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When Thailand was an island – the phylogeny and biogeography of mite harvestmen (Opiliones, Cyphophthalmi, Stylocellidae) in Southeast Asia

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ABSTRACT

Aim To develop a comprehensive explanation for the biological diversity of Southeast Asia, especially in the Wallacea and Sundaland regions. This study focuses on a group of arachnids, mite harvestmen, which are thought to be an extremely old group of endemic animals that have been present in the region since most of its land supposedly formed part of the northern rim of the supercontinent Gondwana.

Location Eastern Himalayas, Thai-Malay Peninsula, Sumatra, Borneo, Java, Sulawesi, and New Guinea.

Methods Approximately 5.6 kb of sequence data were obtained from 110 Southeast Asian Cyphophthalmi specimens. Phylogenetic analyses were conducted under a variety of methods and analytical parameters, and the optimal tree was dated using calibration points derived from fossil data. Event based and paralogy-free subtree biogeographical analyses were conducted.

Results The Southeast Asian family Stylocellidae was recovered as monophyletic, arising on what is now the Thai-Malay Peninsula and diversifying into three main clades. One clade (*Meghalaya*, here formally placed in Stylocellidae) expanded north as far as the eastern Himalayas, a second clade entered Borneo and later expanded back across the Sundaland Peninsula to Sumatra, and a third clade expanded out of Borneo into the entire lower part of Sundaland. Molecular dating suggested that Stylocellidae separated from other Cyphophthalmi 295 Ma and began diversifying 258 Ma, and the lineage that inhabits mostly Borneo today began diversifying between 175 and 150 Ma.

Main conclusions The topology and molecular dating of our phylogenetic hypothesis suggest that Stylocellidae originated on Gondwana, arrived in Southeast Asia via the Cimmerian palaeocontinent, and subsequently diversified north, then south. Their present distribution in the Indo-Malay Archipelago is explained largely by a diversification over the Sundaland Peninsula before western Sulawesi departed and the peninsula was extensively inundated.

Keywords

Arachnida, Arthropoda, Borneo, Cyphophthalmi, Opiliones, Southeast Asia, Sulawesi, Sundaland, vicariance biogeography, Wallacea.

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INTRODUCTION

Evidence that Southeast Asia is composed of Eurasian and Indo-Australian landmasses was found very early by biologists, who noticed striking transitions in its biota. Even before the explorations of Alfred Russel Wallace (Wallace, 1890), it was known that a major biotic break was located in the Malay

Archipelago. Salomon Müller described the limits of Oriental and Australian biotic incursions into the islands (Müller, 1846; Simpson, 1977), and Sclater (1858) drew the south-eastern limit of his 'Regio Indica' at Borneo and Java, not Sulawesi (George, 1987). However, Wallace (1859) positioned the break more precisely and recognized that there was no simple explanation at the time for its unintuitive location. Additional

biotic breaks, such as Huxley's (1868) and Lydekker's (1896), were designated, summarized (Scrivenor *et al.*, 1943) and debated (Mayr, 1944), but after the development of plate tectonics they lost much of their appeal (Simpson, 1977). Nonetheless, they remain in use as a framework for understanding biogeographical patterns in the region (e.g. Atkins *et al.*, 2001; Kitching *et al.*, 2001; Brown & Guttman, 2002; Balke & Ribera, 2004), and Southeast Asia is now seen as a place where poor dispersers from the approaching Australian plate moved as far north as Sulawesi and Lombok, and those from Eurasia dispersed south-east to Sulawesi and Bali (George, 1981). Good dispersers from Eurasia, such as most plants and insects, dominate the entire region and extend out to Pacific islands (Gressitt, 1982; Baker *et al.*, 1998).

Despite Southeast Asia's abundance of organisms and islands, however, finding a repeated signal of geological events beyond the encroachment of the Indo-Australian plate has been difficult (Holloway, 2003). A hierarchy of Southeast Asian landmass associations, expressed as a single area cladogram, would be a more intriguing pattern to extrapolate and explore. Just such a hypothesis was suggested by Wallace (1863) and used as a theoretical model by Nelson & Platnick (1981). Unfortunately, a convincing area cladogram for the region has been elusive, notwithstanding proposals for certain taxa (e.g. Scott, 1997; Ridder-Numan, 1998; Brown *et al.*, 2006a; Sharma & Giribet, 2009) and combined analyses of multiple studies (van Welzen *et al.*, 2003). This is not to say that Southeast Asia holds little promise for biogeographers, for studies of the region regularly identify areas of endemism (Evans *et al.*, 2003), ancient dispersal routes (Abegg & Thierry, 2002), past ecological conditions (Quek *et al.*, 2007; Outlaw & Voelker, 2008) and patterns of taxonomic distributions and species richness (Kitching *et al.*, 2001; Nijman & Meijaard, 2008).

Improved understanding of Southeast Asia's physical history (Hall, 2001, 2002; Metcalfe, 2001) suggests that an area cladogram for the region is actually unrecoverable, for moving plates and fluctuating sea levels have resulted in ephemeral terrestrial connections between landmasses used by biotas originating and diversifying at different times. Indeed, what today appears to be an archipelago was, as recently as 9500 years ago, a single large peninsula, 'Sundaland', that incorporated the Thai-Malay Peninsula, Sumatra, Java and Borneo. Going back even further, but just to the beginning of the Cenozoic, western Sulawesi was included as well. Sundaland's complicated Cenozoic history has already been invoked as causing a lack of structure and resolution in phylogenetic and phylogeographical studies (Ruedi & Fumagalli, 1996; Maekawa *et al.*, 2001; Cannon & Manos, 2003; Gorog *et al.*, 2004), or at least being the explanation for close relationships among lineages on different islands (Brown *et al.*, 2006b; de Bruyn & Mather, 2007; Shih *et al.*, 2009).

For poor dispersers especially, the presence of particular lineages on any of the Indo-Malay islands depends greatly on Sundaland's contours when their ancestor arrived. Birds, mammals and other Eurasian Cenozoic species that resist crossing large bodies of water are all predictably rare on

Sulawesi, which rifted from the southernmost end of Sundaland before their arrival (Metcalfe, 2001; Hall, 2002; Morley, 2003). However, it is unsurprising that the same groups show multiple, lineage-specific patterns of colonization on Sumatra, Java and Borneo, all of which had changing connections to each other and the peninsula during the Cenozoic (Hall, 2001).

Nonetheless, even if we consider Java, Borneo, Sumatra and (for older biota) Sulawesi more tightly linked than their present appearance suggests, their collective relationship to the Thai-Malay Peninsula and its relationship to Eurasia is punctuated by at least two major events that should show a signature in very old biotic distributions. First is the arrival of south-western Borneo (which rifted from north-western Australia during the Jurassic) at the tip of Sundaland during the Cretaceous (145–80 Ma) (Metcalfe, 2009). At that time, species on the south-eastern end of the composite Sumatra–Thai-Malay Peninsula could enter proto-Borneo but nowhere else: Java did not yet exist, western Sulawesi was both submerged and not yet accreted with the peninsula, and the North Palawan terrane was also submerged. Borneo's size, topography, palaeoclimate and position between the Philippines and the Thai-Malay Peninsula have allowed it to greatly influence the region's biota (Moss & Wilson, 1998; Brandon-Jones, 2001; Beck & Khen, 2007), and perhaps the signature of its very appearance can be found in certain groups.

The second event that may be evident in old taxonomic groups is the rifting of microcontinents from the coast of Gondwana 350–140 Ma. Southeast Asia's topology is especially influenced by an early Permian rifting that generated a long palaeocontinent, Cimmeria, which moved north for 35 Myr and accreted with Eurasia by the Triassic (Metcalfe, 2002). Plant fossils suggest that Cimmeria was not completely submerged (van Waveren *et al.*, 2007), and the terrane became what is today the Thai-Malay Peninsula. Most Southeast Asian taxa are not old enough to have come to Asia from early Gondwanan microcontinents, but possible hypotheses for the origin of Southeast Asian *Vireya* rhododendrons (Brown *et al.*, 2006a), pachychilid freshwater land snails (Glaubrecht & von Rintelen, 2003; Kohler & Glaubrecht, 2007) and caddisflies in the genus *Apsilochorema* (Mey, 1998) include rafting on at least the terranes that broke away from Gondwana in the Mesozoic.

Detecting such ancient events in an extant group of organisms is hindered by finding one that is both old and unlikely to disperse, especially over water, but successful and mobile enough to be species rich and widespread; such a group is the Cyphophthalmi, a suborder of Opiliones (daddy-long-legs or harvestmen) that has been shown to be an excellent model for Gondwanan and even global vicariance (Boyer & Giribet, 2007; Boyer *et al.*, 2007). Its Southeast Asian family, Stylocellidae, has numerous species on every major landmass of continental origin or connection (Shear, 1993), and they have already been shown to form clades that suggest a peninsular origin and early diversification on Borneo (Schwendinger & Giribet, 2005; Boyer *et al.*, 2007; Clouse & Giribet, 2007). It is a diverse family ripe for a taxonomic revision, with only 34 of its scores of species described and its generic-level organization antiquated.

Stylocellidae has been divided into four genera. The eyeless peninsular genus *Fangensis* has received focused attention – its diversity, possible paraphyly and placement as sister to the rest of the family being first hypothesized by Schwendinger & Giribet (2005) and later supported by Clouse & Giribet (2007) and Boyer *et al.* (2007). At the other extreme is a second eyeless genus, *Miopsalis*, which is both poorly understood and has little chance of remaining a valid name in the future: erected for a single, tiny species, the type of which we have not been able to inspect (Giribet, 2000), the name has been used recently only as an informal moniker for miniaturized species (Clouse & Giribet, 2007). However, it has become increasingly clear that miniaturization happens in various lineages and is associated with a reduction of eye lenses (Clouse & Giribet, 2007). *Stylocellus* is currently the default genus for moderately sized species with prominent eye lenses, but their widely diverse morphologies shed doubt on the coherence of the genus. Fourth, *Leptopsalis* is currently in synonymy with *Stylocellus* but may prove useful, as its single species (*Leptopsalis beccarii*) is from the especially diverse Sumatran fauna.

The Indian genus *Meghalaya* is currently not placed in any family but has certain morphological similarities (e.g. prominent eye lenses) that could affiliate it with Stylocellidae (Giribet *et al.*, 2007). We include in this study *Meghalaya* specimens from north-east India, as well as the first Chinese Cyphophthalmi ever collected (by J. A. Miller and D. H. Kavanaugh and kindly lent by C. E. Griswold, all of the California Academy of Sciences) from nearby Yunnan Province. The Chinese specimens also resemble *Meghalaya* and appear to confirm the suspicions of Giribet *et al.* (2007) that the genus could be widespread in the eastern Himalayan region.

Stylocellid biogeography does have complications, with species from the Thai-Malay Peninsula being found in both early and derived lineages, at least three species on New Guinea (which, under our current understanding, would require transoceanic dispersal) (Clouse & Giribet, 2007) and the possible inclusion of the more distant eastern Himalayan species. Still, given their promise as a powerful biogeographical model group, we set out to construct the most comprehensive phylogeny of Stylocellidae to date and test whether it contains any signal of early events in Southeast Asia's geological history.

MATERIALS AND METHODS

Specimen collection

Almost all known museum specimens of Cyphophthalmi from Southeast Asia were assembled and augmented by recent collections (see Appendix S1 in the Supporting Information). Recently collected specimens appropriately preserved for DNA extraction were selected to maximize geographical and morphological coverage (Fig. 1). The only major landmass in the region for which we had no modern collections was the Philippine island of Palawan, known to have at least one cyphophthalmid species (*Stylocellus tarumpitao*). To maintain

the biogeographical focus of this study, Southeast Asian terminals were not given taxonomic designations unless previously part of a systematic examination (Schwendinger & Giribet, 2005; Clouse & Giribet, 2007), and no new species-level names were assigned here.

DNA sequencing

Fragments of six markers were amplified and sequenced following previously described protocols (Giribet *et al.*, 2002; Boyer *et al.*, 2005; Schwendinger & Giribet, 2005; Clouse & Giribet, 2007; Sharma & Giribet, 2009): 16S rRNA (c. 470 bp), 18S rRNA (c. 1760 bp), 28S rRNA (c. 2100 bp), cytochrome *c* oxidase subunit I (COI: c. 814 bp), histone H3 (327 bp) and histone H4 (160 bp). Fragment lengths varied for ribosomal markers and, among some of the outgroups, for COI; the total amount of sequence data analysed was approximately (unaligned) 5.6 kb per complete taxon. We used primers described in previous studies (Xiong & Kocher, 1991; Folmer *et al.*, 1994; Colgan *et al.*, 1998; Pineau *et al.*, 2005; Schwendinger & Giribet, 2005; Edgecombe & Giribet, 2006) and added two custom primers for 18S and 28S rRNA: respectively, 4FC (5'-CCAGCAGCCGCG[G]TAATTC-3') and 28SbC (5'-TCGGA[G]GGAACCAGCTAC-3') (novel base identities in brackets). Sequences were combined for multiple specimens of the same collection if found to be identical for shared variable regions. Length-variable sequences (i.e. all markers except histone H3 and histone H4) were divided into fragments along conserved regions. When more than half of a fragment's bases were not sequenced, the whole fragment was removed from the taxon in question and treated as missing data. Sequences new from this study have been deposited in GenBank under accession numbers GQ488025–GQ488465 (see Appendix S2).

Data descriptions

The percentage of complete fragments, with histone H3 and histone H4 each treated as single fragments, was computed for each terminal, and uncorrected *p*-distances between all terminals were calculated using PAUP* (Swofford, 2001). In order to explore variance in DNA quality we calculated a lag time between specimen collection and DNA extraction for each Southeast Asian terminal. It was expected that older collections would have more opportunities to experience harmful temperatures and preservatives (Vink *et al.*, 2005). For sequences formed by combining specimens from more than one collection, the shortest lag time was used.

Phylogenetic analysis

DNA sequences were analysed by direct optimization in POY 4, build 2885 (Varón *et al.*, 2008) under the parsimony criterion (Wheeler, 1996). Outgroups represented other cyphophthalmid families and opilionid suborders. Outgroup COI sequences were amplified using a more internal primer than

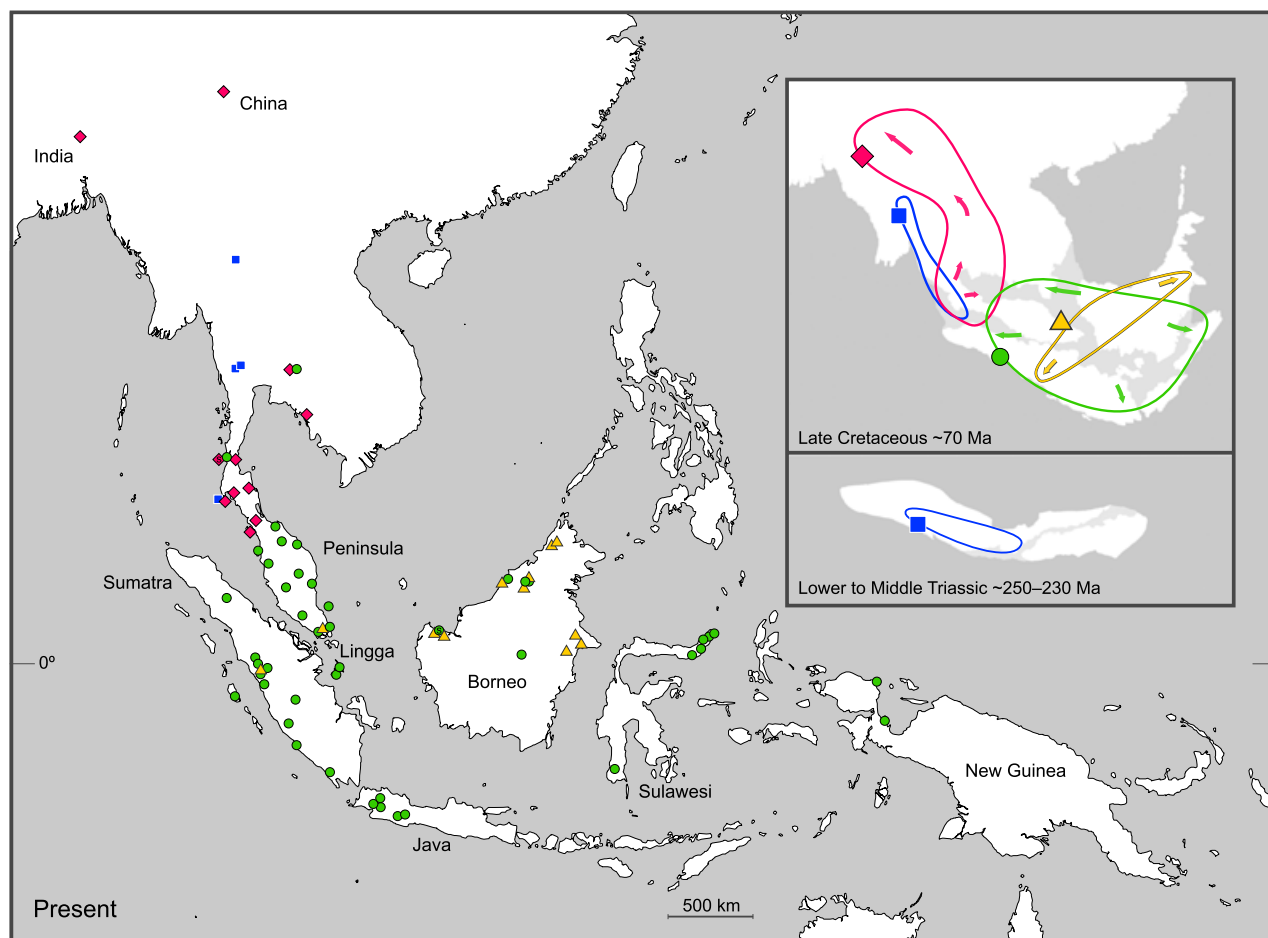


Figure 1 Collection localities for all Southeast Asian Cyphophthalmi species in the expanded terminal set: *Fangensis* (squares), clade A (diamonds), clade B (triangles) and clade C (circles). For clades A and C, points containing the letter 'S' are species found sister to the remaining members of the clade. The inset shows hypothetical range expansions for each clade on a reconstruction of Southeast Asia at the end of the Mesozoic around 70 Ma (top) and on the Sibumasu terrane in the Lower to Middle Triassic c. 250–230 Ma (bottom).

stylocellid sequences and were thus about 157 bases shorter (in addition to being naturally shorter by six bases in *Hesperonemastoma modestum*). Also, the only outgroups with histone H4 were *Cyphophthalmus teyrovskyi*, *Protolophus singularis* and *Suzukielus sauteri*.

Considering the power of 18S and 28S rRNA in recovering stylocellid monophyly and well-supported clades within the family (Schwendinger & Giribet, 2005; Clouse & Giribet, 2007), terminals were chosen for analysis if they had at least 50% of 18S rRNA and either (1) at least 50% of 28S rRNA or (2) any portion of 28S rRNA and another marker. One hundred and twenty-two terminals passed this criterion: six non-cyphophthalmid Opiliones; 21 Cyphophthalmi representing the families Pettalidae, Troglosironidae, Neogoveidae and Sironidae; and 95 Cyphophthalmi from Southeast Asia. An additional terminal set – ‘expanded’ – consisted of those with any portion of 28S rRNA, adding an additional 15 Southeast Asian species to the analysis. Terminals that were found to have similar DNA sequences, morphology and localities were given the same terminal name plus a letter designation (such as ‘Peninsula sp. 17a’ and ‘Peninsula sp. 17b’).

Tree searches were conducted using the automated search function in `poxy` [search (max_time:dd:hh:mm)], which will attempt as many builds (i.e. Wagner trees), swaps [i.e. tree bisection–reconnection (TBR) branch swapping], ratchets (see Nixon, 1999) and tree fusings (see Goloboff, 1999) within the specified time, using nine sets of transformation costs. Transformation cost sets had different ratios of indel-to-transversion costs and transversion-to-transition costs (see Wheeler, 1995). Searching was conducted in several rounds, lasting up to several days each, for each marker separately and all data combined. The shortest trees found from the different transformation costs in the previous round were read into the memory and used for fusing at the end of the new search. When searching failed to find trees that were shorter and/or different in topology from the previous round, searching was stopped for that partition. After the sixth and final round, tree scores were used in a modified incongruence length difference (ILD) test to choose the optimal parameter set (see Appendix S3). This was determined to be the set in which indels cost two, transversions cost two and transitions cost one (‘121’). A final search was conducted using the optimal transformation

cost set to check if iterative pass optimization [set(iterative:exact)] (Wheeler, 2003) would affect tree scores enough to change the optimal transformation cost set; it did not.

Support was calculated in *POY* using dynamic homology, a method where unaligned homologous fragments, not bases, are resampled for each pseudoreplicate. Five searches were carried out on each of 100 pseudoreplicates, each starting from one of five trees read into the memory – the optimal tree and four suboptimal trees found in earlier rounds of searching. Nodal stability was evaluated by searching for clades from the optimal tree in the shortest trees found using the other transformation cost sets; clade searching was done using the program *CLADESCAN* (Sanders, 2010).

To conduct a phylogenetic analysis under the maximum likelihood criterion, all data were aligned using the online version of the program *MAFFT* (Katoh *et al.*, 2002) under default conditions (gap-opening penalty 1.53, algorithm determined by the program depending on marker size). The resulting alignment was used in a maximum likelihood bootstrap search in *RAXML* (randomized ‘axelerated’ maximum likelihood) (Stamatakis *et al.*, 2008) available through the Cyberinfrastructure for Phylogenetic Research (CIPRES) project (<http://www.phylo.org/>). Data were partitioned between markers, the model of evolution was allowed to vary among partitions, and invariant sites were not allowed; all free parameters were determined by the program, including empirical base frequencies, and the general time-reversible (GTR) substitution model was used for each partition.

Tree searches were also conducted in *POY* for the expanded terminal set, finding the shortest trees under each transformation cost set and then fusing them under the optimal set determined earlier (121). Bootstrap support for this tree was also calculated in *POY* under dynamic homology: 50 pseudoreplicates were searched 10 times each, starting with 10 different suboptimal trees read into the memory.

Molecular dating

Nodes in the shortest tree found under parsimony using the 121 transformation cost set were dated using *r8s* 1.71 (Sanderson, 2003) and a calibration point based on a fossil member of the opilionid suborder Eupnoi from the early Devonian (400 Ma) (Dunlop *et al.*, 2004). Given this fossil and the fact that stem-group scorpions (the likely sister group of Opiliones; Giribet *et al.*, 2002) date from the mid-Silurian, 430 Ma (Dunlop, 2007), the root’s date was fixed at 425 Ma, and the non-cyphophthalmid Opiliones were given a minimum age of 400 Ma. The analysis was repeated with the root age fixed at 401, 450, 475 and 500 Ma to assess the sensitivity of descendant nodes to this date. The presence of short branches at the bases of key clades made a cross-validation of methods, penalty functions and algorithms impossible (because it would require pruning those clades), so, for consistency with other studies (Boyer *et al.*, 2007) and upon the recommendation of the developer (Sanderson, 2003), we used the penalized likelihood method, an additive penalty function,

and the truncated Newton algorithm. Cross-validation was used to find the appropriate smoothing function, 320.

Biogeographical analysis

Ancestral regions for each node in the phylogeny based on the normal terminal set were reconstructed using dispersal–vicariance analysis in *DIVA* 1.1 (Ronquist, 1996, 1997). This program uses a model for biogeographical reconstruction in which extinction and speciation by dispersal cost 1 and speciation resulting from vicariance and duplication cost 0. The endemic areas used were the large islands of the Indo-Malay Archipelago and the Thai-Malay Peninsula, as indicated by species monikers. The localities for India sp. 1 and China sp. 1 were treated as a single area, the eastern Himalayas; we considered it to be a more accurate description of the border area where they are found, and it is on a scale similar to other regions in this study.

We also conducted paralogy-free subtree analyses (Nelson & Ladiges, 1996) on the phylogenies based on the normal and expanded terminal sets. To identify subtrees we used the ‘transparent method’ of Ebach *et al.* (2005), where subtrees are constructed starting at the terminals and working down, recording only new areas and using only those subtrees with three or more terminals. The nodes of all subtrees (Table 1) were then coded for the presence (1) or absence (0) of each area (see Brown *et al.*, 2006a for an example). The resulting matrix was analysed under parsimony in *TNT* (Goloboff *et al.*, 2008), and a strict consensus was calculated when more than one most parsimonious tree was found.

RESULTS

The percentage of data fragments obtained for each Southeast Asian terminal ranged fairly evenly from 100% to 11% (Fig. 2a), with sequencing success diminishing with specimen age (Fig. 2b). Uncorrected *p*-distances between all terminals (including outgroups) for all data averaged 5% (standard deviation 4%) and ranged up to 21% (Fig. 2a, shown as

Table 1 Paralogy-free subtrees used to construct the area cladograms for the Southeast Asian Cyphophthalmi shown in Fig. 5 and their clades of origin in the phylogeny shown in Fig. 3.

Paralogy-free subtrees	Clade of origin
<i>Normal terminal set</i>	
(Lingga, (Java, (New Guinea, Sumatra)))	C2
(Sumatra, (Sulawesi, (Peninsula, Borneo)))	C5
(Peninsula, (Java, Sumatra))	C6
<i>Expanded terminal set</i>	
(Peninsula, (Borneo, Sumatra))	B1
(Java, (New Guinea, Sumatra))	C2
(Peninsula, (New Guinea, Sulawesi))	C3
(Sulawesi, (Peninsula, Borneo))	C5
(Peninsula, (Java, Sumatra))	C6

a percentage of the maximum, and Table 2). Within Cyphophthalmi, distances for all data also averaged 5% ($\pm 3\%$) and were as high as 13%. Among the Southeast Asian species, *p*-distances for all data averaged 3% ($\pm 2\%$) and reached a maximum of 10%. Among the Southeast Asian species, the two mitochondrial markers – COI at $20 \pm 3\%$, followed by 16S rRNA at $16 \pm 5\%$ – were the most variable. The nuclear markers varied as follows: histone H3 ($9 \pm 3\%$),

Table 2 Average uncorrected *p*-distances (bold) among different sets of terminals for markers separately and combined (All), \pm the standard deviation, followed by the maximum.

	All terminals	Cyphophthalmi	Southeast Asian
COI	0.26 \pm 0.06, 0.39	0.24 \pm 0.06, 0.39	0.20 \pm 0.03, 0.28
16S	0.26 \pm 0.09, 0.41	0.25 \pm 0.09, 0.40	0.16 \pm 0.05, 0.26
H3	0.14 \pm 0.04, 0.24	0.13 \pm 0.04, 0.22	0.09 \pm 0.03, 0.14
H4	0.06 \pm 0.03, 0.18	0.06 \pm 0.03, 0.18	0.06 \pm 0.03, 0.15
28S	0.06 \pm 0.04, 0.31	0.05 \pm 0.03, 0.15	0.03 \pm 0.02, 0.11
18S	0.01 \pm 0.01, 0.08	0.01 \pm 0.01, 0.03	0.004 \pm 0.003, 0.02
All	0.05 \pm 0.04, 0.21	0.05 \pm 0.03, 0.13	0.03 \pm 0.02, 0.10

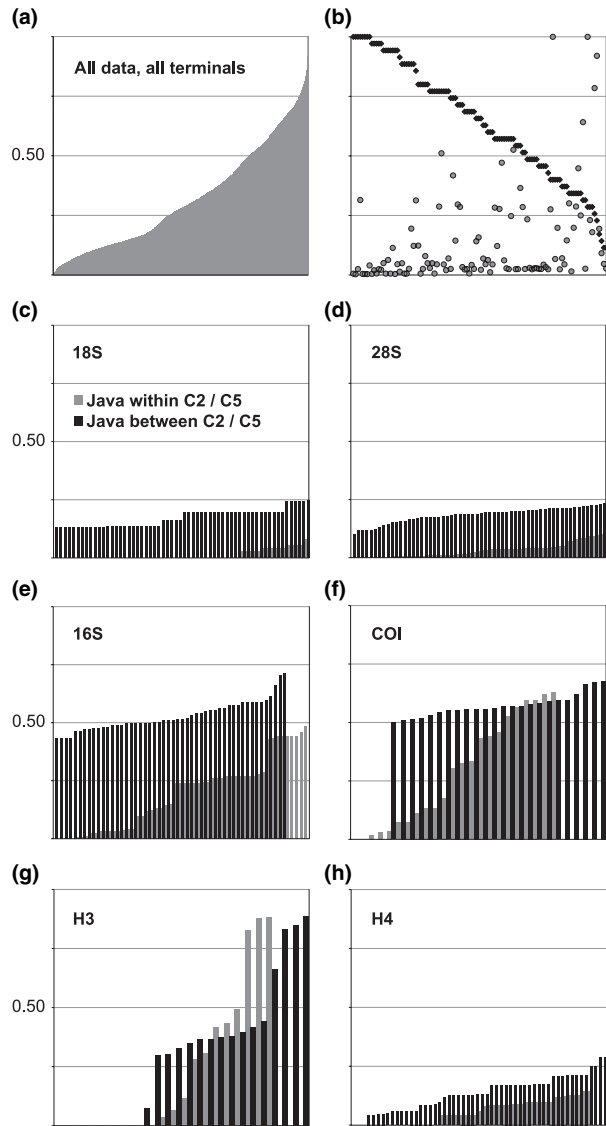


Figure 2 Characteristics of opilionid sequence data analysed. (a) Pairwise distances among all terminals (Southeast Asian Cyphophthalmi and all outgroups), for all markers combined, as a percentage of the maximum distance. (b) Southeast Asian Cyphophthalmi species versus the percentage of all data fragments obtained for each, arranged from most covered to least (black diamonds), with the minimum lag time between specimen collection and DNA extraction (as a fraction of the maximum, 17 years; grey circles). (c–h) Pairwise distances among Javanese species, as a percentage of the maximum for all Southeast Asian species, arranged from closest to most distant and compared within (grey bars) and between (black bars) clades C2 and C6.

histone H4 ($6 \pm 3\%$), 28S rRNA ($3 \pm 2\%$) and 18S rRNA ($0.4 \pm 0.3\%$).

Four trees, 23,987 steps long and differing only in the resolution of six species from northern Sulawesi, were found using the transformation cost set 121 (Fig. 3) in *POY*. The trees were otherwise resolved and recovered the following: the cyphophthalmid families Pettalidae, Troglisironidae and Neogoveidae as monophyletic; Sironidae as a paraphyletic grade between (Neogoveidae + Troglisironidae) and Stylocellidae; and a clade containing all specimens collected from India to New Guinea (Stylocellidae) (Fig. 4). Within Stylocellidae, the genus *Fangensis* was not monophyletic, with *F. leclerci*, *F. spelaeus* and *F. cavernarus* forming a monophyletic group sister to a clade consisting of the more southern *F. insulanus* and the remaining stylocellids. The non-*Fangensis* stylocellids fell into three main clades: a clade consisting of species from the Thai-Malay Peninsula and the eastern Himalayas (Fig. 3, clade A), which probably corresponds to the as yet monotypic *Meghalaya*; a clade composed of most Bornean species and one from Sumatra (B); and, sister to clade B, a large clade that has three Bornean species but is otherwise comprised of all the species from Sulawesi, Java and New Guinea, as well as most species on Sumatra and many on the Thai-Malay Peninsula (C). The topology and biogeographical character of the major clades did not change when the data were analysed for the expanded terminal set, except for the inclusion of a species from the southern tip of the Thai-Malay Peninsula (Peninsula sp. 28) inside clade B.

Support and stability were high for most clades in the optimal tree using the normal terminal set but nearly entirely absent for nodes near the base of clade C (which, as a whole, was well supported) (Fig. 3). All four species of *Fangensis* formed a clade only under cost schemes with low transversion and indel costs. Clade A was recovered under all cost schemes; the only significant change came under the highest transversion and indel costs (441), which placed Peninsula sp. 21, not 16, as sister to the rest of the clade. Clades B and C were found under all cost schemes except 221. Clade (B+C) was recovered under all but two cost schemes, those with high indel-to-transversion costs. Clade (A+B) was never recovered.

The maximum likelihood bootstrap search in *RAXML* recovered many of the same relationships but did find

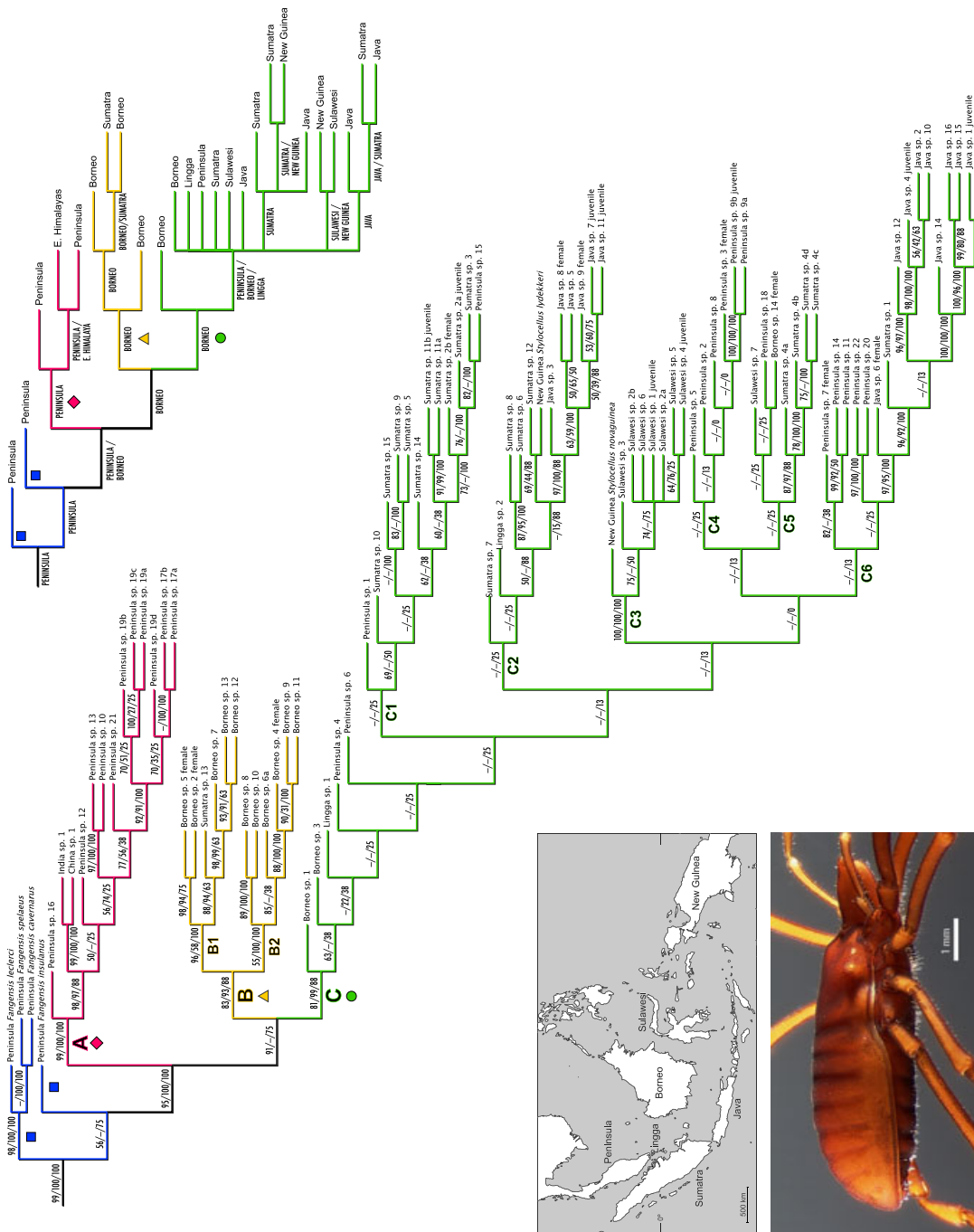


Figure 3 Strict consensus of the most parsimonious trees (showing only Southeast Asian Cyphophthalmi) for combined molecular data of the normal terminal set under the cost transformation scheme 121 (transitions = 1, transversions = 2, indels = 2). Values on nodes are, from left to right, bootstrap support using dynamic homology in *poxy*, maximum likelihood bootstrap and sensitivity (expressed as the fraction of the eight other transformation cost matrices in which the consensus trees had the same node). Dashes indicate < 50% bootstrap support in *poxy* (left) or the absence of the clade in the shortest tree found under maximum likelihood (middle). The upper right inset shows the same phylogeny with terminal names replaced by distributions and unsupported nodes and redundant areas collapsed. Ancestral distributions were reconstructed in DIVA 1.1 (Ronquist, 1996) using the resolved phylogeny. The lower left inset shows a map of the region and a lateral view of Borneo sp. 12 (anterior right, posterior left).

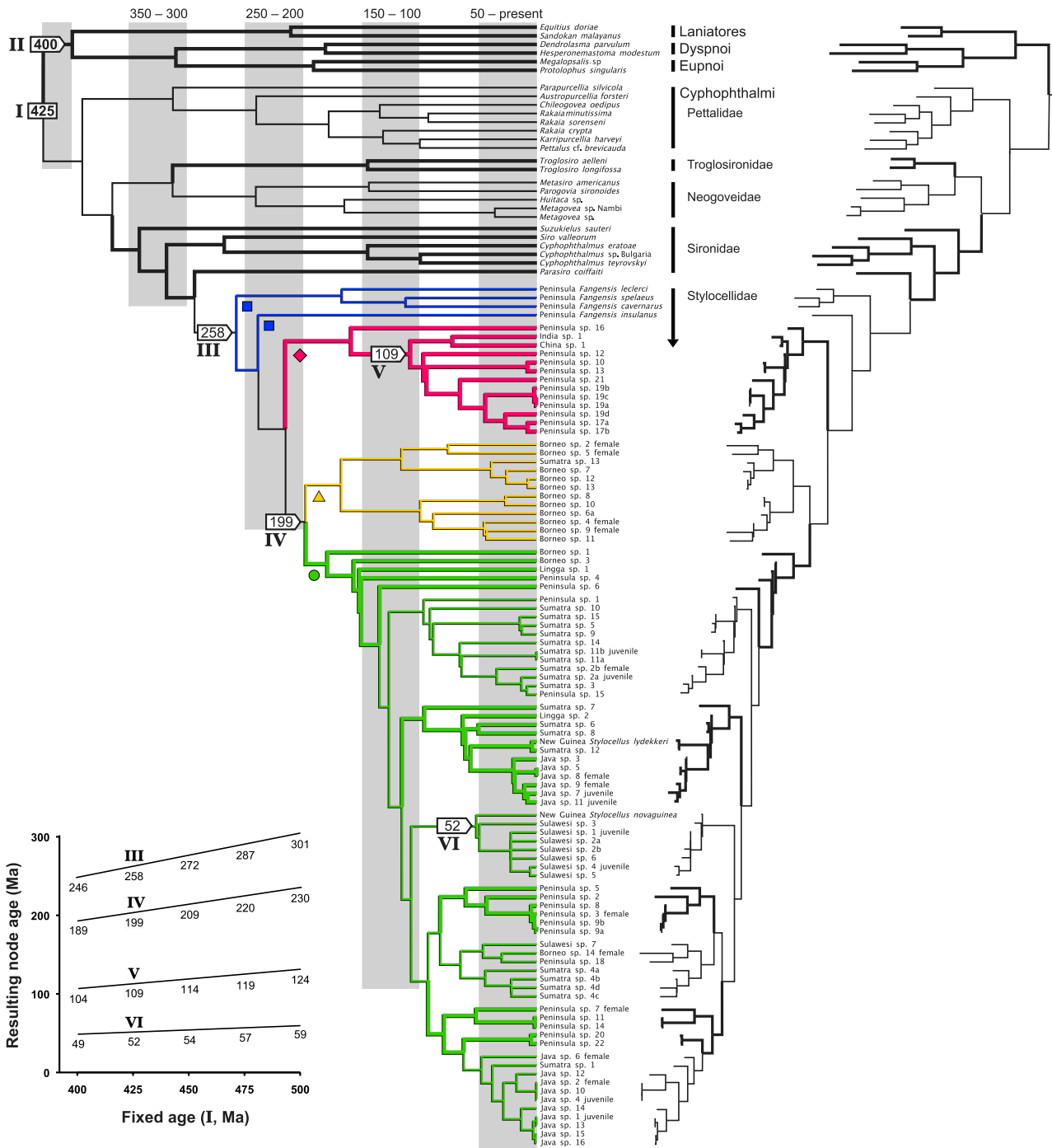


Figure 4 Strict consensus of the most parsimonious trees for the normal terminal set of Cyphophthalmi and outgroup Opiliones found under the transformation cost scheme 121 (the same as Fig. 3, but showing outgroups). The tree topology is identical in the left and right projections, the difference being that parsimony branch lengths on the right have been converted to node ages on the left. Node ages are calculated based on a fixed age at the root of 425 Ma (I) and a minimum age for non-cyphophthalmid Opiliones of 400 Ma (II). The inset shows changes in ages for nodes III–VI when the fixed age (I) is varied from 401 to 500 Ma. Symbols on branches in Stylocellidae (squares, diamond, triangle, circle) match collection localities in Fig. 1 and clades identified in Fig. 3.

Fangensis to be monophyletic (with bootstrap support of 98%) and clades A and B to be sister to each other (a topology never found under parsimony; bootstrap support 84%).

Among the outgroups, Sironidae was the least stable, not being monophyletic under any transformation cost scheme. A

sironid was found as sister to Stylocellidae under four of the eight transformation cost schemes besides the optimal one: *Parasiro coffaiti* under 111, 141 and 241 and *Suzukiellus sauteri* under 211. Pettalidae was recovered as sister to the remaining Cyphophthalmi under all cost schemes except 221, when it was

recovered as sister to Stylocellidae. Under parsimony with the optimal cost set, the clade ('Sironidae' + Stylocellidae) (sironids forming a paraphyletic grade) had 50% bootstrap support, and the clade (*P. coiffaiti* + Stylocellidae) had 73% support. The maximum likelihood bootstrap search recovered Sironidae in a clade with Troglосironidae and Neogoveidae with 73% support, and this clade was found as sister to Stylocellidae with 79% support.

The shortest tree for the expanded terminal set was 59 steps longer (24,046 weighted steps) than that from the normal terminal set, and it had high bootstrap support for several relationships, despite terminals with few data (see Appendix S4). Clades A and (B+C) had high support and stability, but clades B and C were recovered mostly only under low indel-to-transversion ratios. At higher indel-to-transversion ratios, Borneo sp. 16 and sp. 18 tended to move out of Clade B (the former going to clade C), and Borneo sp. 1 and sp. 14 placed outside clade C. Peninsula sp. 28, the one peninsular species from the expanded terminal set that was recovered inside clade B, formed a clade with Sumatra sp. 13 and sp. 17 and Borneo sp. 16 mostly under low indel-to-transversion ratios; under all other cost schemes, it was found in a clade with two other taxa, Sumatra sp. 13 and sp. 17. The two species that had the fewest data – Peninsula sp. 26 and Borneo sp. 17 – were recovered in clades with high bootstrap support.

Fixing the origin of Opiliones at 425 Ma and constraining the origin of the non-cyphophthalmid Opiliones to at least 400 Ma, the Southeast Asian clade was estimated to have split from Sironidae 295 Ma and diversified 258 Ma (Fig. 4). These dates varied, respectively, from 344 to 280 Ma and 301 to 246 Ma as the fixed date for the Opiliones was repositioned from 500 to 401 Ma, but all of them are consistent with an old age for the group.

The optimization of ancestral distributions on the phylogeny from the normal terminal set using *diva* suggests that the stylocellid ancestor was distributed solely on the landmass now known as the Thai-Malay Peninsula. The family subsequently expanded to Borneo with the ancestors of clades (A(B+C)) or (B+C). The distribution for the ancestor of clade A is optimized to the Peninsula, and clades B and C to Borneo. Within clade C, the ancestral distribution is suggested to have

either remained on Borneo or expanded from Borneo to the Lingga-Riau Archipelago or Thai-Malay Peninsula, then out to Sumatra, Sulawesi, New Guinea and Java. Clade B appears to have had an independent dispersal to Sumatra as well.

For the paralogy-free subtree analysis, three subtrees were extracted from the phylogeny of the normal terminal set and five from the phylogeny of the expanded terminal set (Table 1); they resolved, respectively, to one and two shortest area cladograms (Fig. 5). These indicated a close relationship between the Thai-Malay Peninsula and Borneo and between Sumatra and Java.

DISCUSSION

Previous studies of the Southeast Asian Cyphophthalmi were less complete, including fewer terminals and markers than used here, but the relationships they suggested are confirmed here: (1) a Southeast Asian clade consistent with what has been called the family Stylocellidae, (2) species in the genus *Fangensis* as sister to the remaining members of the family, (3) *F. insulanus* tending to render *Fangensis* paraphyletic, (4) a clade comprised mostly of Bornean species, and, sister to that clade, (5) a clade containing all the species found on the Lingga-Riau Archipelago, Java, Sulawesi and New Guinea (Schwendinger & Giribet, 2005; Boyer *et al.*, 2007; Clouse & Giribet, 2007). Also suggested in previous studies, although unknown at the time, was the inclusion of *Meghalaya* in Stylocellidae, which we demonstrate here by the placement of eastern Himalayan species in clade A. In one previous molecular phylogeny we included two *Meghalaya* species (Peninsula sp. 17b and sp. 19d) and recovered them as sister to clade B using maximum likelihood (Boyer *et al.*, 2007), but we did not connect them morphologically to the Indian species being described at the same time (Giribet *et al.*, 2007). *Meghalaya* is here formally assigned to Stylocellidae.

The optimization of distributions using a dispersal and vicariance model supports the ancestral area reconstruction most readily suggested by the paraphyletic grades and sister-group relationships in the phylogeny: the first ancestral locality is the Thai-Malay Peninsula, then Borneo to the south and the eastern Himalayas to the north, and finally Sumatra, Sulawesi, New Guinea and Java to the south-east. An important unresolved ancestral distribution is that of clade (A(B+C)), left as Borneo or the Peninsula, but this could indicate an intermediate place of origin now inundated. The unresolved origin of clade C after Borneo sp. 3 could mean the same, especially considering the often early placement of species from the Lingga-Riau Archipelago. *diva* has been noted to produce less resolved or plausible reconstructions when species distributions are clearly influenced by vicariance (Sanmartín, 2007). This is true for *Fangensis* and clades A and B, which are mostly restricted to certain regions, but clade C is not so clear, and thus we preferred *diva* for an event-based analysis.

Our general area cladograms have clear weaknesses but are consistent with hypothesized past events in the region. Subtrees were based on unsupported nodes and unstable taxa,

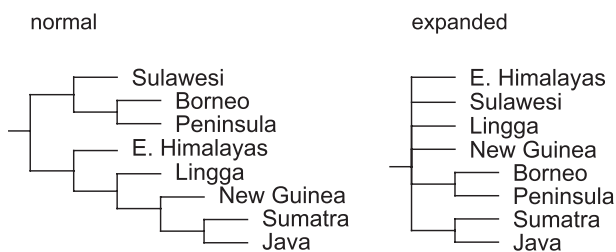


Figure 5 Summary area cladograms of the Southeast Asian Cyphophthalmi based on paralogy-free subtrees in the phylogeny based on the normal terminal set (left) and the expanded terminal set (right, strict consensus of two equally parsimonious trees). Subtrees are listed in Table 1.

and they came mostly from clade C, limiting their information to landmass relationships of the past 150–200 Ma. We also defined overlapping regions at a scale quite different from actual species ranges (all of Borneo, for example, rather than just Gunung Mulu National Park). Nonetheless, the repeated area relationships in clade C offer a rare opportunity for biogeographical analysis in Cyphophthalmi, clades of which are usually restricted to a single region. What emerges most clearly from our subtree analysis is a close relationship between Borneo and the Peninsula, as well as between Sumatra and Java. This result is consistent with geological reconstructions that show both landmass pairs to have maintained terrestrial connections since the appearance of the second member until as recently as 5–10 Ma (Hall, 1998). The placement of Sulawesi as sister to (Peninsula + Borneo) in the more resolved area cladogram agrees with geological reconstructions, Borneo being the only landmass with which Sulawesi had a terrestrial connection. Thus, although some regions are unresolved, and the backtracking of lineages muddles the branching order, the area cladograms do recover the oldest and most persistent landmass relationships.

The fact that *Fangensis* and clade A did not diversify into the Indo-Malay Archipelago deprives us of a more robust analysis of those landmass relationships, but their restriction to the Peninsula and areas north is consistent with molecular dating that places their origin to a time when the other landmasses did not yet exist. The low vagility of Cyphophthalmi, which leads to phylogenies remarkably free of dispersal noise (Boyer & Giribet, 2007; Boyer *et al.*, 2007), also limits a discussion of their biogeography mostly to a pairing between their phylogeny and geological history. Nonetheless, the match between Stylocellidae and proposed geological events is striking, especially considering the complexity of Southeast Asia.

Gondwanan origin

The topology of our optimal tree is incompatible with the two most intuitive hypotheses for the origin of Stylocellidae. First, they are not derived from eastern Himalayan species to the north, which apparently diversified out of the Thai-Malay Peninsula, not the reverse. Second, Stylocellidae are not closely related to Pettalidae in nearby Sri Lanka or Australia, a sister-group relationship between the two families being found under only one suboptimal transformation cost scheme.

The peninsular ancestral terrane of Stylocellidae and the results of our molecular dating together suggest a Gondwanan origin for the family. One scenario for the diversification of Stylocellidae out of Sironidae (as shown in our optimal tree) that does not require a recent long-distance dispersal is for it to have happened when their current homelands (Southeast Asia and Laurasia) were connected by a shoreline across North Africa and Arabia along the southern Palaeo-Tethys coastline during the Sakmarian and Asselian Ages in the early Permian (299–284 Ma) (Metcalfe, 1998, 2001, 2002). This date agrees well with our molecular dating, which puts the split between Sironidae and Stylocellidae at 295 Ma. (However, alternative

sister group relationships for Stylocellidae exist, e.g. Boyer *et al.*, 2007; and Giribet *et al.*, 2009.) Our dating for the start of stylocellid diversification at 258 Ma is also highly concordant with the rifting of the Sibumasu terrane, which became the eastern end of the long, northward-moving Cimmerian palaeocontinent and is now the region extending from north-east India to the southern Thai-Malay Peninsula. This terrane is hypothesized to have separated from Gondwana around the Wuchiapingian Age of the late Permian (260–254 Ma) (Metcalfe, 2002).

The dating of our phylogeny relies heavily on a single fossil, *Eophalangium sheari* from the early Devonian Rhynie cherts in Scotland (Dunlop *et al.*, 2004). Because it so clearly belongs to the opilionid suborder Eupnoi, superfamily Phalangioidea, we have used its approximate date of 400 Ma as a minimum age for all the non-cyphophthalmid Opiliones. Eupnoi has been hypothesized to be sister to the remaining non-cyphophthalmid suborders (Giribet *et al.*, 2002), but even if it is recovered as more derived, as it was here, the minimum date of 400 Ma for the sister to the Cyphophthalmi does not change. With Eupnoi appearing by 400 Ma, Opiliones as a whole evolving after Scorpiones (Giribet *et al.*, 2002), and stem-group Scorpiones emerging around 430 Ma (Dunlop, 2007), it is difficult not to constrain the origin of Opiliones to the early Silurian. Our initial investigations of molecular dating revealed that the controlling factor in dating nodes was the most recent ancestral fixed age; thus, inputting 400 Ma as a minimum age for the non-cyphophthalmid Opiliones had a trivial effect on Cyphophthalmi, but fixing the age of Opiliones at 425 Ma determined the ages of all descendant nodes. Two cyphophthalmid fossils have been described: (1) a European fossil younger than 40 Ma, probably a member of either *Siro* Latreille, 1796 or *Cyphophthalmus* Joseph, 1868 (Dunlop & Giribet, 2003) (considered synonymous genera when described), and (2) a 100-Myr-old specimen of unclear affinities from Myanmar (Poinar, 2008). However, unless these fossils could be reliably used to fix the age of a node between the ancestors of Opiliones and Stylocellidae, our explorations showed they would have little effect on the ages of nodes important to the history of the Southeast Asian species, so no attempt was made to include them in the dating of our phylogeny. Varying the fixed age at the root had the same proportional effect along the tree, i.e. varying it by 99 Ma around 450 Ma, 22%, caused about a 20% change in descendant nodes. Thus, if one decreases the origin of Opiliones up to 401 Ma, giving Eupnoi only 1 Myr to evolve, Stylocellidae still date from before 245 Ma.

Our dating here does vary significantly from a previous study of world-wide cyphophthalmid biogeography (Boyer *et al.*, 2007), but there are clear reasons why that analysis underestimated ages. We previously found a date for the origin of Stylocellidae of 148–83 Ma, and a date for the origin of Cyphophthalmi of 312–174 Ma (Boyer *et al.*, 2007). Those dates were found by calibrating the molecular phylogeny to combinations of three geological events that imposed minimum ages on certain clades in the absence of dispersal. For

each combination tested, one of the dates had to be set as a fixed age, even though the resulting fixed clade affected by the geological event could have arisen much earlier. Thus, although the analysis made the point that the Cyphophthalmi are very old, even when dated using conservative methods, it was recognized at the time that these dates were too young to agree with the Eupnoi fossil. A more recent study using a much larger sampling of non-Cyphophthalmi species and multiple calibration points also suggests that Cyphophthalmi are older than 300 Ma and that a small subset of Stylocellidae split from the other Cyphophthalmi almost 300 Ma and diversified at around 200 Ma (Giribet *et al.*, 2009), a result consistent with the findings of this study.

Sibumasu diversification

The Sibumasu terrane, what is now mostly the Thai-Malay Peninsula (Metcalf, 1998), is clearly indicated here as the ancestral terrane from which Stylocellidae diversified. The initial lineages in the family, *Fangensis* and clade A (*Meghalaya*), are found exclusively from the central peninsula northward, and even among these, the earlier species – all of *Fangensis* and Peninsula sp. 16 – are found exclusively on the Sibumasu terrane. The eastern Himalayan species appear to have shown a remarkable range extension through a continuous landmass, although the Thai-Malay Peninsula was likely to have been closer to Indochina and oriented in a more east–west direction before the collision of India with Eurasia (Hall, 1998). We have entertained the possibility that India picked up Cyphophthalmi during a possible collision with the peninsula and northern Sumatra around 40 Ma (Ali & Aitchison, 2008), a scenario not unlike that with the African-aligned *Metasiro americanus* in Florida (Boyer *et al.*, 2007). However, the discovery of a cyphophthalmid fossil dating back to 100 Ma from an area between the locations of the Indian and Chinese species (26°20' N, 96°36' E) (Poinar, 2008) makes the collision hypothesis unlikely, assuming the fossil is a stylocellid. The fossil is currently placed in Sironidae, but its quality makes such a determination difficult and its affinity remains untested phylogenetically. The lack of sironid collections from the area adds doubt to that assignment, for the closest sironids occur in Anatolia (Turkey) in the west and on Honshu Island (Japan) in the north-east. Members of clade A had ample time – 100 Myr – to migrate north by the time of this fossil, for dinosaur migrations to the peninsula indicate accretion of the Sibumasu terrane with Laurasia before the Jurassic (Buffetaut *et al.*, 2005).

The emergence of Borneo

The phylogenetic tree presented here suggests that the arrival of Borneo in the Cretaceous allowed a southward expansion of clade (B+C). It began diversifying in western Borneo, where the island first accreted to the peninsula, as evidenced by the locality of Borneo sp. 1, the sister to the remainder of clade C and the oldest lineage after the common ancestor of (B+C).

Then, starting in the Cretaceous, clade B diversified deep into northern and eastern Borneo, and clade C diversified over the Sundaland Peninsula. Clade B also shows evidence of the Cenozoic connection between western Borneo, the southern tip of the Thai-Malay Peninsula and central Sumatra, as it contains species from Sumatra and peninsular Malaysia, all of which are derived.

Clade C

Like clade B, clade C appears to have expanded out of Borneo and across the lower peninsula (including what is today the Lingga-Riau Archipelago) and into Sumatra. Low support among constituent clades within C hinders reconstructions of the sequence of their diversification after expanding out of Borneo, but clearly there were multiple lineages on Borneo, the peninsula and Sumatra. Other lineages, later in the Cenozoic, gave rise to the clades on Java, Sulawesi and New Guinea as those landmasses became available.

As a whole, clade C is stable and well supported, and its more derived internal lineages – many of which are found in certain areas of particular islands, such as those in northern Sulawesi or Java – are also generally well supported. But the relationships among clades that originated between 100 and 50 Ma are remarkably unstable. Not only do they receive little support from resampling or sensitivity analyses, but they are also inclined to move within clade C when new terminals are added (see Appendix S3). Even when unsupported nodes are used, the paralogy-free subtree analysis recovered only the most persistent and stable area relationships in the region, (Peninsula + Borneo) and (Sumatra + Java).

This lack of support within clade C is, in hindsight, not surprising, as it could be a natural consequence of events in the region when it first diversified. Repeated inundations, exposures, accretions and upliftings in Sundaland have, up to the present day, provided a variety of landmass configurations in rapid succession for the different lineages of clade C to colonize, leaving their relationships clouded. A lack of support among broadly distributed Sundaic lineages has been inferred for other groups, and the recent break-up of the peninsula has been invoked as an explanation (Ruedi & Fumagalli, 1996; Maekawa *et al.*, 2001; Gorog *et al.*, 2004). Sharma & Giribet (2009) found a lack of support in a Sundaland clade using eight molecular markers, so a lack of data is not necessarily the cause, and here we use a selection of markers that are demonstrably able to resolve nodes in related lineages arising at the same time.

The various terranes also show their age and connectivity by their relative representation in the main lineages. The Thai-Malay Peninsula has all the species of *Fangensis* as well as species from clades A, B and C. Sumatra has representatives in clades B and C, as does Borneo. More recent landmasses in the region – Java by volcanic growth and Sulawesi by plate collision – have representatives only from clade C. Within clade C, however, the various lineages still suggest that Sumatra, the peninsula, and even Java had multiple invasions

and diversifications. The Javanese lineages in C2 and C6 prompted further examination, simply because the specimens are so similar morphologically and are found in such close proximity on the island; in spite of not being found to be monophyletic under either parsimony or maximum likelihood, it seemed unlikely that their sequences could be very different. In clade C6, a Sumatran (*Sumatra* sp. 1) species falls within a highly supported lineage of Javanese species, although this is not remarkable given that it is found on the southern end of the island, near Java. We investigated if there was any transformation cost scheme that recovered the Javanese species (and *Sumatra* sp. 1) in C2 and C6 as monophyletic, and there was not. Moreover, we examined the pairwise distances of the Javanese sequences of each marker within and between members of clades C2 and C6, and we found that the between-clade distances were distinctly higher, especially for the more conserved markers 18S and 28S rRNA (Fig. 2c–h).

Sulawesi

Western Sulawesi is hypothesized to have accreted with what is now eastern Borneo around the end of the Mesozoic, then to have rifted away again after only 10 Myr, the Makassar Straits opening during the Eocene (Hall, 2002). Given our phylogeny, we would expect the lineage most likely to migrate into western Sulawesi to be closely related to the early Bornean lineages in clade C and subsequently to give rise to the northern Sulawesi lineage when it accreted. Borneo sp. 14, which has a broad range in central and northern Borneo, was recovered as close to Sulawesi sp. 7 from western Sulawesi in the optimal tree, and under different cost schemes it landed among the early splits in clade C with the other Bornean species. Borneo sp. 14 lacked only 11% of the data, rendering missing data an unlikely explanation for its instability. Thus, we find it interesting that within clade C there is a close relationship between one of its few Bornean members and the species from western Sulawesi, and that there is a proclivity for this Bornean species to also fall among the early Bornean lineages. But as usual among island lineages in clade C, our analyses never found a properly sequenced, supported and timed relationship among Borneo, western Sulawesi and northern Sulawesi.

Relationships among the northern Sulawesi species are largely unresolved, again highlighting problems resolving clade C. The species are not identical in morphology or sequence data, and they are spread over a large enough area that we would expect a certain degree of speciation in Cyphophthalmi. The northern Sulawesi species would have most likely dispersed there from western Sulawesi during the past 15 Ma (Hall, 2002) – a date very close to our estimated 22 Ma for their main diversification (Fig. 4) – and perhaps the accessibility of virgin territory allowed them to quickly generate a large amount of diversity not easily connected to other species in clade C. A similar phenomenon can be seen with the Javanese species and some in Sumatra, and it seems reasonable

that land and sea-level changes in the archipelago, like the sudden accessibility of northern Sulawesi, created bursts of speciation in various lineages that have clouded relationships in the entire group. Radiations have occurred in other groups of Cyphophthalmi, notably in New Zealand and the Balkan Peninsula (Boyer & Giribet, 2009; Muriene *et al.*, 2010).

New Guinea

New Guinean Cyphophthalmi are known only from the Bird's Head region of west Papua, collecting efforts in Papua New Guinea yielding none, and no Cyphophthalmi-focused expeditions having yet been conducted in the island's interior. We have previously discussed the anomalous nature of New Guinean species (Clouse & Giribet, 2007), and again here we find the following: (1) no close relationship between *Stylocellus novaguinea* and *Stylocellus lydekkeri*; (2) a close relationship between *S. lydekkeri* and Sumatra sp. 12, Java sp. 3 and New Guinea sp. 1 (the unnamed female collected with *S. novaguinea*); and (3) a close relationship between *S. novaguinea* and the northern Sulawesi species. With their relationships now dated, we have further insight into the possibility that the New Guinean species are the only cases of transoceanic dispersal in the entire suborder. For *S. novaguinea*, its split from the Sulawesi species at 52 Ma was not caused by moving to New Guinea (for New Guinea was even further away and submerged then), and all we are able to conclude is that the species made its journey in the Cenozoic from terranes that are now part of Sulawesi. *Stylocellus lydekkeri* appears to have shared a common ancestor with Sumatra sp. 12 only 3.9 Ma, leaving open few explanations for its presence in New Guinea other than transoceanic dispersal. Given the unfavourable oceanic currents for a natural dispersal (Gordon & Fine, 1996), and the double coincidence of two species from different areas dispersing to no landmass other than the same region of New Guinea, an idiosyncrasy associated with human trade is, by process of elimination, becoming the most likely vector, although difficult to demonstrate scientifically.

CONCLUSIONS

Despite high biodiversity and dynamic tectonics in Southeast Asia, finding a consistent signal of geological events in its biota has been difficult. Much of this is because the events have been too numerous and complex to leave a clear signature on the most commonly studied organisms, many of which arose at different times in the Cenozoic and have significant dispersal capabilities. Cyphophthalmi, which are known poor dispersers and yet widespread and species rich (Giribet & Kury, 2007), are here shown to be excellent models for studying the ancient history of Southeast Asia. They appear to represent a rare case of a modern organism in Southeast Asia that did not immigrate from Eurasia (Kreier & Schneider, 2006; Marivaux *et al.*, 2006; Prasaig *et al.*, 2007) or Australia (Ladiges *et al.*, 2003; Wallace & Deitz, 2006; Muellner *et al.*, 2008) but was already on an ancestral Southeast Asian terrane that rifted

from Gondwana. Multiple lines of evidence presented here support this conclusion: the Thai-Malay Peninsular location of the earliest lineages, the derived position of the eastern Himalayan species, the close relationship between the South-east Asian species and Sironidae but not Pettalidae, and the estimates of clade ages from molecular dating. We have suggested this before (Schwendinger & Giribet, 2005; Boyer *et al.*, 2007; Clouse & Giribet, 2007), but this marks the most comprehensive test of this hypothesis to date. In addition, our phylogeny is consistent with the notion that Borneo arrived later and presented fertile ground for new diversifications in the family, as did the subsequent appearances of Sulawesi and Java. With a framework for their history in hand, we anticipate investigating the relationships of the Stylocellidae further in order to ask more detailed questions about the history of Southeast Asia.

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REFERENCES

- Abegg, C. & Thierry, B. (2002) Macaque evolution and dispersal in insular south-east Asia. *Biological Journal of the Linnean Society*, **75**, 555–576.
- Ali, J.R. & Aitchison, J.C. (2008) Gondwana to Asia: plate tectonics, paleogeography and the biological connectivity of the Indian sub-continent from the Middle Jurassic through latest Eocene (166–35 Ma). *Earth-Science Reviews*, **88**, 145–166.
- Atkins, H., Preston, J. & Cronk, Q.C.B. (2001) A molecular test of Huxley's line: *Cyrtandra* (Gesneriaceae) in Borneo and the Philippines. *Biological Journal of the Linnean Society*, **72**, 143–159.
- Baker, W.J., Coode, M.J.E., Dransfield, J., Dransfield, S., Harley, M.M., Hoffmann, P. & Johns, R.J. (1998) Patterns of distribution of Malesian vascular plants. *Biogeography and geological evolution of SE Asia* (ed. by R. Hall and J.D. Holloway), pp. 243–258. Backhuys Publishers, Leiden, The Netherlands.
- Balke, M. & Ribera, I. (2004) Jumping across Wallace's line: *Allodessus* Guignot and *Limbodessus* Guignot revisited (Coleoptera: Dytiscidae, Bidessini) based on molecular-phylogenetic and morphological data. *Australian Journal of Entomology*, **43**, 114–128.
- Beck, J. & Khen, C.V. (2007) Beta-diversity of geometrid moths from northern Borneo: effects of habitat, time and space. *Journal of Animal Ecology*, **76**, 230–237.
- Boyer, S.L. & Giribet, G. (2007) A new model Gondwanan taxon: systematics and biogeography of the harvestman family Pettalidae (Arachnida, Opiliones, Cyphophthalmi), with a taxonomic revision of genera from Australia and New Zealand. *Cladistics*, **23**, 337–361.
- Boyer, S.L. & Giribet, G. (2009) Welcome back New Zealand: regional biogeography and Gondwanan origin of three endemic genera of mite harvestmen (Arachnida, Opiliones, Cyphophthalmi). *Journal of Biogeography*, **36**, 1084–1099.
- Boyer, S.L., Karaman, I. & Giribet, G. (2005) The genus *Cyphophthalmus* (Arachnida, Opiliones, Cyphophthalmi) in Europe: a phylogenetic approach to Balkan Peninsula biogeography. *Molecular Phylogenetics and Evolution*, **36**, 554–567.
- Boyer, S.L., Clouse, R.M., Benavides, L.R., Sharma, P., Schwendinger, P.J., Karunaratna, I. & Giribet, G. (2007) Biogeography of the world: a case study from cyphophthalmid Opiliones, a globally distributed group of arachnids. *Journal of Biogeography*, **34**, 2070–2085.
- Brandon-Jones, D. (2001) Borneo as a biogeographic barrier to Asian–Australasian migration. *Faunal and floral migrations and evolution in SE Asia–Australasia* (ed. by I. Metcalfe, J.M.B. Smith, M. Morwood and I. Davidson), pp. 365–372. A.A. Balkema, Lisse.

- Brown, G.K., Nelson, G. & Ladiges, P.Y. (2006a) Historical biogeography of *Rhododendron* section *Vireya* and the Malaysian Archipelago. *Journal of Biogeography*, **33**, 1929–1944.
- Brown, G.K., Craven, L.A., Udovicic, F. & Ladiges, P.Y. (2006b) Phylogeny of *Rhododendron* section *Vireya* (Ericaceae) based on two non-coding regions of cpDNA. *Plant Systematics and Evolution*, **257**, 57–93.
- Brown, R.M. & Guttman, S.I. (2002) Phylogenetic systematics of the *Rana signata* complex of Philippine and Bornean stream frogs: reconsideration of Huxley's modification of Wallace's Line at the Oriental–Australian faunal zone interface. *Biological Journal of the Linnean Society*, **76**, 393–461.
- de Bruyn, M. & Mather, P.B. (2007) Molecular signatures of Pleistocene sea-level changes that affected connectivity among freshwater shrimp in Indo-Australian waters. *Molecular Ecology*, **16**, 4295–4307.
- Buffetaut, E., Suteethorn, V., Tong, H.Y. & Kosir, A. (2005) First dinosaur from the Shan–Thai Block of SE Asia: a Jurassic sauropod from the southern peninsula of Thailand. *Journal of the Geological Society*, **162**, 481–484.
- Cannon, C.H. & Manos, P.S. (2003) Phylogeography of the Southeast Asian stone oaks (*Lithocarpus*). *Journal of Biogeography*, **30**, 211–226.
- Clouse, R.M. & Giribet, G. (2007) Across Lydekker's Line – first report of mite harvestmen (Opiliones : Cyphophthalmi : Stylocellidae) from New Guinea. *Invertebrate Systematics*, **21**, 207–227.
- Colgan, D.J., McLauchlan, A., Wilson, G.D.F., Livingston, S.P., Edgecombe, G.D., Macaranas, J., Cassis, G. & Gray, M.R. (1998) Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Australian Journal of Zoology*, **46**, 419–437.
- Dunlop, J.A. (2007) Paleontology. *Harvestmen: the biology of Opiliones* (ed. by R. Pinto-da-Rocha, G. Machado and G. Giribet), pp. 247–265. Harvard University Press, Cambridge, MA.
- Dunlop, J.A. & Giribet, G. (2003) The first fossil cyphophthalmid (Arachnida, Opiliones) from Bitterfeld amber, Germany. *Journal of Arachnology*, **31**, 371–378.
- Dunlop, J.A., Anderson, L.I., Kerp, H. & Hass, H. (2004) A harvestman (Arachnida: Opiliones) from the Early Devonian Rhynie cherts, Aberdeenshire, Scotland. *Transactions of the Royal Society of Edinburgh – Earth Sciences*, **94**, 341–354.
- Ebach, M.C., Humphries, C.J., Newman, R.A., Williams, D.M. & Walsh, S.A. (2005) Assumption 2: opaque to intuition? *Journal of Biogeography*, **32**, 781–787.
- Edgecombe, G.D. & Giribet, G. (2006) A century later – a total evidence re-evaluation of the phylogeny of scutigermorph centipedes (Myriapoda : Chilopoda). *Invertebrate Systematics*, **20**, 503–525.
- Evans, B.J., Supriatna, J., Andayani, N., Setiadi, M.I., Cannatella, D.C. & Melnick, D.J. (2003) Monkeys and toads define areas of endemism on Sulawesi. *Evolution*, **57**, 1436–1443.
- Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R.C. (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**, 294–299.
- George, W. (1981) Wallace and his line. *Wallace's line and plate tectonics* (ed. by T.C. Whitmore), pp. 3–8. Clarendon Press, London.
- George, W. (1987) Complex origins. *Biogeographical evolution of the Malay archipelago* (ed. by T.C. Whitmore), pp. 119–131. Clarendon Press, London.
- Giribet, G. (2000) Catalogue of the Cyphophthalmi of the world (Arachnida, Opiliones). *Revista Ibérica de Aracnología*, **2**, 49–76.
- Giribet, G. & Kury, A.B. (2007) Phylogeny and biogeography. *Harvestmen: the biology of Opiliones* (ed. by R. Pinto-da-Rocha, G. Machado and G. Giribet), pp. 62–87. Harvard University Press, Cambridge, MA.
- Giribet, G., Edgecombe, G.D., Wheeler, W.C. & Babbitt, C. (2002) Phylogeny and systematic position of Opiliones: a combined analysis of chelicerate relationships using morphological and molecular data. *Cladistics*, **18**, 5–70.
- Giribet, G., Sharma, P.P. & Bastawade, D.B. (2007) A new genus and species of Cyphophthalmi (Arachnida: Opiliones) from the north-eastern states of India. *Zoological Journal of the Linnean Society*, **151**, 663–670.
- Giribet, G., Vogt, L., Pérez González, A., Sharma, P. & Kury, A.B. (2009) A multilocus approach to harvestman (Arachnida: Opiliones) phylogeny with emphasis on biogeography and the systematics of Laniatores. *Cladistics*, doi:10.1111/j.1096-0031.2009.00296.x.
- Glaubrecht, M. & von Rintelen, T. (2003) Systematics, molecular genetics and historical zoogeography of the viviparous freshwater gastropod *Pseudopotamis* (Cerithioidea, Pachychilidae): a relic on the Torres Strait Islands, Australia. *Zoologica Scripta*, **32**, 415–435.
- Goloboff, P.A. (1999) Analyzing large data sets in reasonable times: solutions for composite optima. *Cladistics*, **15**, 415–428.
- Goloboff, P.A., Farris, J.S. & Nixon, K.C. (2008) TNT, a free program for phylogenetic analysis. *Cladistics*, **24**, 774–786.
- Gordon, A.L. & Fine, R.A. (1996) Pathways of water between the Pacific and Indian oceans in the Indonesian seas. *Nature*, **379**, 146–149.
- Gorog, A.J., Sinaga, M.H. & Engstrom, M.D. (2004) Vicariance or dispersal? Historical biogeography of three Sunda shelf murine rodents (*Maxomys surifer*, *Leopoldamys sabanus* and *Maxomys whiteheadi*). *Biological Journal of the Linnean Society*, **81**, 91–109.
- Gressitt, J.L. (1982) Pacific-Asian biogeography with examples from the Coleoptera. *Entomologia Generalis*, **8**, 1–11.
- Hall, R. (1998) The plate tectonics of Cenozoic SE Asia and the distribution of land and sea. *Biogeography and geological evolution of SE Asia* (ed. by R. Hall and J.D. Holloway), pp. 99–132. Backhuys Publishers, Leiden, The Netherlands.

- Hall, R. (2001) Cenozoic reconstructions of SE Asia and the SW Pacific: changing patterns of land and sea. *Faunal and floral migrations and evolution in SE Asia–Australasia* (ed. by I. Metcalfe, J.M.B. Smith, M. Morwood and I. Davidson), pp. 35–56. A.A. Balkema, Lisse.
- Hall, R. (2002) Cenozoic geological and plate tectonic evolution of SE Asia and the SW Pacific: computer-based reconstructions, model and animations. *Journal of Asian Earth Sciences*, **20**, 353–431.
- Holloway, J.D. (2003) Biological images of geological history: through a glass darkly or brightly face to face? *Journal of Biogeography*, **30**, 165–179.
- Huxley, T.H. (1868) On the classification and distribution of the Alectoromorphae and Heteromorphae. *Proceedings of the Zoological Society of London*, 294–319.
- Katoh, K., Misawa, K., Kuma, K. & Miyata, T. (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, **30**, 3059–3066.
- Kitching, L., Eastwood, R. & Hurley, K. (2001) Butterflies and Wallace's Line: faunistic patterns and explanatory hypotheses within the south-east Asian butterflies. *Faunal and floral migrations and evolution in SE Asia–Australasia* (ed. by I. Metcalfe, J.M.B. Smith, M. Morwood and I. Davidson), pp. 269–286. A.A. Balkema, Lisse.
- Kohler, F. & Glaubrecht, M. (2007) Out of Asia and into India: on the molecular phylogeny and biogeography of the endemic freshwater gastropod *Paracrostoma* Cossmann, 1900 (Caenogastropoda: Pachychilidae). *Biological Journal of the Linnean Society*, **91**, 627–651.
- Kreier, H.P. & Schneider, H. (2006) Phylogeny and biogeography of the staghorn fern genus *Platynerium* (Polypodiaceae, Polypodiidae). *American Journal of Botany*, **93**, 217–225.
- Ladiges, P.Y., Udovicic, F. & Nelson, G. (2003) Australian biogeographical connections and the phylogeny of large genera in the plant family Myrtaceae. *Journal of Biogeography*, **30**, 989–998.
- Lydekker, R. (1896) *A geographical history of mammals*. Cambridge University Press, Cambridge.
- Maekawa, K., Kon, M., Araya, K. & Matsumoto, T. (2001) Phylogeny and biogeography of wood-feeding cockroaches, genus *Salganea* Stål (Blaberidae: Panesthiinae), in Southeast Asia based on mitochondrial DNA sequences. *Journal of Molecular Evolution*, **53**, 651–659.
- Marivaux, L., Bocat, L., Chaimanee, Y., Jaeger, J.J., Marandat, B., Srisuk, P., Tafforeau, P., Yamee, C. & Welcomme, J.L. (2006) Cynocephalid dermopterans from the Palaeogene of South Asia (Thailand, Myanmar and Pakistan): systematic, evolutionary and palaeobiogeographic implications. *Zoologica Scripta*, **35**, 395–420.
- Mayr, E. (1944) Wallace's Line in the light of recent zoogeographic studies. *Quarterly Review of Biology*, **19**, 1–44.
- Metcalfe, I. (1998) Palaeozoic and Mesozoic geological evolution of the SE Asian region: multidisciplinary constraints and implications for biogeography. *Biogeography and geological evolution of SE Asia* (ed. by R. Hall and J.D. Holloway), pp. 25–42. Backhuys Publishers, Leiden, The Netherlands.
- Metcalfe, I. (2001) Palaeozoic and Mesozoic tectonic evolution and biogeography of SE Asia–Australasia. *Faunal and floral migrations and evolution in SE Asia–Australasia* (ed. by I. Metcalfe, J.M.B. Smith, M. Morwood and I. Davidson), pp. 15–34. A.A. Balkema, Lisse.
- Metcalfe, I. (2002) Permian tectonic framework and palaeogeography of SE Asia. *Journal of Asian Earth Sciences*, **20**, 551–566.
- Metcalfe, I. (2009) Late Palaeozoic and Mesozoic tectonic and palaeogeographical evolution of SE Asia. *Late Palaeozoic and Mesozoic ecosystems in SE Asia* (ed. by E. Buffetaut, G. Cuny, J. Le Loeuff and V. Suteethorn), pp. 7–23. The Geological Society of London, London.
- Mey, W. (1998) The distribution of *Apsilochorema* Ulmer, 1907: biogeographic evidence for the Mesozoic accretion of a Gondwana microcontinent to Laurasia. *Biogeography and geological evolution of SE Asia* (ed. by R. Hall and J.D. Holloway), pp. 91–98. Backhuys Publishers, Leiden, The Netherlands.
- Morley, R.J. (2003) Interplate dispersal paths for megathermal angiosperms. *Perspectives in Plant Ecology Evolution and Systematics*, **6**, 5–20.
- Moss, S.J. & Wilson, E.J. (1998) Biogeographic implications from the Tertiary palaeogeographic evolution of Sulawesi and Borneo. *Biogeography and geological evolution of SE Asia* (ed. by R. Hall and J.D. Holloway), pp. 133–164. Backhuys Publishers, Leiden, The Netherlands.
- Muellner, A.N., Pannell, C.M., Coleman, A. & Chase, M.W. (2008) The origin and evolution of Indomalayan, Australasian and Pacific island biotas: insights from Aglaieae (Meliaceae, Sapindales). *Journal of Biogeography*, **35**, 1769–1789.
- Müller, S. (1846) Über den Character der Thierwelt auf den Inseln des indischen Archipels, ein Beitrag zur zoologischen Geographie. *Archiv für Naturgeschichte*, **12**, 109–128.
- Murienne, J., Karaman, I. & Giribet, G. (2010) Explosive evolution of an ancient group of Cyphophthalmi (Arachnida: Opiliones) in the Balkan Peninsula. *Journal of Biogeography*, **37**, 90–102.
- Nelson, G. & Ladiges, P.Y. (1996) Paralogy in cladistic biogeography and analysis of paralogy-free subtrees. *American Museum Novitates*, **3167**, 1–58.
- Nelson, G. & Platnick, N. (1981) *Systematics and biogeography: cladistics and vicariance*. Columbia University Press, New York.
- Nijman, V. & Meijaard, E. (2008) Zoogeography of primates in insular Southeast Asia: species–area relationships and the effects of taxonomy. *Contributions to Zoology*, **77**, 117–126.
- Nixon, K.C. (1999) The parsimony ratchet, a new method for rapid parsimony analysis. *Cladistics*, **15**, 407–414.
- Outlaw, D.C. & Voelker, G. (2008) Pliocene climatic change in insular Southeast Asia as an engine of diversification in *Ficedula* flycatchers. *Journal of Biogeography*, **35**, 739–752.

- Pineau, P., Henry, M., Suspene, R., Marchio, A., Dettai, A., Debruyne, W., Petit, T., Lecu, A., Moisson, P., Dejean, A., Wain-Hobson, S. & Vartanian, J.P. (2005) A universal primer set for PCR amplification of nuclear histone H4 genes from all animal species. *Molecular Biology and Evolution*, **22**, 582–588.
- Poinar, G. (2008) *Palaeosiro burmanicum* n. gen, n. sp., a fossil Cyphophthalmi (Arachnida: Opiliones: Sironidae) in Early Cretaceous Burmese amber. *Advances in arachnology and developmental biology. Papers dedicated to Prof. Dr. Božidar Čurčić* (ed. by S.E. Makarov and R.N. Dimitrijević), pp. 267–274. Institute of Zoology, Belgrade; Bulgarian Academy of Sciences (BAS), Sofia; Faculty of Life Sciences, Vienna; Serbian Academy of Sciences and Arts (SASA), Belgrade; and UNESCO MAB Committee, Serbia.
- Prasaia, P., Hundsdoerfer, A.K. & Fritz, U. (2007) Phylogeny and taxonomy of endangered, South and South-east Asian freshwater turtles elucidated by rntDNA sequence variation (Testudines: Geoemydidae: *Batagur*, *Callagur*, *Hardefla*, *Kachuga*, *Pangshura*). *Zoologica Scripta*, **36**, 429–442.
- Quek, S.P., Davies, S.J., Ashton, P.S., Itino, T. & Pierce, N.E. (2007) The geography of diversification in mutualistic ants: a gene's-eye view into the Neogene history of Sundaland rain forests. *Molecular Ecology*, **16**, 2045–2062.
- Ridder-Numan, J.W.A. (1998) Historical biogeography of *Spatholobus* (Leguminosae-Papilionoideae) and allies in SE Asia. *Biogeography and geological evolution of SE Asia* (ed. by R. Hall and J.D. Holloway), pp. 259–278. Backhuys Publishers, Leiden, The Netherlands.
- Ronquist, F. (1996) *Dispersal–vicariance analysis (DIVA) version 1.1*. Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden. Computer program and documentation available at ftp.uu.se or ftp.systbot.uu.se.
- Ronquist, F. (1997) Dispersal–vicariance analysis: a new approach to the quantification of historical biogeography. *Systematic Biology*, **46**, 195–203.
- Ruedi, M. & Fumagalli, L. (1996) Genetic structure of Gymnures (genus *Hylomys*; Erinaceidae) on continental islands of Southeast Asia: historical effects of fragmentation. *Journal of Zoological Systematics and Evolutionary Research*, **34**, 153–162.
- Sanders, J.G. (2010) Cladscan, a program for automated phylogenetic sensitivity analysis. *Cladistics*, **26**, 114–116.
- Sanderson, M.J. (2003) r8s: inferring absolute rates of molecular evolution and divergence times in the absence of a molecular clock. *Bioinformatics*, **19**, 301–302.
- Sanmartín, I. (2007) Event-based biogeography: integrating patterns, processes, and time. *Biogeography in a changing world* (ed. by M.C. Ebach and R.S. Tangney), pp. 135–159. CRC Press, Boca Raton.
- Schwendinger, P.J. & Giribet, G. (2005) The systematics of the south-east Asian genus *Fangensis* Rambla (Opiliones : Cyphophthalmi : Stylocellidae). *Invertebrate Systematics*, **19**, 297–323.
- Sc Slater, P.L. (1858) On the general geographical distribution of the members of the class Aves. *Journal of the Proceedings of the Linnean Society: Zoology*, **2**, 130–145.
- Scott, B. (1997) Biogeography of the Helicoidea (Mollusca: Gastropoda: Pulmonata): land snails with a Pangean distribution. *Journal of Biogeography*, **24**, 399–407.
- Scrivenor, J.B., Burkill, I.H., Smith, M.A., Corbet, A.S., Airy Shaw, H.K., Richards, P.W. & Zeuner, F.E. (1943) A discussion on the biogeographic division of the Indo-Australian archipelago, with criticism of the Wallace and Weber lines and of any other dividing lines and with an attempt to obtain uniformity in the names used for the divisions. *Proceedings of the Linnean Society of London*, **154**, 120–165.
- Sharma, P. & Giribet, G. (2009) Sandokanid phylogeny based on eight molecular markers – the evolution of a southeast Asian endemic family of Laniatores (Arachnida, Opiliones). *Molecular Phylogenetics and Evolution*, **52**, 432–447.
- Shear, W.A. (1993) New species in the oilionid genus *Stylocellus* from Malaysia, Indonesia and the Philippines (Opiliones, Chyphophthalmi, Stylocellidae). *Bulletin of the British Arachnological Society*, **9**, 174–188.
- Shih, H.T., Yeo, D.C.J. & Ng, P.K.L. (2009) The collision of the Indian plate with Asia: molecular evidence for its impact on the phylogeny of freshwater crabs (Brachyura: Potamidae). *Journal of Biogeography*, **36**, 703–719.
- Simpson, G.G. (1977) Too many lines; the limits of the Oriental and Australian zoogeographic regions. *Proceedings of the American Philosophical Society*, **121**, 107–120.
- Stamatakis, A., Hoover, P. & Rougemont, J. (2008) A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology*, **57**, 758–771.
- Swofford, D.L. (2001) *PAUP*. Phylogenetic analysis using parsimony (*and other methods), version 4*. Sinauer Associates, Sunderland, MA.
- Varón, A., Vinh, L.S., Bomash, I. & Wheeler, W.C. (2008) POY 4.0.2885. American Museum of Natural History, New York.
- Vink, C.J., Thomas, S.M., Paquin, P., Hayashi, C.Y. & Hedin, M. (2005) The effects of preservatives and temperatures on arachnid DNA. *Invertebrate Systematics*, **19**, 99–104.
- Wallace, A.R. (1859) Letter from Mr. Wallace concerning the geographical distribution of birds. *Ibis*, **1**, 449–454.
- Wallace, A.R. (1863) On the physical geography of the Malay Archipelago. *Journal of the Royal Geographical Society*, **33**, 217–234.
- Wallace, A.R. (1890) *The Malay Archipelago*, 10th edn. Periplus (HK) Ltd, Hong Kong.
- Wallace, M.S. & Deitz, L.L. (2006) Australian treehoppers (Hemiptera: Membracidae: Centrotinae: Terentiini): phylogeny and biogeography. *Invertebrate Systematics*, **20**, 163–183.
- van Waveren, I.M., Iskandar, E.A.P., Booi, M. & van Konijnenburg-van Cittert, J.H.A. (2007) Composition and palaeogeographic position of the Early Permian Jambi flora from Sumatra. *Scripta Geologica*, **135**, 1–28.
- van Welzen, P.C., Turner, H. & Hovenkamp, P. (2003) Historical biogeography of Southeast Asia and the West Pacific,

or the generality of unrooted area networks as historical biogeographic hypotheses. *Journal of Biogeography*, **30**, 181–192.

Wheeler, W. (1996) Optimization alignment: the end of multiple sequence alignment in phylogenetics? *Cladistics*, **12**, 1–9.

Wheeler, W.C. (1995) Sequence alignment, parameter sensitivity, and the phylogenetic analysis of molecular data. *Systematic Biology*, **44**, 321–331.

Wheeler, W.C. (2003) Iterative pass optimization of sequence data. *Cladistics*, **19**, 254–260.

Xiong, B. & Kocher, T.D. (1991) Comparison of mitochondrial DNA sequences of seven morphospecies of black flies (Diptera: Simuliidae). *Genome*, **34**, 306–311.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Collection locality information for all *Cyphophthalmi* and outgroup *Opiliones* analysed in this study.

Appendix S2 GenBank accession numbers for all sequence data analysed.

Appendix S3 Results from the modified incongruence length difference (ILD) test.

Appendix S4 Phylogeny of the expanded terminal set.

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