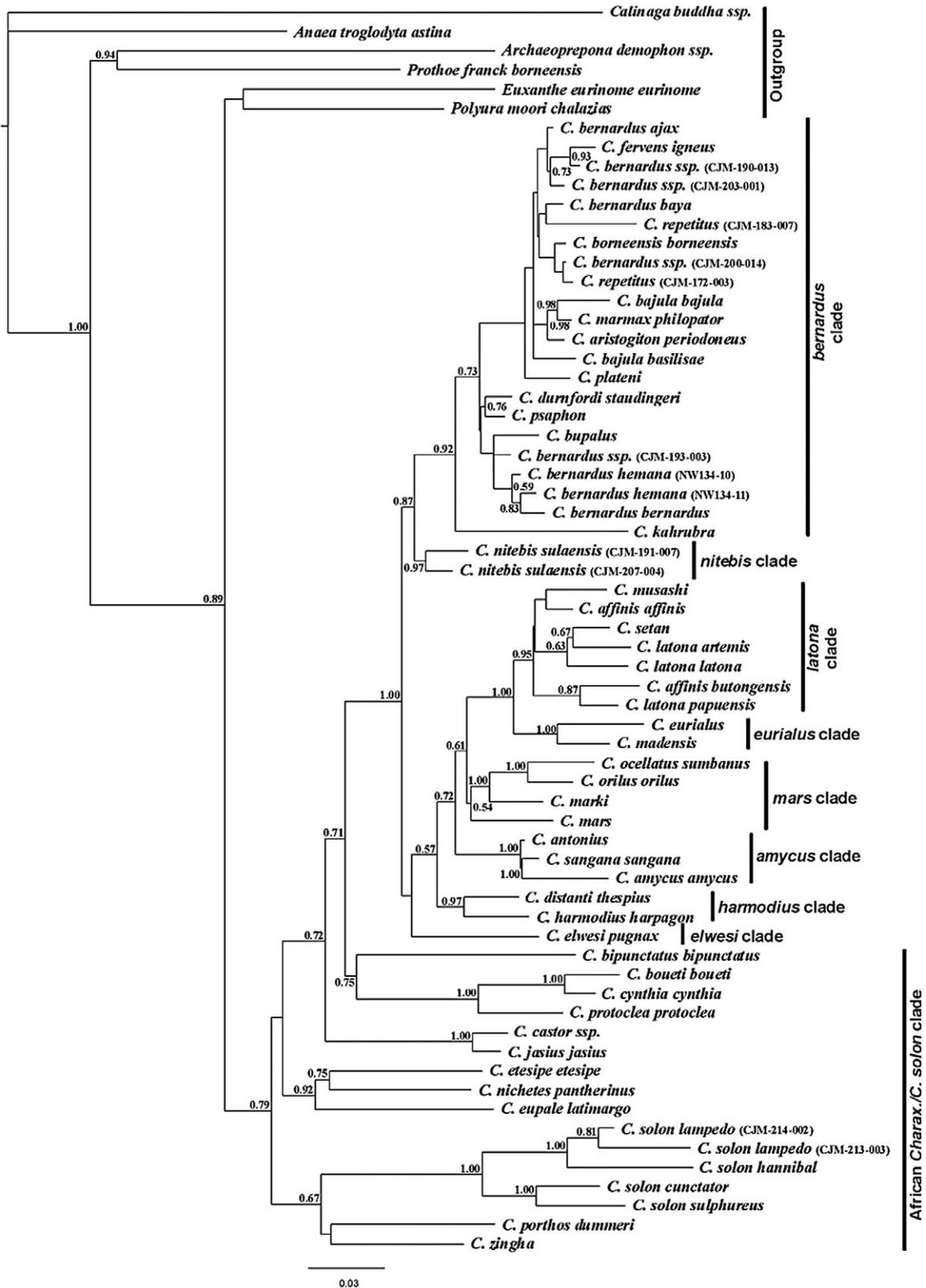


Figure 3. Fifty percent majority rule phylogram from the Bayesian analysis of the combined *COI*, *ND5* and *wingless* dataset. Numbers to the left of nodes are the posterior probabilities of those nodes.

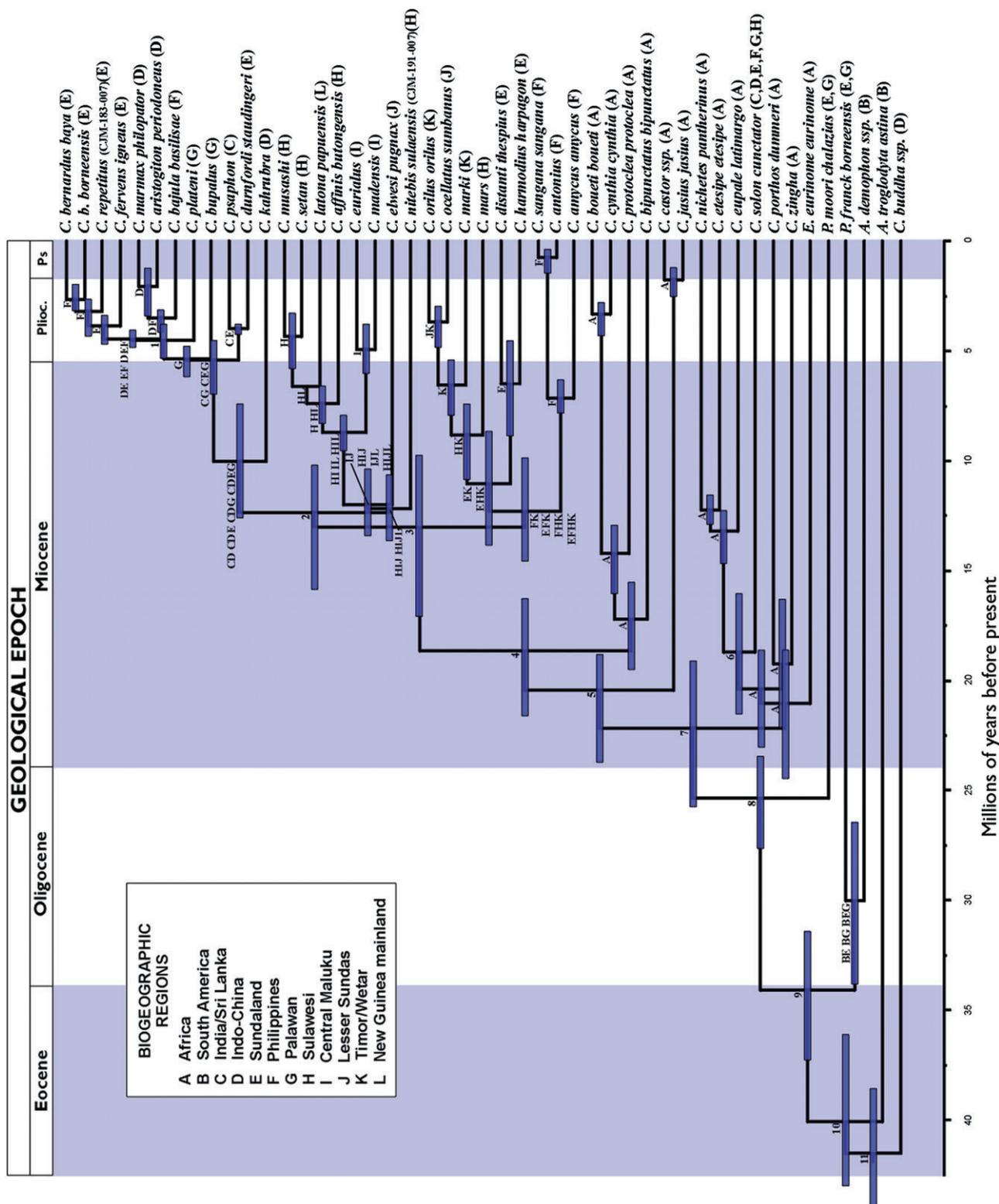


Figure 4. Chronogram based on the Bayesian topology with associated posterior credibility limits. Results of the dispersal-vicariance analysis, with unrestricted ancestral areas, are shown for each node. Certain nodes revealed too many ancestral distributions to fit on the figure and are listed in the Appendix (Table A4).

approximately 8 Mya and that between the Buru endemic *Charaxes madensis* Rothschild and *Charaxes eurialus* Cramer, from Seram, at approximately 5 Mya. Sudden diversification of the *bernardus* clade in Oriental/Sundaland occurred at approximately 5 Mya.

DISCUSSION

SYSTEMATIC IMPLICATIONS

The systematics of African *Charaxes* have been the focus of numerous comprehensive studies over the past few decades and the putative species groups are characterized almost exclusively on the morphology of the adult hind wings (Van Someren, 1975). A molecular study by Aduse-Poku *et al.* (2009) conformed closely with that of the above mentioned classification. These authors synonymized both *Euxanthe* and *Polyura* with *Charaxes*. The systematics of *Polyura* and *Euxanthe* were covered by Smiles (1982) and Smiles (1985), respectively. Conversely, the systematics of Indo-Pacific *Charaxes*, as a group, remain essentially unknown, despite being amongst the most popular insects in the region. A new classification is proposed herein, based on phylogenetic relationships. Although morphological characters were not used as part of this analysis, broad correlations between phylogenetic groupings, genetic divergence and phenotype are obvious (Fig. 2; see Appendix, Table A2).

Eight distinctive clades, representing all Indo-Pacific *Charaxes* except *C. solon*, were resolved, all of which are endemic to the region. *Charaxes solon* was one of three Indo-Pacific *Charaxes* used in the dataset of Aduse-Poku *et al.* (2009), which also included *Charaxes marmax* and *C. bernardus*. *Charaxes solon* was recovered as a sister to various African *Charaxes* in the present study and as an immediate sister to the monotypic *Charaxes* subgroup *jahlusa* in the study by Aduse-Poku *et al.* (2009). The latter study, however, retained *C. solon* in its own species group. The remaining clades/species groups (*elwesi*, *harmodius*, *amycus*, *mars*, *eurialus*, *latona*, *nitebis*, *bernardus*) form a monophyletic group and their specific compositions are outlined in the Appendix (Table A2). As well as genetic affinity, the majority of these groupings are in accordance with morphological similarities, especially wing pattern and colour. In particular, high genetic and phenotypic divergence is evident in the former seven above-mentioned clades, whereas the currently recognized 12 species in the *bernardus* species group are essentially of similar morphotype (orange–brown uppersides, with similarly patterned undersides) and show generally low to negligible pairwise sequence divergence between species. For example, *COI* pairwise divergence between sympatric, sibling Bornean

C. bernardus and *Charaxes borneensis* does not exceed 1.4%, whereas, for sister species *C. orilus* and *C. ocellatus*, the difference is 3.5% for the same gene.

Charaxes solon represents an early lineage which has diversified into two main sub-lineages, one of which occurs in South-East Asia and Sundaland, and the other in Sulawesi and the Philippines. Strong phenotypic similarities are exemplified by genetic differentiation.

We sampled three specimens of typical ‘*marmax*’ and three ‘*aristogiton*’ for *COI* in the present study and revealed no consistent sequence differentiation. We also found, from an examination of long series in the Natural History Museum (London) and Naturalis Museum (Leiden), that the diagnostic characters of *C. marmax* and *Charaxes aristogiton* proposed in various studies (Pinratana & Eliot, 1996) were highly variable, with numerous intergrades. Such characters assessed included the extent of the fore and hind wing upperside black apical and terminal region, the fused (or nonfused) state of the hind wing apical spots, the underside ground colour, and intensity of the hind wing underside postmedian band. Indeed, the distributions of the two taxa in Indo-China are remarkably parallel, with nominate *marmax* and *aristogiton* occurring in Sikkim and Assam to Myanmar, Thailand, Laos, whereas *Charaxes marmax philopator* Fruhstorfer and *Charaxes aristogiton peridoneus* Fruhstorfer co-occur in Vietnam. For the above reasons, we suggest that *C. aristogiton* be regarded as a junior synonym of *C. marmax*.

We found evidence for a well-supported sister relationship (MP = 95%) between the sympatric *Charaxes bupalus* and *Charaxes plateni*, both of which are endemic to Palawan. This is intriguing because the former taxon shows a superficial resemblance to *C. borneensis*, which is widespread in Sundaland. Indeed, D’Abrera (1985) combined *C. bupalus* as a subspecies of *C. borneensis*.

The nonmonophyly of *C. bernardus* with respect to other *Charaxes* taxa in the *bernardus* clade may be attributed to high variation in the *COI* gene fragment, with generally low pairwise divergence between members of this group. *Charaxes bernardus* may possibly represent a taxon which is currently undergoing diversification, corroborated by the numerous localized forms of this insect and the overall high morphological variation of adults. One exception is noted below, where a distinctive taxon, until recently referred to as the Bornean race of *C. bernardus*, is herein separated, based on phenotypic and genetic differences (see Appendix, Table A3).

HISTORICAL BIOGEOGRAPHY AND DIVERSIFICATION

Charaxes was shown by Aduse-Poku *et al.* (2009) to have originated in Africa and the same study reported

estimated times of divergence between the African and the Asian (*solon* and *bernardus*) *Charaxes* species groups in the range 13–17 Mya, which agrees quite closely with our own estimate of the divergence between African (including *solon* clade) and Indo-Pacific *Charaxes* at approximately 18 Mya.

The zoogeographical distributions of each Indo-Pacific *Charaxes* species were mapped on our phylogeny to produce a taxon-area cladogram (Fig. 4). The Indo-Pacific *C. solon* clearly nests within African *Charaxes*, where it is sister to clades (*C. eupale* + *C. etesipe* + *C. nichetes*) and (*Charaxes porthos* + *Charaxes zingha*). All remaining Indo-Pacific *Charaxes* clades are distributed in either Wallacea (*mars*, *nitebis*, *elwesi*, *eurialus*, and *latona* clades), the Philippines (*amycus* clade) or the Oriental region (*bernardus* and *harmodius* clades), with almost no overlapping of taxa between the three regions. The exceptions are the widespread *C. solon* and also *C. elwesi*, *C. bernardus*, and *Charaxes harmodius* on the island of Bali, which itself is both part of Sundaland and the Lesser Sunda Islands. A single species, *C. latona* also occurs to the east of Wallacea, in Maluku, New Guinea, and far northern Queensland. Within Indo-Pacific *Charaxes*, speciation events between Wallacean taxa are notably older than those between species in the Oriental region and the Philippines, and longer branches indicate extended isolation of Wallacean *Charaxes*. Such a pattern was also recorded for other groups of butterflies co-occurring in Wallacea (C. Müller & L. B. Beheregaray, unpubl. data).

The identification/postulation of old Wallacean lineages in the genus *Charaxes* is intriguing. Although few studies appear to have recognized the temporal importance of Wallacea for the evolution of butterflies in the region, Vane-Wright & de Jong (2003) implied in their reproduced phylogenies of various Indo-Pacific genera that certain endemic Sulawesi taxa represented basal lineages, suggesting 'a relatively ancient element among the Sulawesi butterflies' (Vane-Wright & de Jong, 2003: 29). Additionally, de Boer (1995), in his reconstructed phylogenies of Indo-Pacific cicadas, showed that genera and tribes endemic to Sulawesi were essentially of basal, ancient lineages. Indeed, in their area cladogram, they linked 'SE Asia' with Sulawesi (i.e. an ancient affinity). However, they did not provide any estimation of divergence times. Sulawesi is the largest island in Wallacea and its fauna have also been the subject of most biogeographical studies pertaining to Wallacea (Van Balgooy, 1987; Vane-Wright, 1990).

Although we were unable to determine the origin of the Wallacean clades, it is clear that the Wallacean lineages are old, relative to the Sundanian *bernardus* clade, comprising all Indo-Pacific *Charaxes* that occur

to the west of Wallace's Line. Vane-Wright & de Jong (2003) inferred that both faunal and cladistic analysis point to a Sulawesi butterfly fauna of mainly Asian (Sundaland) origin, with no special relationship with Borneo. Vane-Wright & de Jong (2003) postulated that low generic endemism, coupled with the high levels of species endemism, suggests a long but not excessive period of evolution in isolation, probably 'seeded' in geological West Sulawesi by species derived from Asia and Sundaland. Vane-Wright & de Jong (2003) also suggested that, subsequent to the collision of the Asian and Australian plates bringing east and west Sulawesi together, continuing faunal exchange has mainly been a one-way route, into Sulawesi.

GEOLOGICAL VICARIANCE

Phylogenetic patterns within *Charaxes*, in conjunction with the DIVA analysis, imply that geological processes have led to the vicariant diversification and speciation of several Indo-Pacific clades and the species they comprise. Broadly, three primary vicariant events that are likely attributable to geological processes are recognized, namely: (i) the allopatric diversification of five Wallacean clades (*nitebis*, *latona*, *mars*, *elwesi*, *eurialus*) from a common ancestor; (ii) separation of the Philippine endemics in the *amycus* clade from the *mars* and *harmodius* clades; and (iii) formation of a cluster of sibling species within the *bernardus* clade, with ranges in either Indo-China or south-east Asia (essentially Sundaland), separated by the Isthmus of Kra in Peninsula Thailand.

During the early Miocene, approximately 25 Mya, the Philippine Sea Plate began to rotate clockwise and ultimately resulted in the accretion of fragments (micro-continents) from the northern Australian margin into the south-east Asian margin, notably in Sulawesi (Hall, 1996, 2002, 2009). The Wallacean fragments were likely shifted after being sliced from their original continental block along the Yapen–Sorong Fault Zone (or similar palaeo-faults), a major fault extending east to west from northern Irian Jaya to Sulawesi (Hamilton, 1979; Letouzey *et al.*, 1983; Dow & Sukamto, 1984; McCaffrey & Abers, 1991; Charlton, 1996; Kreemer *et al.*, 2000), after its inception during Oligocene to Middle Pliocene time (Lee & Lawver, 1995; Charlton, 1996; Hall, 1996; Packham, 1996; Hall & Wilson, 2000). On the basis of stratigraphic similarities, Villeneuve *et al.* (1998, 2001) proposed that several Wallacean micro-fragments constituted a single continental fragment, named the 'Kolonodale block', which collided with Sulawesi during Late Oligocene–Early Miocene time. A further fragment, the 'Lucipara' block, distinct from the

Kolonodale Block (Villeneuve *et al.*, 1998; Cornée *et al.*, 2002), collided during the Middle Miocene (Smith & Silver, 1991; Honthaas *et al.*, 1997; Villeneuve *et al.*, 1998). Finally, the Banggai Sula micro-continent has been colliding with north-east Sulawesi subsequent to Late Miocene–Early Pliocene time (Davies, 1990; Villeneuve *et al.*, 2000, 2001). A ‘Banda’ micro-continent may have been an amalgamation of these blocks during the Miocene (F. Hirschberger, pers. comm.) and possibly harboured the ancestor of Wallacean *Charaxes*.

At the time (middle Miocene, 15 Mya) these Australian continental crust (Wallacean) fragments began colliding with West Sulawesi, subduction at the Banda Trench was initiated and subsequently lead to back-arc spreading and the inception of the Banda Basins (Banda Sea) (Réhault *et al.*, 1994; Honthaas *et al.*, 1998; Hirschberger *et al.*, 2000; Hirschberger *et al.*, 2005), as a consequence of rollback of the Java Trench subduction hinge (Hall, 2009). Geochemical, petrological, and geophysical data (namely magnetic lineation data) support an age of 7–12.5 Mya and 3.5–6.5 Mya, respectively, for the north west–south east opening of the North and South Banda basins (Réhault *et al.*, 1994; Honthaas *et al.*, 1998; Hirschberger *et al.*, 2000, 2001, 2005). The active spreading stopped during Middle Pliocene time in response to the collision between the Australian continent and the Banda Arc dated at approximately 3 Mya (Carter, Audley-Charles & Barber, 1976; Hamilton, 1979; Bowin *et al.*, 1980; Abbott & Chamalaun, 1981; Audley-Charles, Ballantyne & Hall, 1988; Richardson & Blundell, 1996). Such back-arc spreading and fragmentation of an early micro-continent has essentially generated Wallacea and additionally has probably shaped the biogeographic patterns that we see today in Wallacean *Charaxes*. Back-arc spreading at approximately 13 Mya coincides with the initial divergence of the Wallacean clade, which progressively diversified as the Banda microcontinent was dismembered, leading to the subsequent vicariant splitting of Sulawesi/Timor respective endemics *C. mars* and *C. marki*, as well as *C. eurialus* and *C. madensis* in Buru/Seram.

The sister relationship of *C. mars* (Sulawesi) and *C. marki* (Timor) (as demonstrated under BI) is congruent with that between *Cethosia myrina* Felder (Sulawesi) and *Cethosia leschenaulti* Godart (Timor) in a phylogenetic study (C. Müller & L. B. Beheregaray, unpubl. data). Such a Sulawesi–Timor connection was recognized in an assessment of the *Parantica tityoides* group (Ackery & Vane-Wright, 1984), although the correlation could not be explained. Müller & Beheregaray (unpubl. data) suggested that both Sulawesi and part of Timor were part of a micro-continent during the Miocene and their separation lead to the

allopatric evolution of the fore-mentioned taxa. Timor is a fused composite, comprising mostly Australian Plate margin but the upper nappes are ‘Asian’ (R. Hall, pers. comm.; F. Hirschberger, pers. comm.). The Timorese fossil mammal, *Anthracothema verhoeveni* Von Koenigswald belongs to a genus otherwise known from mainland Asia (Von Koenigswald, 1967). The above-mentioned fossil is from Mota Bour, within the Bobonaro Complex, which is the type, and so far unique, locality for *Charaxes marki*.

The occurrence of the *amycus* clade of Philippine endemics raises questions regarding their origin, which, according to our DIVA analysis, was the result of a vicariant event. In all our analyses, this clade nested within the Wallacean clade as a relatively early lineage, implying a relatively early connection between Wallacea and the Philippines. The Philippines have been connected, via island arcs, to both Sundaland (Borneo) and Sulawesi at various times during the late Tertiary. Moss & Wilson (1998) implied that island-hopping routes between Borneo, the Philippines, and Sulawesi included the Sulu, Sangihe, and Cagayan arcs. Inger (1954) considered that there were two periods during the Tertiary, when faunal exchange between Sulawesi and the Philippines may have been possible: in the late Miocene when the south-eastern portion of the Philippines were relatively close to the north-eastern part of Sulawesi and during the Pliocene when a chain of volcanoes (the Sangihe arc) extended northward from Sulawesi towards the Philippines (Hall, 1998). The Sangihe Arc is long-lived and is likely to have been a link between the Philippines and Wallacea (Sulawesi) intermittently during the Cenozoic. However, the Philippines proper shares the overwhelming majority of its butterfly genera with the Malay Peninsula and the Greater Sunda Islands. The major exception involves a few small genera shared exclusively with Sulawesi (Vane-Wright, 1990). There is a much higher species level affiliation between Sulawesi and the Moluccas (i.e. the Wallacean connection). Sulawesi has 200 endemic butterflies out of a total of approximately 460, but shares only two butterfly species exclusively with Borneo and only eight exclusively with the Philippines (R. Vane-Wright, pers. comm.).

The Isthmus of Kra represents a significant range divide within the *bernardus* clade of *Charaxes*, with *C. marmax*, *C. aristogiton*, and *Charaxes kahruha* restricted to the north (Indo-China), whereas *C. borneensis* and *Charaxes durnfordi* occur south of the boundary (Sundaland). The widespread *C. bernardus* occupies both Indo-China and Sundaland. The Isthmus of Kra has long been considered a floral and avifaunal biogeographic transition zone between two areas of endemism (Wells, 1976; Hughes, Round & Woodruff, 2003; Woodruff, 2003). Essentially, this

boundary separates Sundaic species from Indo-Chinese. Current hypotheses propose that climatic changes as well as geological influences (such as a marine seaway across the isthmus most recently during the early Pliocene) account for this pattern (Hughes *et al.*, 2003; Woodruff, 2003; Reddy, 2008). Palaeomagnetic data indicates Post-Cretaceous clockwise rotations in Thailand and northern Malaysia (Schmidtke, Fuller & Haston, 1990; Fuller *et al.*, 1991) whereas counter-clockwise rotations (McElhinny, Haile & Crawford, 1974; Haile *et al.*, 1983; Schmidtke *et al.*, 1990; Fuller *et al.*, 1991) are reported from Tertiary and older rocks further south. Therefore, it is evident that a north Malaya block has been separated from a south Malaya block at the Khlong Marui fault, within the Isthmus of Kra.

CLIMATIC VICARIANCE

DIVA analysis in the present study revealed several vicariant speciation events that are likely a reflection of climate dynamics, rather than geological events, because they span areas of relative tectonic stability and the events are distinctly young (Pliocene and Pleistocene). For *Charaxes* in the Indo-Pacific, the Pliocene marked a period of peak diversification within the *bernardus* clade, particularly in Sundaland. The most prominent response to climate change in the past has likely been sea level fluctuation.

The evolution of the *bernardus* clade at the species level appears to have been largely vicariant in nature, although several species are parapatric/sympatric in distribution and therefore, for such species, no inferences can be made from the area cladogram (Fig. 4). The Palawan endemic sister species pair, *C. bupalus* Honrath and *C. plateni* Staudinger, may also have been isolated from the remainder of Sundaland but, in this case, this pair form an early lineage with respect to the tight clade of closely related Sundaic species in the *bernardus* clade. We consider that the splitting of the three Philippine endemic *Charaxes* within the *amycus* clade may have been climate induced because they are generally all lowland species and their ranges are partly overlapping (with the exception of *Charaxes sangana*). Although not assessed in this work, fine-scale subspecific evolution in *Charaxes* has undoubtedly been promoted by climate fluctuations, in both the *bernardus* clade species in Sundaland, as well as *C. ocellatus* and *C. elwesi* in the Lesser Sunda Islands, where these islands were connected and fragmented repeatedly during the Pleistocene.

Although the broad-scale consensus in many biogeographical assessments is pronounced Pleistocene sea-level fluctuations promoting explosive diversification, much earlier significant sea level changes have been

recorded. Monk, De Fretes & Reksodiharjo-Lilley (1997) stated that Indonesia experienced several major falls in sea level during the late Oligocene and Pliocene. Of these lowering sea levels, at least the last one in the Pleistocene Period was well known to connect islands of the Great Sundas with an extension to Southeast Asia, whereas most of the others appear to have let Borneo remain isolated resulting in high endemism on that island (Natus, 2005). Hutchison (1989) considered that the Sunda platform was for the largest part above sea level. Conversely, the same study suggested that, several times during the late Tertiary, the sea levels were much higher than present, which would ultimately have increased the separation of the Greater Sunda Islands, comprising Sundaland (Hutchison, 1992). Several studies have suggested that the well-documented Pleistocene sea-level fluctuations promoted vicariant speciation (Corbet, 1946; Buch, 1994), as opposed to host plant co-evolution (Maschwitz *et al.*, 1984; Farrell, 1998). However, as with our *Charaxes* dataset, more examples have recently arisen which imply that the Pliocene period was temporally more important than the Pleistocene (Klicka & Zink, 1997; Ward, 1999; Hewitt, 2000; Moritz *et al.*, 2000; Taberlet & Cheddadi, 2002; Gorog, Sinaga & Engstrom, 2004; Jiggins *et al.*, 2006) Indeed, the Pliocene was a time of peak diversification among African *Charaxes* (Aduse-Poku *et al.*, 2009).

DISPERSAL

Our DIVA analysis revealed several examples of dispersal in *Charaxes*, as both early and more recent divergences. One of the most important is the split of the Oriental monotypic *solon* clade from an otherwise African clade in the mid Miocene, approximately 17 Mya. This coincides remarkably well with the split of all other Indo-Pacific *Charaxes* from African stock. Such colonization in the east, from African ancestors, can be explained as a combination of dispersal and vicariance through fragmentation by climate dynamics. Interestingly, land connection between the Africa and Asia is considered to have formed at this time, during the mid Miocene (Willis & McElwain, 2002). It is therefore most likely that some descendants of the African *Charaxes* colonized Asia across the Arabian Peninsula, as also proposed for the nymphalid genus *Junonia* (Kodandaramaiah & Wahlberg, 2007). Contraction of tropical forest into isolated fragment subsequent to an intense cooler and drier climate in the mid and late Miocene perhaps caused a permanent isolation in the populations in Africa and Asia. Conditions between forests refugia in these continents might have been both harsh and persisted long enough to have created allopatric sister species. In

this regard, the divergence can be attributed to initial dispersal (colonization), followed by vicariance.

Colonization of Wallacea by *Charaxes* is likely to have been through either the Philippines or via dispersal from Sundaland. The Makassar Straits separated Borneo and Sulawesi as early as 42 Mya (mid Eocene), well before the origins of even ancestral Indo-Pacific *Charaxes*. Indeed, O[h]tsuka (1996) concluded that there is almost no influence of Sulawesi on the butterflies of Borneo. The presence of Pliocene 'Asian' stegodont fossils in Sulawesi is almost certainly attributed to dispersal because elephants have been shown to swim long distances (R. Hall, pers. comm.). Conversely, influx from the Philippines may have been either dispersal (via island hopping) or vicariant (when the fore-mentioned volcanic arcs were eroded, enlarging the sea barriers). On the basis of the total absence of species of freshwater fish in Sulawesi other than those likely to have been introduced by humans, Cranbrook (1981) concluded that there has been no direct, unbroken connection between Sulawesi and the principle landmass of the Sunda shelf.

Palawan represents a pivotal point between Sundaland and the Philippines and, in a biogeographic sense, has been interpreted in a number of ways. It is particularly interesting in our DIVA analysis because the *bernardus* clade, with the exception of *C. durnfordi* and *Charaxes psaphon*, are shown to have dispersed from a Palawan ancestor, owing to the phylogenetic position of the Palawan endemics *C. bupalus* and *C. plateni*. Such colonization by various organisms from Palawan has been recorded by other workers (Inger, 1954; Brown & Alcalá, 1955; Alcalá & Brown, 1998). Vicariance may be arguably more responsible for the connection between Palawan and the Philippines (possibly Mindoro), as suggested by our DIVA analysis of the *amycus* clade of Indo-Pacific *Charaxes*. The possibility that a land bridge (Hall, 1998) existed between northern Palawan and southern Mindoro is consistent with some geological evidence (Rangin, Stephan & Müller, 1985). Palawan is not at all the simple extension of north Borneo (or the Sunda Shelf) as the prevailing zoogeographical perspective would suggest (Widmann, 1998; Kennedy *et al.*, 2000). The presence of two endemic *Charaxes* (i.e. *C. bupalus* and *C. plateni*) attests to this.

Within Wallacea, almost all of the splitting events as revealed by our DIVA analysis indicate that vicariance has shaped very clear, largely allopatric patterns of diversification. Only one node was shown to be the result of dispersal, being the colonization of the Lesser Sundas by what is now *C. elwesi* from a common ancestor with *C. orilus* in Timor/Wetar, during the Pliocene at approximately 3.6 Mya. This speciation event coincides closely with the time that the Australian margin collided with the Banda Arc and the

generation of some of the more easterly islands of the Lesser Sundas close to Timor, where the distance would have been reduced to less than one hundred kilometres between the two biogeographic zones.

The only example of radiation away from Wallacea is that of *C. latona*, which occurs marginally within Wallacea (Sula Islands) and throughout northern Maluku and New Guinea, to the east. Indeed, *C. latona* is the only species in the genus that occurs to the east of Wallacea. Diversification above species level east of Wallacea has been inhibited by the lack of sea barriers, maintaining genetic flow between various populations of *C. latona*, as well as a lack of time for genetic drift to manifest between such populations. Probably, *C. latona* entered northern Maluku via Obi as this island was pushed east via strike-slip faulting along the Sorong Fault, from the late Miocene/early Pliocene to recent times. During the early stages of its migration, Obi was likely juxtaposed with Taliabu Island and other islands in the Sula group. Whether *C. latona* diverged from the Sulawesi endemic *Charaxes affinis* as a result of vicariance (the splitting of Obi from the 'Banda' microcontinent) or by dispersal along the narrow bridge between Sulawesi and the Sula Islands is unknown because *C. latona* is not monophyletic with respect to *C. affinis* in our analyses. *C. latona* then likely dispersed throughout New Guinea once land connections with Maluku were established. It is very unlikely that *C. latona* originated in New Guinea and spread westward into Wallacea to the Sula Islands. Some studies (Michaux, 1994; Moss & Wilson, 1998) have suggested that the motion of the Sula block acted as a raft, carrying New Guinea biota to Sulawesi. However, the Sula block was likely submerged during its mobilization from New Guinea. Indeed, the butterfly fauna of the Sula Islands is strictly of Sulawesi affinity and a biodiversity assessment at all altitudes of that island revealed only endemics that have relatives in the latter biogeographic zone (Müller, 2004).

CONCLUSIONS

Phylogenetic relationships within Indo-Pacific *Charaxes* recognize eight distinct lineages that show significant concordance between morphology and genotype. Biogeographical analysis of the genus has provided an insight into the history of the region and the processes that have shaped the evolution of these groups.

After the genus originated in Africa, patterns of radiation and diversification may be directly correlated with inferred tectonic and climatic events that spanned the late Tertiary. During the early Miocene, approximately 17 Mya, both the *solon* clade and the ancestor of all remaining Indo-Pacific *Charaxes* separated from their African ancestors, likely the *jahlusa*

and *candiope* clades, respectively. Such colonization was probably through dispersal via the Arabian Peninsula and was terminated as a result of vicariance following the fragmentation of suitable forested habitat.

The earliest lineage among Indo-Pacific *Charaxes* (excepting *C. solon*) appears to have diverged subsequent to the initial fragmentation of the 'Banda' micro-continent during the mid Miocene, approximately 13 Mya, as a result of complex back-arc spreading in the Flores Basin and later the Banda Sea Basins. Shortly afterwards, at approximately 12.5 Mya, the large *bernardus* clade separated from the remainder of Indo-Pacific *Charaxes*. Diversification in the *bernardus* group was predominantly controlled by climatic changes during the Pliocene and possibly as recently as the Pleistocene, particularly affecting lineages in Sundaland (Greater Sunda Islands) as well as those separated by the Isthmus of Kra. The peak of divergence among Indo-Pacific *Charaxes* appears to be of mid Miocene to late Pliocene age, from approximately 3–13 Mya. Speciation events between Wallacean taxa are notably older than those between species in the Oriental region and the Philippines, indicating extended isolation of *Charaxes* lineages in Wallacea.

As corroborated by the high levels of local endemism in the genus, inter-island dispersal has been a relatively infrequent, stochastic event. Conversely, vicariance, through geological and climatic processes, has been largely responsible for the diversification of *Charaxes* in the Indo-Pacific, most notably in Wallacea. Sea separating the aerial fragments acted as a powerful barrier to dispersal and is ever-present today. For these reasons, there is no overlapping of the clades between either Wallacea (*mars*, *nitebis*, *elwesi*, *eurialus*, and *latona* clades), the Philippines (*amycus* clade) or the Oriental region (*bernardus* and *harmodius* clades), with the exception of the widespread *C. solon* and partial overlap of the *bernardus* and *elwesi* clades on the island of Bali.

Wallacea remains a diversity hotspot and its conservation is of paramount importance. Patterns and theories elucidated from this study of Indo-Pacific *Charaxes* agree with our analysis of *Cethosia* and are models for future Indo-Pacific biogeographic studies.

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APPENDIX

Table A1. Exemplar taxa used in the present study, with collection localities and GenBank accession numbers

Tribe	Taxon name	Author	Voucher Code	Locality	COI	ND5	Wingless
<i>Outgroup</i>							
Calimaginae	<i>Calinoga buddha</i> ssp.	Moore, 1857	NW64-3	UK: Stratford Butterfly Farm	AY090208	GU585289	AY090141
Charaxinae	<i>Anaea troglodyta astina</i>	(Fabricius, 1793)	NW152-18	DOMINICAN REPUBLIC: Pedernales	GQ256760	GU585291	GQ2566650
	<i>Archaeoprepona demophon</i> ssp.	(Linnaeus, 1758)	NW81-9	UK: Stratford Butterfly Farm	AY090220	GU585290	AY090153
	<i>Charaxes bipunctatus</i> <i>bipunctatus</i>	Rothschild, 1894	KAP-222	GHANA: Bobiri	GQ256780	GU585331	GQ256667
	<i>Charaxes boueti boueti</i>	Feisthamel, 1850	KAP-050	GHANA: Bobiri	GQ256786	GU585332	GQ256672
	<i>Charaxes castor</i> ssp.	(Cramer, [1775])	NW78-3	UK: Stratford Butterfly Farm	AY090219	–	AY090152
	<i>Charaxes cynthia cynthia</i>	Butler, 1866	NW107-11	UGANDA	GQ256794	GU585333	GQ256678
	<i>Charaxes etesipe etesipe</i>	(Godart, 1824)	NW164-2	UGANDA: Kibale Forest	GQ256800	GU585334	GQ256684
	<i>Charaxes eupale latimargo</i>	Joicey & Talbot, 1921	NW164-3	UGANDA: Kibale Forest	GQ256807	GU585335	GQ256690
	<i>Charaxes jaisius jaisius</i>	(Linnaeus, 1767)	NW147-3	ITALY: Canneto	GQ256824	GU585327	GQ256703
	<i>Charaxes nichetes</i> <i>pantherinus</i>	Rousseau-Decelle, 1934	NW114-14	ZAMBIA: Lesombo River	GQ256842	GU585336	GQ256718
	<i>Charaxes porthos dummeri</i>	Joicey & Talbot, 1922	NW118-11	UGANDA: Kibale Forest	GQ256858	GU585337	GQ256733
	<i>Charaxes protoclea protoclea</i>	Feisthamel, 1850	KAP-251	GHANA: Bia	GQ256860	GU585338	GQ256735
	<i>Charaxes zingha</i>	(Stoll, 1780)	NW133-1	GHANA: Boi-Tano	GQ256878	GU585339	GQ256751
	<i>Euxanthe eurinome eurinome</i>	(Cramer, [1775])	NW131-10	GHANA: Ankasa	EU141357	GU585292	EU141238
	<i>Polyura moori chalezias</i>	Fruhstorfer, 1914	NW121-24	INDONESIA: Bali	EU528325	GU585293	EU528282
	<i>Prothoe franck borneensis</i>	Fruhstorfer, 1913	CJM-187-001	INDONESIA: Long Laai, Kalimantan	GU585249	GU585301	GU585340
<i>Ingroup</i>							
Charaxinae	<i>Charaxes affinis affinis</i>	Butler, 1865	CJM-169-003	INDONESIA: Toraja, central Sulawesi	GU585259	–	–
	<i>Charaxes affinis butongensis</i>	Tsukada, 1991	CJM-170-005	INDONESIA: Buton Is.	GU585278	GU585304	GU585359
	<i>Charaxes amycus amycus</i>	C. & R. Felder, 1861	CJM-175-002	PHILIPPINES: Mindoro	GU585279	GU585306	–
	<i>Charaxes antoniuss</i>	Hanafusa, 1985	CJM-179-002	PHILIPPINES: Leyte Is.	GU585265	GU585296	GU585348
	<i>Charaxes aristogiton</i> <i>periodoneus</i>	Fruhstorfer, 1914	CJM-205-003	THAILAND: Chiangmai	GU585252	GU585324	GU585357

<i>Charaxes bajula bajula</i>	Staudinger, 1889	CJM-201-001	PHILIPPINES: Palawan	GU585251	-	GU585361
<i>Charaxes bajula basilisae</i>	Schröder & Treadaway, 1982	CJM-210-003	PHILIPPINES: Panay Is.	GU585256	GU585322	GU585358
<i>Charaxes bernardus ajax</i>	Fawcett, 1897	CJM-208-001	INDONESIA: Sumatra	GU585250	-	-
<i>Charaxes bernardus baya</i>	Moore, 1857	CJM-198-020	INDONESIA: Bali Is.	GU585253	GU585316	GU585345
<i>Charaxes bernardus bernardus</i>	(Fabricius, 1793)	NW134-12	VIETNAM: Cuc Phuong	GQ256779	GU585330	GQ256666
<i>Charaxes bernardus hemana</i>	Butler, 1870	NW134-10	BANGLADESH	GQ256777	GU585328	GQ256664
<i>Charaxes bernardus hemana</i>	Butler, 1870	NW134-11	BANGLADESH	GQ256778	GU585329	GQ256665
<i>Charaxes bernardus</i> ssp.	Undescribed	CJM-200-014	INDONESIA: Sengayan, Kalimantan	GU585273	GU585312	-
<i>Charaxes bernardus</i> ssp.	Undescribed	CJM-203-001	INDONESIA: Sengayan, Kalimantan	-	GU585317	-
<i>Charaxes bernardus</i> ssp.	Undescribed	CJM-190-013	INDONESIA: Gupak, Kalimantan	GU585274	GU585320	GU585349
<i>Charaxes bernardus</i> ssp.	Undescribed	CJM-193-003	INDONESIA: Sengayan, Kalimantan	-	GU585323	GU585364
<i>Charaxes borneensis</i>	Butler, 1869	CJM-195-003	INDONESIA: Sengayan, Kalimantan	GU585254	GU585318	GU585342
<i>Charaxes bupalus</i>	Staudinger 1889	CJM-188-001	PHILIPPINES: Palawan	GU585280	GU585314	GU585347
<i>Charaxes distantii thespius</i>	Fruhstorfer, 1914	CJM-186-001	INDONESIA: Long Laai, Kalimantan	GU585267	GU585294	-
<i>Charaxes durnfordi staudingeri</i>	Rothschild, 1894	CJM-212-001	INDONESIA: West Java	GU585281	GU585319	GU585343
<i>Charaxes elwesi pugnax</i>	Tsukada & Nishiyama, 1979	CJM-189-001	INDONESIA: Sumba is.	GU585282	-	-
<i>Charaxes euriatalus euriatalus</i>	(Cramer, [1775])	CJM-196-001	INDONESIA: Seram Is.	GU585277	GU585307	-
<i>Charaxes fervens igneus</i>	Tsukada, 1991	CJM-206-002	INDONESIA: Simeulue Is.	GU585255	GU585321	GU585350
<i>Charaxes harmodius harpagon</i>	Staudinger, 1885	CJM-194-001	PHILIPPINES: Palawan	GU585283	GU585295	GU585344
<i>Charaxes kahruha</i>	(Moore, [1895])	CJM-185-001	THAILAND: Chiangmai	GU585272	-	-
<i>Charaxes latona artemis</i>	Rothschild, 1900	CJM-167-003	INDONESIA: Taliabu Is.	-	GU585302	-
<i>Charaxes latona latona</i>	Butler, 1865	CJM-178-002	INDONESIA: Batjan	-	GU585298	-
<i>Charaxes latona papuensis</i>	Butler, 1869	CJM-181-007	PAPUA NEW GUINEA: Watut River, Morobe Prov.	GU585285	-	-
<i>Charaxes madensis</i>	Rothschild, 1899	CJM-177-002	INDONESIA: Buru Is.	-	GU585308	GU585355

Table A1. Continued

Tribe	Taxon name	Author	Voucher Code	Locality	COI	ND5	Wingless
	<i>Charaxes marki</i>	Lane & Müller, 2006	CJM-148-001	EAST TIMOR, Maur Region	GU585263	GU585300	GU585341
	<i>Charaxes marmax philopator</i>	Fruhstorfer, 1914	CJM-197-004	THAILAND: Chiangmai	GU585257	GU585313	GU585360
	<i>Charaxes mars</i>	Staudinger, 1885	CJM-199-004	INDONESIA: Seryong, central Sulawesi	GU585264	GU585297	GU585356
	<i>Charaxes musashi</i>	Tsukada, 1991	CJM-192-001	INDONESIA: Seryong, central Sulawesi	GU585284	GU585305	GU585353
	<i>Charaxes nitebis nitebis</i>	Hewitson, 1862	CJM-207-004	INDONESIA: Luwuk, Sulawesi	GU585288	–	–
	<i>Charaxes nitebis sulaensis</i>	Rothschild, 1900	CJM-191-007	INDONESIA: Peleng Is.	GU585287	–	GU585366
	<i>Charaxes ocellatus sumbanus</i>	Rothschild, 1896	CJM-171-001	INDONESIA: Sumba Is.	GU585261	GU585325	GU585351
	<i>Charaxes orilus orilus</i>	Butler, 1869	CJM-173-001	INDONESIA: Kapan, West Timor	GU585262	GU585299	GU585352
	<i>Charaxes plateni</i>	Staudinger, 1889	CJM-209-002	PHILIPPINES: Palawan	GU585258	GU585315	GU585346
	<i>Charaxes psaphon</i>	Westwood, 1847	CJM-204-002	SRI LANKA	GU585286	–	–
	<i>Charaxes repetitus</i>	Butler, 1896	CJM-172-003	INDONESIA: Sengayan, Kalimantan	GU585276	–	–
	<i>Charaxes repetitus</i>	Butler, 1896	CJM-183-007	INDONESIA: Sengayan, Kalimantan	GU585275	–	GU585363
	<i>Charaxes sangana sangana</i>	Schroder & Treadaway, 1988	CJM-202-001	PHILIPPINES: Sanga Sanga Is.	GU585266	GU585326	–
	<i>Charaxes setan</i>	Detani, 1983	CJM-166-001	INDONESIA: Peleng Is.	GU585260	GU585303	GU585354
	<i>Charaxes solon cunctator</i>	Fruhstorfer, 1914	CJM-174-001	THAILAND: Chiangmai	GU585270	GU585309	GU585362
	<i>Charaxes solon hannibal</i>	Butler, 1869	CJM-184-005	INDONESIA: Salawatang, Sulawesi	GU585271	–	GU585365
	<i>Charaxes solon lampedo</i>	Hübner, 1816	CJM-213-003	PHILIPPINES: Negros	GU585269	GU585311	–
	<i>Charaxes solon lampedo</i>	Hübner, 1816	CJM-214-002	PHILIPPINES: Palawan	GU585268	GU585310	–
	<i>Charaxes solon sulphureus</i>	Rothschild & Jordan, 1898	NW134-13	BANGLADESH	CQ256866	–	CQ256739

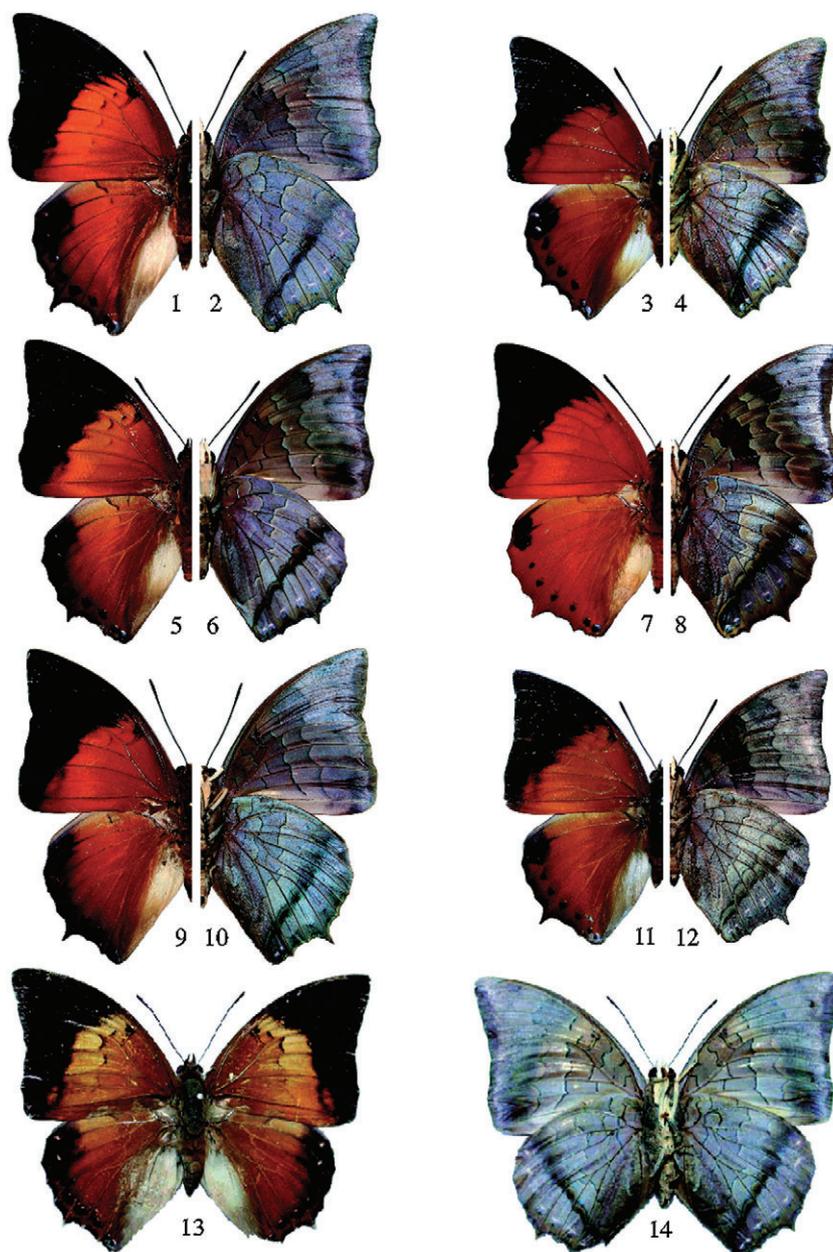
Table A2. Indo-Pacific *Charaxes* species groups and composition

Species group/ clade	Taxon
African/ <i>solon</i>	<i>C. solon</i> (Fabricius, 1793)
<i>elwesi</i>	<i>elwesi</i> Joicey & Talbot, 1922
<i>mars</i>	<i>marki</i> Lane & Müller, 2006 <i>mars</i> Staudinger, 1885 <i>ocellatus</i> Fruhstorfer, 1896 <i>orilus</i> Butler, 1869
<i>nitebis</i>	<i>nitebis</i> Hewitson, 1862
<i>harmodius</i>	<i>distanti</i> Honrath, 1885 <i>harmodius</i> C. & R. Felder, 1867
<i>amycus</i>	<i>amycus</i> C. & R. Felder, 1861 <i>antonius</i> Semper, 1878 <i>sangana</i> Schroder & Treadaway, 1988
<i>eurialus</i>	<i>eurialus</i> (Cramer, [1775]) <i>madensis</i> Rothschild, 1899
<i>latona</i>	<i>affinis</i> Butler, 1865 <i>latona</i> Butler, 1865 <i>musashi</i> Tsukada, 1991 <i>setan</i> Detani, 1983
<i>bernardus</i>	<i>aristogiton</i> C. & R. Felder, 1867 <i>bajula</i> Staudinger, 1889 <i>bernardus</i> (Fabricius, 1793) <i>borneensis</i> Butler, 1869 <i>bupalus</i> Staudinger 1889 <i>durnfordi</i> Distant, 1884 <i>fervens</i> Butler, 1889 <i>kahruba</i> (Moore, [1895]) <i>marmax</i> Westwood, 1847 <i>plateni</i> Staudinger, 1889 <i>psaphon</i> Westwood, 1847 <i>repetitus</i> Butler, 1896

Appendix A3.

CHARAXES REPETITUS (BUTLER), 1896 STAT. REV.

(FIG. A1, 1; A1, 2; A1, 5; A1, 6; A1, 9; A1, 10; A1, 13; A1, 14)



Charaxes repetitus Butler, 1896. *Journal of the Linnean Society of London (Zoology)*, **25**, 392.

Charaxes bernardus repetitus Butler – d’Abrera, 1985: 398; Otsuka, 1988: plate 75.

Type locality. Sarawak, East Malaysia (Borneo): Holotype male Figs A1, 13 & 14).

Other material examined. 33 males, labelled INDONESIA, approximately 65 km south Malinau, north-east Kalimantan, July, 2000, July-Aug, 2003.

Diagnosis. Larger than *C. bernardus*: (Figs A1: 3, 4, 7, 8, 11, 12) (FW length 43–47 mm, $N = 33$), dark apical region is broader, underside is unicolourous by comparison to *bernardus*. A paler median band is present on the fore wing upperside, and the overall ground colour above is very tawny brown and not orange as in *C. bernardus*. The inner margin of the hindwing upperside is cream-grey, unlike *bernardus* which is orange. Wing shape is consistent, while that of *bernardus* is variable.

Appendix A3. *Continued*

Charaxes repetitus differs from *C. bernardus*, as well as *C. borneensis*, from changes of several bases in *COI* (approximately 1.5% divergence).

Distribution. Known only from Borneo.

Remarks. Butler, in the original description, considered *C. repetitus* to be the Bornean representative of *C. polyxena*. The type was not illustrated by Butler and seemingly has never been figured, such that no correlation has been made in recognizing the two sympatric species. In fact, both *C. repetitus* and *C. bernardus* fly together in Borneo, where the former appears to be very localized. The subspecies of *C. bernardus* in Borneo is rendered undescribed.

Charaxes repetitus is a much larger, more robust insect than that of *C. bernardus*. Behaviourally, *C. repetitus* is distinct from *C. bernardus*. The former species does not appear to be attracted to bait (carrion, dung, urine), unlike *C. bernardus*, which is readily lured. Additionally, nearly all known specimens are from open hill-tops. *C. bernardus* may frequent the same hill-tops, but is more commonly observed on hill-tops with a partially closed canopy.

A distinct difference was noted in the appearance times of the two taxa on hill tops in Borneo. *Charaxes bernardus* was first to appear at approximately 10.30 h and leaving around 13.30 h. *Charaxes repetitus* would appear shortly afterwards, around 14.00 h and persist until 15.30 h, whereas *C. borneensis* would overlap partially with *C. repetitus*, flying later into the afternoon. This difference in timing has been recorded in hill-topping behaviour in closely-related sympatric African *Charaxes* by Henning (1989), who noted that males of *Charaxes legeri* Plantrou arrive between 09.00 and 09.40 h and leave between 10.10 and 10.50 h, whereas *C. jasius epijasius* (Reiche) (arrived between 11.25 and 13.30 h and departs at approximately 15.00 h). This behaviour is not unique to *Charaxes* and fine scale non-overlapping late afternoon flight durations were noted in species of sympatric green *Arhopala* Boisduval species of the *aurea* species group (*Arhopala trogon* (Distant), *Arhopala caeca* (Hewitson), *Arhopala borneensis* Bethune-Baker) also on mountain tops in Borneo (*C. Müller*, pers. observ.). This behaviour is presumed to maintain isolation between species.

Table A4. Unrestricted ancestral areas for nodes, as a result of the dispersal-vicariance analysis

Node 1	DG EG DEG FG DFG EFG DEFG
Node 2	CIJ CDIJ CEIJ CDEIJ CGIJ CDGIJ CEGIJ CDEGIJ CHIJ CDHIJ CEHIJ CDEHIJ CGHIJ CDGHIJ CEGHIJ CDEGHIJ CIJL CDIJL CEIJL CDEIJL CGIJL CDGIJL CEGIJL CDEGIJL CHIJL CDHIJL CEHIJL CDEHIJL CGHIJL CDGHIJL CEGHIJL CDEGHIJL
Node 3	CFHIJK CDFHIJK CEFHIJK CDEFHIJK CFGHIJK CDFHIJK CEFGHIJK CDEFGHIJK CFHIJKL CDFHIJKL CEFHIJKL CDEFHIJKL CFGHIJKL CDFGHIJKL CEFGHIJKL CDEFGHIJKL
Node 4	ACFHIJK ACFHIJK ACEFHIJK ACDEFHIJK ACFGHIJK ACFGHIJK ACEFGHIJK ACDEFGHIJK ACFHIJKL ACFHIJKL ACEFHIJKL ACDEFHIJKL ACFGHIJKL ACFGHIJKL ACEFGHIJKL ACDEFGHIJKL
Node 5	ACFHIJK ACFHIJK ACEFHIJK ACDEFHIJK ACFGHIJK ACFGHIJK ACEFGHIJK ACDEFGHIJK ACFHIJKL ACFHIJKL ACEFHIJKL ACDEFHIJKL ACFGHIJKL ACFGHIJKL ACEFGHIJKL ACDEFGHIJKL
Node 6	AC AD ACD AE ACE ADE ACDE AF ACF ADF ACDF AEF ACEF ADEF ACDEF AG ACG ADG ACDG AEG ACEG ADEG ACDEG AFG ACFG ADFG ACDFG ACFG ACEFG ADEFG ACDEFG AH ACH ADH ACDH AEH ACEH ADEH ACDEH AFH ACFH ADFH ACDFH AEFH ACEFH ADEFH ACDEFH AGH ACGH ADGH ACDGH AEGH ACEGH ADEGH ACDEGH AFGH ACFGH ADFGH ACDFGH ACFGH ACEFGH ADEFGH ACDEFGH
Node 7	ACFHIJK ACFHIJK ACEFHIJK ACDEFHIJK ACFGHIJK ACFGHIJK ACEFGHIJK ACDEFGHIJK ACFHIJKL ACFHIJKL ACEFHIJKL ACDEFHIJKL ACFGHIJKL ACFGHIJKL ACEFGHIJKL ACDEFGHIJKL
Node 8	ACEFHIJK ACEFHIJK ACFGHIJK ACFGHIJK ACEFGHIJK ACEFGHIJK ACEFHIJKL ACEFHIJKL ACFGHIJKL ACFGHIJKL ACEFGHIJKL ACEFGHIJKL
Node 9	ACEFGHIJKL ABCEFGHIJK ACDEFGHIJK ABCDEFHIJK ACEFGHIJKL ABCEFGHIJKL ACDEFGHIJKL ABCDEFHIJKL
Node 10	ABCEFGHIJK ABCDEFHIJK ABCEFGHIJKL ABCDEFHIJKL
Node 11	ABCDEFHIJK ABCDEFHIJKL
