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ORIGINAL ARTICLE

Biogeography of the world: a case study from cyphophthalmid Opiliones, a globally distributed group of arachnids

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ABSTRACT

Aim To test the hypothesis that continental drift drives diversification of organisms through vicariance, we selected a group of primitive arachnids which originated before the break-up of Pangaea and currently inhabits all major landmasses with the exception of Antarctica, but lacks the ability to disperse across oceanic barriers.

Location Major continental temperate to tropical landmasses (North America, South America, Eurasia, Africa, Australia) and continental islands (Bioko, Borneo, Japan, Java, New Caledonia, New Guinea, New Zealand, Sri Lanka, Sulawesi, Sumatra).

Methods Five kb of sequence data from five gene regions for more than 100 cyphophthalmid exemplars were analysed phylogenetically using different methods, including direct optimization under parsimony and maximum likelihood under a broad set of analytical parameters. We also used geological calibration points to estimate gross phylogenetic time divergences.

Results Our analyses show that all families except the Laurasian Sironidae are monophyletic and adhere to clear biogeographical patterns. Pettalidae is restricted to temperate Gondwana, Neogoveidae to tropical Gondwana, Stylocellidae to Southeast Asia, and Troglosironidae to New Caledonia. Relationships between the families inhabiting these landmasses indicate that New Caledonia is related to tropical Gondwana instead of to the Australian portion of temperate Gondwana. The results also concur with a Gondwanan origin of Florida, as supported by modern geological data.

Main conclusions By studying a group of organisms with not only an ancient origin, low vagility and restricted habitats, but also a present global distribution, we have been able to test biogeographical hypotheses at a scale rarely attempted. Our results strongly support the presence of a circum-Antarctic clade of formerly temperate Gondwanan species, a clade restricted to tropical Gondwana and a Southeast Asian clade that originated from a series of early Gondwanan terranes that rifted off northwards from the Devonian to the Triassic and accreted to tropical Laurasia. The relationships among the Laurasian species remain more obscure.

Keywords
Arachnida, Arthropoda, Cyphophthalmi, Gondwana, New Caledonia, Opiliones, Pangea, phylogeny, Southeast Asia, vicariance biogeography.

INTRODUCTION

A profound understanding of the distributions of organisms through space and time led Charles Darwin and Alfred R. Wallace to present jointly the most influential biological hypothesis ever formulated, their theory of evolution through natural selection (Darwin & Wallace, 1858). Today, many evolutionary biologists continue the tradition of Darwin and

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Wallace by focusing on biogeography, the scientific discipline that studies the geographical distributions of living organisms and the ecological and historical causes that explain them (Nelson & Platnick, 1981; Crisci *et al.*, 2003). Although theoretical contributions to historical biogeography are numerous, most empirical studies have focused exclusively on the Southern Hemisphere (Sanmartín & Ronquist, 2004; Giribet & Edgecombe, 2006; Van Bocxlaer *et al.*, 2006). To date, few conclusive empirical studies of the worldwide historical biogeography of terrestrial organisms are available, because the members of clades with global distributions tend to present dispersal abilities that obscure historical biogeographical patterns (e.g. Dumont *et al.*, 2005; Bossuyt *et al.*, 2006). To find a group of land organisms with an ancient global distribution, and therefore suitable for a study of historical biogeography on a global scale, one needs to look among the earliest colonizers of terrestrial environments.

Arachnids were among the very first groups of animals to conquer land during the Siluro-Devonian (Shear, 1991; Dunlop, 1997), when all current landmasses except East Asia and Siberia formed a megacontinent that later fully assembled as Pangaea. Harvestmen (order Opiliones) may be among the earliest arachnids (Shultz, 1990; Wheeler & Hayashi, 1998; Giribet *et al.*, 2002), with Cyphophthalmi constituting the oldest group of harvestmen (Shultz, 1998; Giribet *et al.*, 2002). Their sister group is already found in the 400-million-year-old Devonian Rhynie cherts of Scotland (Dunlop *et al.*, 2003, 2004 (for 2003); Dunlop, 2007). Cyphophthalmi were probably distributed throughout Pangaea, where the group underwent an initial phase of diversification into the major lineages known today (Giribet & Kury, 2007). Since then, their evolutionary history has been driven by vicariance, with only a single putative instance of trans-oceanic dispersal documented (Clouse & Giribet, 2007).

Cyphophthalmi mainly inhabit tropical to temperate rainforests all over the globe – with the exception of islands of oceanic origin – including most of the so-called terrestrial biodiversity hotspots (Myers *et al.*, 2000) (Fig. 1). These harvestmen are currently classified into six families (Pinto-da-Rocha *et al.*, 2007), each restricted to a well-defined biogeographical region (Giribet & Kury, 2007). Pettalidae occurs in the former temperate Gondwana, including Australia, Madagascar, New Zealand, Southern Africa, southern South America and Sri Lanka; Sironidae inhabits the former Laurasia, with representatives in Europe, Japan and North America; Neogoveidae occurs in Florida, tropical South America and tropical West Africa, which constituted tropical Gondwana; Stylocellidae occurs in mainland Southeast Asia as well as the islands of Sundaland and Wallacea, including Borneo, Java, Palawan, Sulawesi, Sumatra and the so-called Bird's Head in western New Guinea; and finally the two monogeneric families Troglosironidae and Ogoveidae occur in New Caledonia and tropical West Africa, respectively (Shear, 1980; Juberthie, 1988; Giribet, 2000; Boyer & Giribet, 2007; Giribet & Kury, 2007). Cyphophthalmi have undergone notable radiations in isolated areas such as the Balkans (Boyer *et al.*, 2005), New Zealand (Forster, 1948, 1952; Boyer & Giribet, 2007) and Sumatra (authors' unpublished data), and their sensitivity to habitat degradation makes them potentially valuable indicators of forest quality.

In order to evaluate the hypothesis that Cyphophthalmi have diversified by vicariance as land masses have drifted away from an ancestral megacontinent, we collected specimens of this cryptic group of arachnids in all continents and most major continental islands where Cyphophthalmi have been reported (Fig. 1), with the exceptions of Madagascar, Corsica and Sardinia. We then performed a phylogenetic analysis of DNA sequence data derived from five molecular loci evolving

Figure 1 Distribution of the cyphophthalmid specimens treated in this study. Locality data were generated with the GIS software ArcMap 9.1. Colours reflect current families: orange for Sironidae, green for Neogoveidae, red for Pettalidae, blue for Stylocellidae and purple for Troglosironidae. The only landmass with Cyphophthalmi not included in our study is Madagascar; the monogeneric West African family Ogoveidae was not included.

at different rates using different methods and techniques and estimated time divergences for certain key splits.

MATERIALS AND METHODS

Species sampling

Specimens were collected from leaf litter and humus samples in all major landmasses where Cyphophthalmi have been reported except for the Mediterranean islands of Corsica and Sardinia, where a field trip in October 2005 by G.G. failed to provide any of the two known *Parasiro* species, and Madagascar, where large surveys of soil fauna by the California Academy of Sciences have not yielded any specimens of the monotypic genera *Ankaratra* and *Manangotria* (Shear & Gruber, 1996). All collections for specimens included in our analyses have been included in Fig. 1, generated with the GIS software ArcMap 9.1 (Rockware, 2005). The sampling represents all families except for the monogeneric family Ogoveidae, and all the non-monotypic genera (except the ogoveid genus *Ogovea*) plus several monotypic genera. Ogoveidae is known from three species in West Africa (Giribet & Prieto, 2003; Giribet, 2007b), but a trip to Bioko in July 2003 by G.G. did not yield any *Ogovea* specimens, despite providing numerous specimens of neogoveids.

Molecular methods

Molecular markers included two nuclear ribosomal genes (complete 18S rRNA, and a 2.2 kb fragment of 28S rRNA), one nuclear protein-encoding gene (histone H3), and two mitochondrial markers, one ribosomal (16S rRNA) and one protein-encoding (cytochrome *c* oxidase subunit I). These markers have proven informative in many evolutionary studies on arthropods, including harvestmen and other arachnids (Hormiga *et al.*, 2003; Prendini *et al.*, 2003, 2005; Boyer *et al.*, 2005; Boyer & Giribet, 2007).

Total DNA was extracted from whole animals using Qiagen's DNEasy® Tissue Kit (Valencia, CA, USA), either by crushing the individual or one appendage in the lysis buffer or by incubating an intact animal or appendage in lysis buffer overnight, then removing the specimen before proceeding with the rest of the manufacturer's extraction protocol, as described by Boyer *et al.* (2005).

Purified genomic DNA was used as a template for PCR amplification of the genes for 18S rRNA, 28S rRNA, 16S rRNA, cytochrome *c* oxidase subunit I (COI hereafter) and histone H3. The complete 18S rRNA (*c.* 1.8 kb) was amplified in three overlapping fragments of *c.* 900 bp each, using primer pairs 1F–5R, 3F–18Sbi and 18Sa2.0–9R (Giribet *et al.*, 1996; Whiting *et al.*, 1997). An additional primer pair internal to 1F–5R was used for sequencing, 4R (Giribet *et al.*, 1996). The first *c.* 2200 bp of 28S rRNA were amplified using the primer sets 28SD1F/28Srd1a–28Sb (Whiting *et al.*, 1997; Park & Ó Foighil, 2000; Edgecombe & Giribet, 2006), 28Sa–28Srd5b (Whiting *et al.*, 1997; Schwendinger & Giribet, 2005) and 28S4.8a–28S7bi (Schwendinger & Giribet, 2005). Sequencing of the 28S rRNA gene was performed with those primers and some additional internal primers: 28Sa (Whiting *et al.*, 1997) and 28Srd4b (Edgecombe & Giribet, 2006). 16S rRNA was amplified and sequenced using the primer pair 16Sar–16Sb (Xiong & Kocher, 1991; Edgecombe *et al.*, 2002). COI was amplified and sequenced using the primer pair LCO1490–HCO2198 (Folmer *et al.*, 1994). The complete coding region of histone H3 was amplified and sequenced using primer pair H3aF–H3aR (Colgan *et al.*, 1998).

Polymerase chain reactions (PCR) (50 μL) included 4 μL of template DNA, 1 μM of each primer, 200 μM of dinucleotide-triphosphates (dNTPs; Invitrogen), 1× PCR buffer containing 1.5 mM $MgCl_2$ (Applied Biosystems, Branchburg, NJ, USA) and 1.25 units of AmpliTaq DNA polymerase (Applied Biosystems). The PCR reactions were carried out using a GeneAmp PCR System 9700 thermal cycler, and involved an initial denaturation step (5 min at 95°C) followed by 35 cycles including denaturation at 95°C for 30 s, annealing (ranging from 42 to 49°C) for 30 s and extension at 72°C for 1 min, with a final extension step at 72°C for 10 min.

The double-stranded PCR products were visualized by agarose gel electrophoresis (1% agarose), and purified using Qiagen QIAQuick spin columns. The purified PCR products were sequenced directly; each sequence reaction contained a total volume of 10 μL including 2 μL of the PCR product, irrespective of PCR yield, 1 μM of one of the PCR primer pairs, 1 μL of ABI BigDye™ 5× sequencing buffer and 0.5 μL of ABI Big Dye™ Terminator v3.0 (Applied Biosystems). The sequence reactions, performed using the thermal cycler described above, involved an initial denaturation step for 3 min at 95°C, and 25 cycles (95°C for 10 s, 50°C for 5 s, 60°C for 4 min). The BigDye-labelled PCR products were cleaned with AGTC® gel filtration cartridges or plates (Edge BioSystems, Galthesburg, MD, USA). The sequence reaction products were then analysed using an ABI Prism 3100 or 3730 genetic analyser.

Chromatograms obtained from the automatic sequencer were read and 'contig sequences' (assembled sequences) were assembled using the sequence editing software sequencher™ 4.7 (Gene Codes Corporation, Am Arbor, MI, USA). Sequence data were edited in MacGDE 2.2 (Linton, 2005). All new sequences have been deposited in GenBank under accession numbers DQ825507–DQ825650, EF108574–EF108596 and EF028095–EF028096 (Table 1).

Phylogenetic analysis

Data analyses were based on a direct optimization approach using parsimony (Wheeler, 1996) and maximum likelihood (Wheeler, 2006) as optimality criteria. DNA sequence data were analysed under the dynamic regime of direct optimization (Wheeler, 1996) in the computer package poy v.3.0.11 (Wheeler *et al.*, 2004). Tree searches were conducted by a combination of random addition sequences with subtree pruning and regrafting (SPR) and tree bisection and reconnection (TBR) branch swapping followed by multiple rounds

Table 1 List of species with MCZ accession numbers, locality data, collection coordinates and GenBank accession numbers for the five markers employed in this study. For more details on collecting localities contact the authors.

	MCZ Accession #	Locality	Coordinates	18S rRNA	28S rRNA	16S rRNA	COI	Histone H3
OUTGROUPS								
Protolophus singularis	DNA101033	California, USA		EF028095	EF028096	EF108581	EF108586	EF108592
Megalopsalis sp.	DNA100783	SI, New Zealand		EF108573	EF108576	EF108582	EF108587	EF108593
Hesperonemastoma modestum	DNA100312	Oregon, USA		AF124942	EF108577	EF108583	EF108588	EF108594
Dendrolasma parvulum	DNA100318	Japan		EF108574	EF108578	EF108584	EF108589	
Equitius doriae	DNA100607	Australia		U37003	EF108579		EF108590	EF108595
Oncopus malayanus	DNA100321	Malaysia		EF108575	EF108580	EF108585	EF108591	EF108596
FAMILY NEOGOVEIDAE								
Huitaca n.sp. Boyacá	DNA101407	Colombia	5°46'46''N, 73°27'13''W	DQ518090	DQ825596	DQ518050	DQ518129	DQ518167
Metagovea n.sp.	DNA101410	Colombia	1°17'06''N, 78°04'25''W	DQ518091	DQ825597			DQ518168
Metasiro americanus	DNA101532	Florida, USA	30°33'53''N, 84°57'05''W	DQ825542	DQ825595	DQ825616	DQ825645	DQ825513
Neogovea n.sp.	DNA101408	Colombia	4°02'41''S, 69°59'23''W	DQ825543	DQ825598	DQ825618	DQ825646	DQ825514
Neogovea n.sp.	DNA101409	Colombia	1°17'06''N, 78°04'25''W	DQ825544	DQ825599	DQ825619	DQ825646	
Neogoveidae sp.	DNA100869	Venezuela	5°39'N, 67°38'W*	DQ825545	DQ825600	DQ825617	DQ825647	
Paragovia n.sp.	DNA101052	Equatorial Guinea	1°39'29''N, 10°18'41''E	DQ825546	DQ825601	DQ825620	DQ825648	DQ825515
Paragovia n.sp.	DNA101057	Equatorial Guinea	2°07'52''N, 9°52'18''E	DQ825547	DQ825602	DQ825621		DQ825516
Paragovia cf. *sironoides*	DNA100462	Equatorial Guinea	2°19'59''N, 9°48'11''E	AY639493	DQ825603	DQ825622	AY639581	AY639459
Paravodia cf. *sironoides*	DNA101053	Equatorial Guinea	1°39'29''N, 10°18'41''E	DQ825548	DQ825604	DQ825623	DQ825649	DQ825517
Paragovia cf. *sironoides*	DNA101056	Equatorial Guinea	1°26'54''N, 9°46'51''E	DQ825549	DQ825605	DQ825624	DQ825650	DQ825518
Paragovia sironoides	DNA101059	Bioko, Equatorial Guinea	3°43'32''N, 8°50'17''E	DQ518092	DQ825606	DQ518051	DQ518131	DQ518169
Paragovia sironoides	DNA101061	Bioko, Equatorial Guinea	3°42'10''N, 8°52'30''E	DQ825550	DQ825607			DQ825519
FAMILY PETTALIDAE								
Aoraki calcarobtusa westlandica	DNA101125	SI, New Zealand	42°06'04''S, 171°24'54''E	DQ518004	DQ518038	DQ518070	DQ518121	DQ518163
Aoraki crypta	DNA101289	NI, New Zealand	37°32'02''S, 175°44'29''E	DQ518000	DQ518043	DQ518068	DQ518120	DQ518156
Aoraki denticulata	DNA100961	SI, New Zealand	41°48'29''S, 172°50'01''E	DQ518001	DQ518040	DQ518069	DQ518126	DQ518158
Aoraki inerma	DNA100967	NI, New Zealand	38°43'45''S, 177°09'46''E	DQ518003	DQ518041			DQ518159
Austropurcellia arcticosa	DNA100951	QLD, Australia	17°09'58''S, 145°24'56''E	DQ517984	DQ518023		DQ518111	DQ518147
Austropurcellia daviesae	DNA100947	QLD, Australia	17°14'44S, 145°38'32''E	DQ517985	DQ518024		DQ518112	DQ518148
Austropurcellia forsteri	DNA100945	QLD, Australia	16°03'41''S, 145°27'44''E	DQ517983	DQ518022	DQ518064	DQ518110	DQ518146
Austropurcellia scoparia	DNA100946	QLD, Australia	16°35'41''S, 145°16'46''E	DQ517982	DQ518021	DQ518065	DQ518108	
Chileogovea oedipus	DNA101413	Chile	40°40'S, 72°36'W*	DQ133721	DQ825571	DQ518055	DQ133745	
Chileogovea sp.	DNA101490	Chile	39°50'W, 73°13'W*	DQ133722	DQ825572	DQ518054	DQ825635	DQ518133
Karripurcellia harveyi	DNA101303	WA, Australia	34°29'42''S, 115°58'31''E	DQ517980	DQ825578	DQ518062	DQ518106	DQ518143
Karripurcellia harveyi	DNA101304	WA, Australia	34°32'23''S, 116°02'27''E	DQ517981	DQ825579	DQ518063	DQ518107	DQ518144
Neopurcellia salmoni	DNA100939	SI, New Zealand	44°06'28''S, 169°21'19''E	DQ517998		DQ518066	DQ825638	DQ518145
Neopurcellia salmoni	DNA100949	SI, New Zealand	43°28'08''S, 170°01'06''E	DQ825539	DQ518037		DQ518019	DQ825512
Parapurcellia monticola	DNA100386	South Africa	29°03'12''S, 29°23'06''E	DQ518973	DQ825575		DQ518098	DQ518135
Parapurcellia silvicola	DNA100385	South Africa	28°44'39''S, 31°08'15''E	AY639494	DQ825574	DQ518053	AY639582	DQ518136
Pettalus cf. *brevicauda*	DNA101227	Sri Lanka	7°15'54''N, 80°35'39''E	DQ517975	DQ518018	DQ518057	DQ518101	DQ518138
Pettalus n.sp. 1	DNA101282	Sri Lanka	7°23'05''N, 80°49'01''E	DQ825537	DQ825576	DQ825613	DQ825636	DQ825510

Table 1 continued

	MCZ Accession #	Locality	Coordinates	18S rRNA	28S rRNA	16S rRNA	COI	Histone H3
Pettalus n.sp. 2	DNA101283	Sri Lanka	7°23'05''N, 80°49'01''E	DQ517974	DQ518016	DQ518056	DQ518100	DQ518137
Pettalus n.sp. 3	DNA101285	Sri Lanka	6°55'30''N, 80°49'10''E	DQ517976	DQ518017	DQ518058	DQ518102	DQ518139
Pettalus n.sp. 4	DNA101286	Sri Lanka	6°55'30''N, 80°49'10''E	DQ517977	DQ518013	DQ518059	DQ518103	DQ518140
Pettalus n.sp. 5	DNA101287	Sri Lanka	6°49'24''N, 80°50'59''E	DQ517978	DQ518014	DQ518060	DQ518104	DQ518141
Pettalus n.sp. 6	DNA101288	Sri Lanka	6°33'19''N, 80°22'13''E	DQ517979	DQ518015	DQ518061	DQ518105	DQ518142
Pettalus n.sp. 7	DNA101574	Sri Lanka	6°23'N, 80°28'E*	DQ825538	DQ825577	DQ825614	DQ825637	DQ825511
Purcellia illustrans	DNA100387	South Africa	33°58'58''S, 18°25'28''E	AY639495	DQ825573	DQ518052	DQ518099	DQ518134
Rakaia antipodiana	DNA100957	SI, New Zealand	43°15'05''S, 171°22'03''E	DQ517988	DQ518031	DQ518072	DQ518115	DQ518151
Rakaia florensis	DNA101295	SI, New Zealand	40°49'57''S, 172°58'8''E	DQ517986	DQ518025	DQ518083	DQ518113	DQ518149
Rakaia lindsayi	DNA101128	StI, New Zealand	43°02'03''S, 171°45'53''E	DQ517995	DQ518027	DQ518081	DQ518118	DQ518154
Rakaia magna australis	DNA100963	SI, New Zealand	42°22'38''S, 172°24'12''E	DQ517991	DQ518034	DQ518076	DQ518124	DQ518152
Rakaia minutissima	DNA101291	NI, New Zealand	39°24'59''S, 175°13'07''E	DQ517987	DQ518026	DQ518082	DQ518114	DQ518150
Rakaia solitaria	DNA101294	NI, New Zealand	41°28'01''S, 175°26'56''E	DQ517997	DQ518029	DQ518075	DQ518119	DQ518155
Rakaia sorenseni sorenseni	DNA100969	SI, New Zealand	46°06'35''S, 167°41'25''E	DQ517993	DQ518036	DQ518079	DQ518116	DQ518153
Rakaia stewartiensis	DNA100944	StI, New Zealand	46°53'36''S, 168°06'14''E	DQ517994	DQ518028	DQ518080	DQ518117	
FAMILY SIRONIDAE								
Cyphophthalmus duricorius	DNA100487	Slovenia	46°01'N, 14°40'E*	AY639461	DQ513120	AY639526	AY639556	
Cyphophthalmus eratoae	DNA100497	Macedonia	41°25'N, 22°16'E*	AY639469	DQ825590	AY639543	AY639561	AY639447
Cyphophthalmus ere	DNA100499	Serbia	43°50'N, 20°03'E*	AY639462	DQ825593	AY639527	AY639557	AY639444
Cyphophthalmus gjorgjevici	DNA100498	Macedonia	41°58'N, 21°21'E*	AY639464	DQ825587	AY639529	AY639559	
Cyphophthalmus gordani	DNA100495	Montenegro	42°27'N, 19°16'E*	AY639467	DQ825592	AY639531		AY639446
Cyphophthalmus martensi	DNA100494	Montenegro	42°24'N, 18°46'E*	AY539471	DQ825589	AY639536	AY639563	AY639449
Cyphophthalmus minutus	DNA100493	Montenegro	42°39'N, 18°40'E*	AY639473	DQ825591	AY639537	AY639565	AY639450
Cyphophthalmus ognjanovici	DNA101039	Bosnia & Hertz.	43°01'N, 18°31'E*	AY639475	DQ825594		AY639567	AY639451
Cyphophthalmus rumijae	DNA101492	Montenegro	42°10'N, 19°20'E*	AY639477	DQ825588	AY639539	AY639569	AY639453
Cyphophthalmus teyrovskyi	DNA100910	Montenegro	42°14'N, 19°04'E*	AY639482	DQ513118	AY639544	AY639571	AY639454
Cyphophthalmus trebinjanum	DNA101038	Bosnia & Hertz.	42°14'N, 19°10'E*	AY639483	DQ513119		AY639572	
Cyphophthalmus zetae	DNA100907	Montenegro	42°56'N, 18°30'E*	AY639485	AY639515	AY639546	AY639574	AY639456
Cyphophthalmus n.sp. Bulgaria1	DNA101342	Bulgaria	41°24'N, 23°13'E*	AY918870	DQ513117		AY918878	AY918880
Cyphophthalmus n.sp. Bulgaria2	DNA101343	Bulgaria	41°35'N, 24°41'E*	AY918871	DQ825586	AY918876	AY918879	AY918881
Paramiopsalis ramulosus	DNA100459	Spain	42°18'54''N, 8°29'12''W	AY639489	DQ513121	AY639550	DQ825641	
Parasiro coiffaiti	DNA101383	Spain	42°09'09''N, 1°55'49''E	AY918872	DQ513122	AY918877	DQ825642	AY918882
Siro acaroides	DNA100488	Oregon, USA	44°35'N, 123°31'W*	AY639490	DQ513128	AY639551	DQ825643	
Siro n.sp., Washington	DNA101614	Washington, USA	45°54'56''N, 123°57'52''W	DQ513139	DQ513125		DQ825644	
Siro exilis	DNA100489	Maryland, USA	39°29'N, 79°26'W*	AY639491	DQ825585		AY639579	
Siro kamaiakensis	DNA101611	Washington, USA	47°44'47''N, 116°42'07''W	DQ513147	DQ513134		DQ513115	
Siro rubens	DNA100457	France	44°05'00''N, 3°34'53''E	AY428818	DQ825584	DQ133735	DQ513111	
Siro valleorum	DNA100461	Italy	45°59'N, 9°52'E*	AY639492	DQ513123	AY639552	AY639580	AY639458
Siro n.sp., Calaveras	DNA101623	California, USA	38°16'38''N, 120°18'19''W	DQ513146	DQ513133			
Siro n.sp., Shasta	DNA101622	California, USA	41°03'49''N, 122°21'37''W	DQ513149	DQ513136			
Suzukielus sauteri	DNA101543	Japan	35°38'03''N, 139°14'28''E	DQ513138	DQ513116	DQ518086	DQ513108	DQ518166

Table 1 continued

	MCZ Accession #	Locality	Coordinates	18S rRNA	28S rRNA	16S rRNA	COI	Histone H3
Suzukielus sauteri	DNA101550	Japan	34°50'00''N, 138°55'54''E	DQ825541	DQ825583	DQ825615	DQ825640	
STYLOCELLIDAE								
Fangensis cavernarus	DNA101460	Thailand	14°23'54''N, 99°04'53''E	DQ133714	DQ133726		DQ133740	DQ518132
Fangensis insulanus	DNA100388	Thailand	7°53'06''N, 98°26'13''E	DQ133710	DQ825551		DQ133737	
Fangensis insulanus	DNA101063	Thailand	7°53'07''N, 98°26'14''E	DQ133711	DQ825552		DQ133738	
Fangensis leclerci	DNA100913/100865 combined	Thailand	19°34'33''N, 99°3'40''E	DQ133713	DQ825553			
Fangensis spelaeus	DNA100669	Thailand	14°17'59''N, 98°58'59''E	DQ133712	DQ825554		AY639583	AY639460
Miopsalis n.sp.	DNA101513	Malaysia	4°00'00''N, 114°49'00''E	DQ133713	DQ133727			
Stylocellus cf. *sedgwicki*	DNA100239	Thailand	6°01'N, 101°50'E*	DQ133716	DQ133728		DQ133742	
Stylocellus n.sp.	DNA100240	Malaysia	5°48'N, 102°24'E*	AF173419	AF173422		DQ133741	
Stylocellus n.sp.	DNA100870	Indonesia	6°49'S, 106°35'E*	DQ825521	DQ825560	DQ825608		
Stylocellus n.sp.	DNA100608	Indonesia		DQ133718	DQ133730			DQ825507
Stylocellus n.sp.	DNA100609	Indonesia		DQ133719	DQ133731		DQ825625	
Stylocellus n.sp.	DNA100610	Indonesia	5°33'S, 104°25'E*	DQ133720	DQ133732		DQ133744	
Stylocellus lydekkeri	DNA101064	Indonesia	2°43'S, 134°30'E*	DQ133717	DQ133729			
Stylocellus n.sp.	DNA101468	Indonesia	1°14'N, 117°46'E*	DQ825524	DQ825561			
Stylocellus n.sp.	DNA101469	Indonesia	3°42'S, 102°30'E*	DQ825522	DQ825557		DQ825626	
Stylocellus n.sp.	DNA101472	Indonesia	3°59'S, 102°21'E*	DQ825523	DQ825558		DQ825627	DQ825509
Stylocellus n.sp.	DNA101474	Indonesia	1°41'S, 101°16'E*	DQ518093	DQ825556			
Stylocellus n.sp.	DNA101478	Indonesia	0°26'S, 100°25°E*	DQ518094	DQ825555		DQ518096	
Stylocellus n.sp.	DNA101483	Malaysia	4°23'49''N, 102°25'50''E	DQ825532	DQ825567	DQ825610	DQ825633	
Stylocellus n.sp.	DNA101486	Malaysia	2°21'22''N, 102°37'49''E	DQ825533	DQ825568		DQ825631	
Stylocellus n.sp.	DNA101488	Malaysia	3°54'41''N, 103°08'50''E	DQ825529	DQ825566		DQ825634	
Stylocellus n.sp.	DNA101489	Malaysia	3°42'59''N, 101°44'19''E	DQ518095	DQ825565	DQ518087	DQ518097	
Stylocellus n.sp.	DNA101490	Malaysia	3°42'59''N, 101°44'19''E	DQ825535	DQ825569			
Stylocellus n.sp.	DNA101492	Malaysia	5°28'N, 100°15'E*	DQ825531				
Stylocellus n.sp.	DNA101494	Thailand	9°55'05''N, 98°56'34''E	DQ825530			DQ825632	
Stylocellus n.sp.	DNA101500	Thailand	7°53'07''N, 98°26'14''E	DQ825534				
Stylocellus n.sp.	DNA101507	Indonesia	0°12'35''S, 104°36'58''E	DQ825528				
Stylocellus novaguinea	DNA101510	Indonesia	0°50'S, 134°02'E*	DQ825536	DQ825570	DQ825609		
Stylocellus n.sp.	DNA101511	Indonesia	1°19'N, 124°49'E*		DQ825559			
Stylocellus n.sp.	DNA101514	Malaysia	1°46'00''N, 110°19'00''E	DQ825525	DQ825562	DQ825611	DQ825628	
Stylocellus n.sp.	DNA101517	Indonesia	1°04'N, 117°50'E*	DQ825527	DQ825564		DQ825630	DQ825508
Stylocellus n.sp.	DNA101519	Indonesia	0°55'N, 117°54'E*	DQ825526	DQ825563	DQ825612	DQ825629	
FAMILY TROGLOSIRONIDAE								
Troglosiro juberthiei	DNA100344	New Caledonia	22°03'S, 166°28'E	DQ825540				
Troglosiro aelleni	DNA100345	New Caledonia	21°11'S, 165°19'E	AY639497	DQ825580	AY639555	AY639584	DQ518164
Troglosiro longifossa	DNA100867	New Caledonia	22°21'S, 166°58'E	DQ518089	DQ825582	DQ518084	DQ825639	DQ518165
Troglosiro ninqua	DNA101577	New Caledonia	21°45'S, 166°09'E	DQ518088	DQ825581	DQ518085	DQ518128	

NI, North Island; QLD, Queensland; SI, South Island; StI, Stewart Island; WA, Western Australia.

*, Latitude and longitude estimated from locality description.

of tree fusing (Goloboff, 1999, 2002) on a small 50-processor cluster at Harvard University (darwin.oeb.harvard.edu).

For parsimony we undertook a sensitivity analysis of 10 parameter sets varying the relative contributions of indels and base transformations (Wheeler, 1995), and used an index of congruence for selecting a favoured parameter set, represented in Table 2. Our sensitivity analysis included a parameter set, designated 3221, which some argue represents a philosophical equivalent to unweighted parsimony (De Laet, 2005).

Nodal support was estimated via jackknifing with a probability of deletion of e^{-1} (Farris *et al.*, 1996; Farris, 1997). The data were analysed in combination and for each independent partition under different analytical parameter sets, in order to perform a sensitivity analysis (Wheeler, 1995; Giribet, 2003). The optimal parameter set was used for generating an implied alignment (Wheeler, 2003; Giribet, 2005), which was later used for estimating the divergence times in r8s v.1.71 (Sanderson, 2006).

We also performed a maximum likelihood analysis to evaluate the effect of models on the phylogeny of Cyphophthalmi and therefore on the biogeographical implications. For maximum likelihood we used a model of sequence evolution equivalent to GTR + indels with corrections for a discrete gamma distribution (Γ) and a proportion of invariant sites (I), as selected in Modeltest v.3.7 (Posada, 2005) under the Akaike information criterion (Posada & Buckley, 2004). As a starting point we used the same pool of trees used for the parsimony tree fusing analysis and proceeded to tree fusing using the likelihood criterion.

Estimating divergence times

The ages of several clades were estimated using a standard likelihood method, assuming a molecular clock, as implemented in the program r8s 1.71 (Sanderson, 1997, 2006). This method requires at least one node of fixed age within the tree. Other ages assigned to clades may be entered as constraints on either maximum or minimum age. Only one fossil cyphophthalmid is known, from the Bitterfeld amber in Germany, dated at between 35 and 22 Ma (Dunlop & Giribet, 2003). Morphologically, this specimen is a member of the *Cyphophthalmus* + *Paramiopsalis* + *Siro* clade. Given the clear ingroup position of this species and its uncertain age, we did not use it as a constraint. Dates were assigned to clades based on biogeographical vicariance events, such as the opening of the Mascarene basin separating Sri Lanka from Madagascar 88–84 Ma. In this case, a minimum age of 84 Ma was assigned to the clade of animals from Sri Lanka. Other dates employed in this analysis include the rifting of Gondwana and Laurasia 165 Ma, assigned to the base of the clade including *Paragovia* and *Metasiro*, and the initial break-up of Gondwana (the rifting of Madagascar + Greater India from Africa 120 Ma; Sanmartín, 2002) assigned to the base of Pettalidae. Using these three dates, we ran a series of analyses, where in each iteration the age of one clade was fixed, leaving the other two dates set as minimum clade ages. We also ran analyses using those fixed dates only without additional constraints (see Table 4).

RESULTS

Phylogenetic analyses

For the parsimony direct optimization analyses, parameter set 221 minimized overall incongruence among partitions and was thus selected as the 'optimal' parameter set for these data (Table 2). After two rounds of tree fusing, the analyses found 50 shortest trees of 27,254 weighted steps. The strict consensus of the 50 trees found under the optimal parameter set is shown in Figure 2. Families have been colour-coded and jackknife values higher than 50% are plotted on each node. This tree shows monophyly of Cyphophthalmi, as well as that of the families Pettalidae (red, clade a), Troglosironidae (purple), Neogoveidae (green) and Stylocellidae (blue, clade c). On the contrary, the family Sironidae (orange) appears to form three independent clades, one containing the genera *Siro*, *Paramiopsalis* and *Cyphophthalmus* (clade d), and two clades for *Suzukielus* (a monotypic genus from Japan) and *Parasiro* (a genus endemic to the western Mediterranean). This tree also shows high geographical structure within the families Pettalidae, Sironidae and Neogoveidae and illustrates poor resolution within Stylocellidae.

A maximum likelihood tree, based on the ribosomal data only, had a $-\log L = 25737.65$ and it shows an alternative resolution at the base of the tree, with stylocellids as the sister group to all other cyphophthalmids, followed by pettalids (fig. 3). This implies that the presence of eyes is a plesiomorphy of the suborder Cyphophthalmi, and not a derived feature as previously thought (Fig. 3).

Table 2 Tree lengths for the different partitions analysed (18S, 18S rRNA; 28S, 28S rRNA; COI, cytochrome *c* oxidase subunit I; 16S, 16S rRNA; H3, histone H3; RIB, nuclear ribosomal data; MOL, five loci combined) and congruence values (ILD) for the combined analysis of the five molecular loci combined at different parameter sets (left column).

	18S	28S	COI	16S	H3	RIB	MOL	ILD
111	596	3405	7301	4300	933	4032	16935	0.0236
121	828	4955	11135	6854	1268	5844	25633	0.0231
141	1271	7589	18106	10869	1907	9021	40838	0.0268
211	615	3793	7396	4738	933	4445	17890	0.0232
221	*859*	*5639*	*11229*	*7655*	*1268*	*6559*	*27254*	*0.0222*
241	1341	8976	18590	12823	1907	10449	44888	0.0279
411	647	4296	7412	5127	933	4967	18851	0.0231
421	919	6553	11264	8385	1268	7524	29139	0.0257
441	1461	10795	18706	14646	1907	12418	48778	0.0259
3221	1212	6799	14684	8800	1866	8082	34185	0.0241

The first numeral used in the parameter set (leftmost) column corresponds to the ratio between indel/transversion and the following two numbers correspond to the ratio between transversion/transition; e.g. 111 is equal weights, 121 corresponds to an indel/transversion ratio of 1 and a transversion/transition ratio of 2:1 – so indels have a cost of 2, transversions have a cost of 2 and transitions have a cost of 1. (For a list of the specific step matrices that this involves see Giribet *et al.*, 2002; Appendix 4.) The optimal ILD value is indicated in italics.

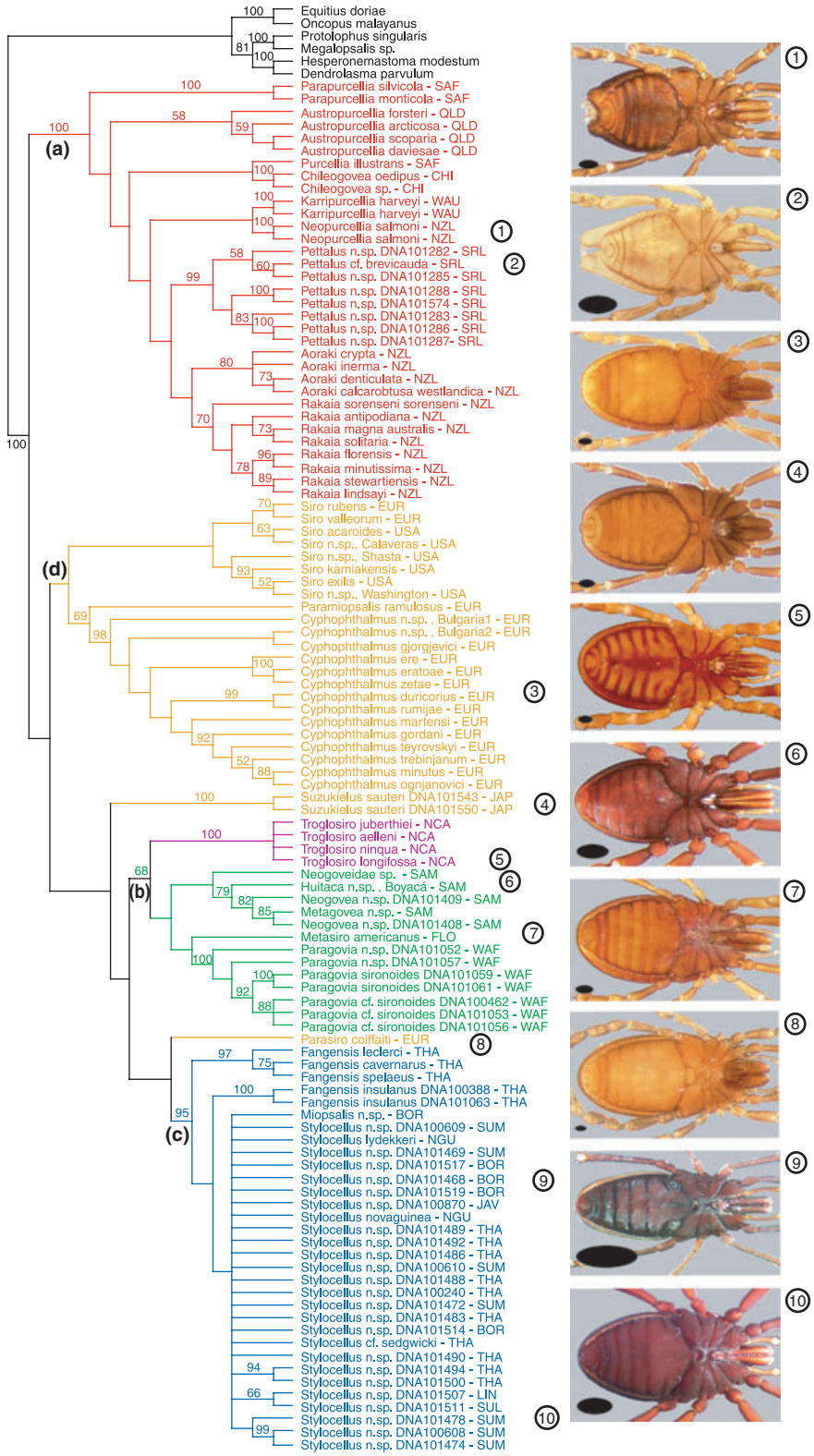

Figure 2 Phylogenetic relationships of cyphophthalmid specimens based on the strict consensus of 50 fundamental trees. Colours correspond to those assigned to each family in Fig. 1. Representative specimens for each clade are illustrated in ventral view; black ellipses are drawn to scale to reflect size differences. Numbers on branches indicate jackknife support values. Landmass abbreviations are: BOR, Borneo; CHI, Chile; EUR, Europe; FLO, Florida; JAP, Japan; JAV, Java; LIN, Lingga Archipelago; NCA, New Caledonia; NGU, New Guinea; NZL, New Zealand; QLD, Queensland; SAF, South Africa; SAM, northern South America; SRL, Sri Lanka; SUL, Sulawesi; SUM, Sumatra; THA, Thailand; USA, United States of America; WAF, West Africa; WAU, Western Australia.

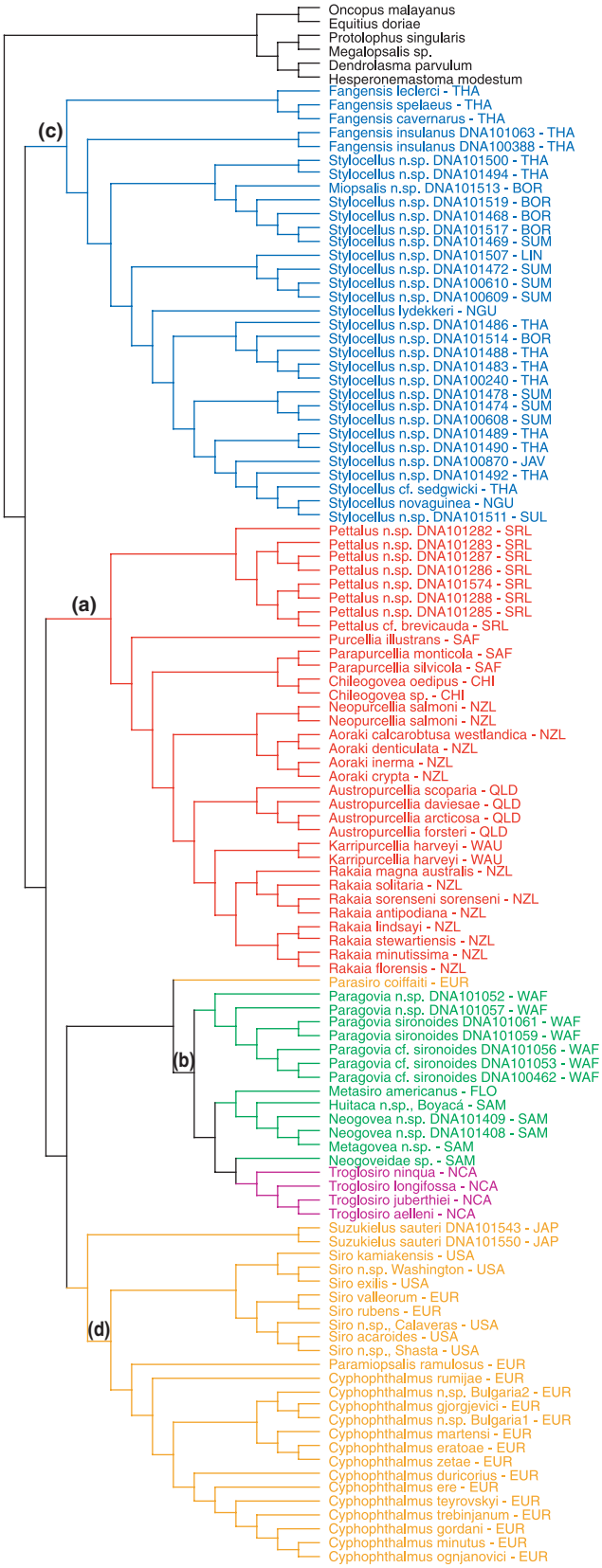

Figure 3 Maximum likelihood tree based on the ribosomal data (–log L = 25737.65) showing an alternative resolution at the base of the Cyphophthalmi tree. Colour coding and abbreviations as in fig. 2.

Parsimony trees obtained under other parameter sets were similar to the one illustrated in Fig. 1, as shown in the Navajo rug (sensitivity plot) (Fig. 4). Monophyly of Pettalidae, Stylocellidae and Troglosironidae is found under all analytical conditions, including the maximum likelihood analysis. Sironidae is monophyletic under several parameter sets when

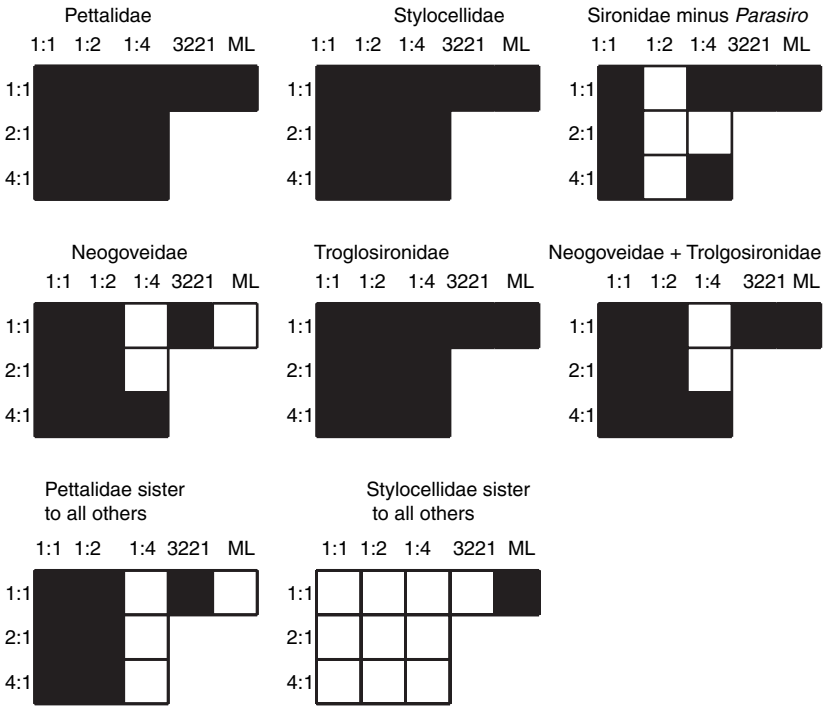

Figure 4 Navajo rugs illustrating the monophyly (black square) or non-monophyly (white square) for selected clades under the explored parameter sets and maximum likelihood (ML) analyses. Ratios represent the weighting scheme of transversion:transition costs (rows) and transversion:gap costs (columns). 3221 represents a weighting scheme that has been argued to be philosophically equivalent to unweighted parsimony.

the Mediterranean genus *Parasiro* is excluded (Fig. 4); this includes the maximum likelihood analysis as well as several parameter sets for the parsimony analysis, but not under the optimal parameter set (Fig. 2). With the exception of Stylocellidae – which is in urgent need of taxonomic evaluation – all the genera of other families included in the analyses are monophyletic.

Biogeography

The combined analysis of all genes using different phylogenetic approaches shows monophyly of four groups of species, each occurring in a major biogeographical region of the globe. Simplified area cladograms reflecting the biogeographical hypotheses implied by Figs 2–3 are provided in Fig. 5. Three of these regions are supported in virtually all analyses including the independent analysis of each gene partition: (1) a temperate (southern; circum-Antarctic) Gondwanan clade, containing all members of the family Pettalidae, (2) a clade uniting the members from tropical (northern) Gondwana (the Neotropical and Afrotropical family Neogoveidae), south-eastern USA (the monotypic genus *Metasiro*) and New Caledonia (the family Troglosironidae), and (3) a Southeast Asian clade, comprising the family Stylocellidae (Figs 2–4). A fourth clade (4) contains all Laurasian species from North America and Europe (family Sironidae) except two unusual Laurasian genera, the monotypic *Suzukielus* from Japan and *Parasiro* from the Western Mediterranean (Figs. 2, 5). The four groups will be referred to hereafter as: (1) temperate Gondwanan (Pettalidae), (2) tropical Gondwanan + New Caledonia (Neogoveidae + Troglosironidae), (3) Southeast Asian (Stylocellidae) and (4) Laurasian (Sironidae).

Date estimates

Results for the age estimates are shown in Table 3. The divergence time estimates suggest that the earliest existence of Cyphophthalmi can be dated between 174 and 312 Ma. Although this age is younger than would be expected phylogenetically – the sister group of Cyphophthalmi was already present in the Devonian (Dunlop *et al.*, 2003, 2004 (for 2003)) – it places the most recent common ancestor to extant

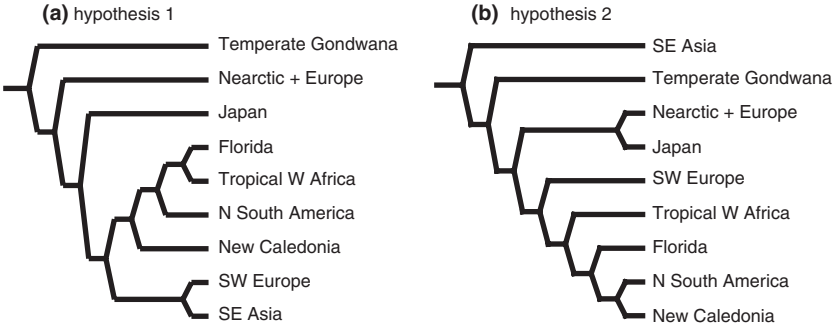

Figure 5 Area cladogram summarizing the hypotheses presented in Fig. 2 (a, hypothesis 1) and Fig. 3 (b, hypothesis 2).

Table 3 Estimated divergence times.

Fixed age	Cypho	Stylo	Troglo+ Neo	Troglo	Neo	MPARA	SAm Neo	PET	SL
PET = 120 + constraints	253	121	195	40	179	165	147		84
PET = 120	174	83	124	28	109	92	91		57
MPARA = 165 + constraints	312	148	221	49	195		163	215	101
MPARA = 165	311	148	221	49	195		163	214	101
SL = 84 + constraints	296	141	213	47	190	165	159	201	
SL = 84	259	123	184	41	162	137	136	178	

PET = Pettalidae; SL = Sri Lanka (*Pettalus*); MPARA = *Metasiro* + *Paragovia;* Cypho = Cyphophthalmi; Stylo = Stylocellidae; Troglo+Neo = Troglosironidae+Neogoveidae; Troglo = Troglosironidae; Neo = Neogoveidae; Meta+Para = *Metasiro*+*Paragovia*; SAm Neo = South American Neogoveidae.

The left column indicates the fixed ages in Ma for different nodes in the tree, based on dates of biogeographical vicariance events. The resulting ages when fixing the age of a given node and using the other dates as minimum ages (+ constraints), or fixing the age of a node without using any other dates as calibration points, are shown in the other columns.

Cyphophthalmi in the Jurassic to Carboniferous, still prior to the break-up of Pangaea. The estimated age for the family Pettalidae is 178–215 Ma, in the Jurassic/Triassic. The split between the families Troglosironidae and Neogoveidae is estimated at 124–221 Ma. Diversification within Neogoveidae occurred at 109–195 Ma, in the Cretaceous/Jurassic, whereas diversification within Troglosironidae is Tertiary, dated at only 28–49 Ma.

DISCUSSION

Relationships among the four major clades suggested by different phylogenetic methods vary little and allow us to draw important conclusions about the biogeography and morphology of ancient Cyphophthalmi. Parsimony analysis recovered the temperate Gondwanan clade at the base of the cyphophthalmid radiation, followed by the Laurasian clade, the Japanese *Suzukielus,* the tropical Gondwanan + New Caledonia clade, and the western Mediterranean *Parasiro* as sister to the Southeast Asian clade (Figs 2 & 5). The maximum likelihood analysis suggested a hypothesis with the Southeast Asian clade as sister to the other cyphophthalmids, with the temperate Gondwanan + New Caledonia clade coming next (Figs 3 & 5). Either resolution suggests that the first Cyphophthalmi offshoot inhabited the Gondwanan portion of Pangaea because stylocellids, although currently in Southeast Asia, inhabit terranes that rifted off from north-eastern Gondwana as separate blocks and accreted to Eurasia in the Mesozoic during the closure of the Palaeotethys Ocean (Rogers & Santosh, 2004). Both hypotheses also suggest that the presence of eyes in Cyphophthalmi, now recognized in most members of the families Stylocellidae and Pettalidae (Sharma & Giribet, 2006; Giribet & Boyer, 2007), but not in those of other families, constitutes a primitive character of the group and not, as was previously thought, a secondarily derived character of stylocellids (e.g., Shear, 1980; Giribet & Boyer, 2002).

The temperate Gondwanan clade

We consistently find monophyly of the temperate Gondwanan family Pettalidae, which currently occurs in Chile, South Africa, Madagascar, Sri Lanka, Western Australia, Queensland and New Zealand. The estimated age of this family is 178–215 Ma, older than the minimum age assigned to the node, indicating diversification within the family prior to the break-up of the supercontinent. This family represents a distinctive example of a Gondwanan group whose distribution may indeed be explained solely by vicariance. Phylogenetic and palaeontological studies have demonstrated that the textbook example of Gondwanan vicariance, the southern beech tree genus *Nothofagus*, actually has a history which includes several major trans-oceanic dispersal events (Cook & Crisp, 2005). A notable illustration of the importance of vicariance in Cyphophthalmi is that all Sri Lankan species are more closely related to other temperate Gondwanan Cyphophthalmi than to members of the Southeast Asian family Stylocellidae, despite the present-day proximity of Sri Lanka to Thailand, Malaysia and Indonesia. The Indian subcontinent rifted from Australia and Africa some 150–160 Ma and collided with Eurasia about 50 Ma (Sanmartín & Ronquist, 2004). Therefore, the close relationship of Sri Lankan Pettalidae to the species from New Zealand, Australia, South Africa and Chile must be ancient indeed.

The Southeast Asian clade

One of the hottest – and perhaps most complicated – topics in biogeography is the origin of the Malay Archipelago (Hall, 2002). The relationship of the Southeast Asian clade to other Cyphophthalmi is not resolved with high support, but a resolution obtained in some analyses indicates a sister relationship with the western Mediterranean *Parasiro* (Fig. 5a). Alternatively, other analyses place stylocellids more basally in the tree, in agreement with our hypothesis that they drifted

northwards and diversified in the Sibumasu terrane (Fig. 5b). The internal relationships of Stylocellidae support a number of postulates about the biogeographical history of the islands in the Malay Archipelago.

Our phylogeny, especially the finding of the Thai genus *Fangensis* as a grade sister to all other species in the family, may imply a southward pattern of cladogenesis from the Thai–Malay Peninsula to the Malay Archipelago, a result that is consistent with postulated historical land connections among the components of Sundaland (Thai–Malay Peninsula, Borneo, Sumatra and Java). The early origin of western Sulawesi as part of Borneo and the submersion of eastern Sulawesi before its collision with western Sulawesi explain the presence of Stylocellidae and the absence of temperate Gondwanan Pettalidae throughout that island (Moss & Wilson, 1998). Consistent with our inferences about the limited trans-oceanic dispersal abilities of these arachnids, the only island in the Philippines from where such animals are known is Palawan, which is also the only Philippine island of continental origin, the rest being volcanic. However, the presence of stylocellid species in the western part of New Guinea can hardly be explained by vicariance, as it seems that New Guinea has never been in contact with Sulawesi. If this were confirmed, it would constitute the first documented case of trans-oceanic dispersal in Cyphophthalmi (Clouse & Giribet, 2007).

The tropical Gondwanan + New Caledonia clade

Morphologically, the members of the tropical Gondwanan clade (Neogoveidae) + the New Caledonian Troglosironidae share striking and unique characters, such as the row of teeth on the claw of the second pair of walking legs (Fig. 6a,b), not found in the members of other families (Fig. 6c,d), and special secretory gland pores in the ventral abdominal region of males. These characters have previously been used to suggest a close relationship between Troglosironidae and Neogoveidae in a cladistic analysis of morphological data (Giribet & Boyer, 2002). The support and stability obtained in our molecular analysis further corroborates this result.

Within Neogoveidae, species from West Africa and South America each form monophyletic groups in most analyses, with the unusual North American species *Metasiro americanus* resolved as the sister group to the West African species. Found in south-eastern USA, *M. americanus* was recently transferred from the Laurasian family Sironidae to Neogoveidae (Giribet, 2007a) as it possesses the distinctive modified second claw found in Neogoveidae and Troglosironidae. Biogeographically, the close relationship of *Metasiro* to West African Neogoveidae is explained by an ancient vicariance event. In the last 35 years geologists have amassed substantial evidence from palaeontological analyses, comparisons of radiometric dates, palaeomagnetic data and stratigraphic correlations (Rowley & Pindell, 1989; Randazzo & Jones, 1997) demonstrating that Florida's basement rocks were originally a part of the West African continental margin near Senegal. This block attached to North America during the formation of Pangaea in the Permo-Carboniferous, at which time south-eastern North America was in close contact with both West Africa and northern South America. The block rifted from Gondwana when the seafloor spread between Africa and North America 180–165 Ma and remained attached to North America, thereby forming present-day Florida (Sanmartín, 2002).

A salient outcome of this study is the relationship of New Caledonia to Tropical Gondwana, which has never been

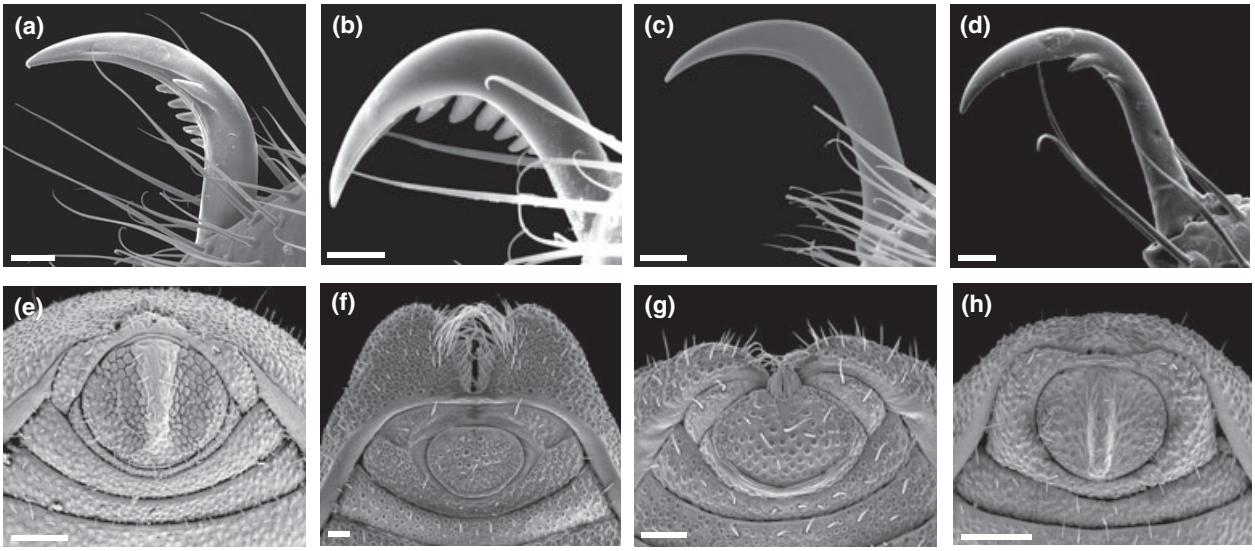

Figure 6 Significant morphological characters employed in cyphophthalmid taxonomy. (a)–(d) Second walking leg claw showing differences in dentition: (a) *Huitaca* sp. Santander (Neogoveidae), scale bar 50 μm; (b) *Troglosiro longifossa* (Troglosironidae), scale bar 20 μm; (c) *Rakaia magna australis* (Pettalidae), scale bar 50 μm; (d) *Parasiro minor* (Sironidae), scale bar 10 μm. (e)–(h) Anal region showing the different modifications of anal plate, sternites 8 and 9 and tergite IX (scale bars 100 μm): (e) *Suzukielus sauteri* (Sironidae); (f) *Pettalus* cf. *brevicauda* (Pettalidae); (g) *Rakaia solitaria* (Pettalidae); (h) *Siro valleorum* (Sironidae).

proposed based on geological or biological data. This is the most stable of the inter-family relationships proposed by our analyses and had been previously suggested based on morphology (Giribet & Boyer, 2002), although alternative views exist (Shear, 1993). The present-day distributions of Troglosironidae on New Caledonia and Neogoveidae in tropical Gondwana may constitute a challenge to our vicariance-based model in so far as New Caledonia and the tropical Gondwanan landmasses are hardly contiguous. However, dispersal to New Caledonia from West Africa or tropical South America (or vice versa) is unlikely because the split between Troglosironidae and Neogoveidae is estimated from our analysis to have taken place 124–221 Ma, preceding the break-up of Gondwana. Diversification of Neogoveidae was around 109–195 Ma, whereas it occurred much more recently within Troglosironidae, dated at only 28–49 Ma, indicating possible diversification after a catastrophic event (Table 3).

An alternative scenario consistent with the ancient split between the families invokes a pan-Gondwanan distribution of this group. Such a pan-Gondwanan distribution is conceivable given that New Caledonia has been interpreted as an ancient island, home to such relictual groups as *Amborella,* the sister genus to all other flowering plants (Mathews & Donoghue, 1999). Moreover, the extensive former distribution of Pettalidae on Gondwana indicates that a similar distribution is possible for tropical Gondwanan Cyphophthalmi. This hypothesized pan-Gondwanan distribution of the tropical Gondwana clade is disputable, given that such a distribution has not been validated by other poorly dispersing taxa of Gondwanan origin. In particular, the biota of New Caledonia is generally related to Australia and New Zealand biogeographically (Walley & Ross, 1991). Further studies of the New Caledonian biota in a broad phylogenetic and biogeographical context may shed light on this enigma.

The Laurasian clades

Our analyses consistently retrieve a Laurasian clade, including species from North America and Europe in the family Sironidae. Within this group, there are two main clades, the trans-Atlantic genus *Siro* from western Europe and both coasts of the USA, and a Mediterranean clade including *Paramiopsalis* and *Cyphophthalmus*. However, the placement of the Japanese genus *Suzukielus* and the Mediterranean genus *Parasiro* requires further discussion. The position of the Japanese species *Suzukielus sauteri* is unstable, as it groups with Sironidae in some analyses and appears sister to the clade formed by species from tropical Gondwana + Southeast Asia in others. Morphologically, this monotypic genus is enigmatic, with its taxonomically important anal region (Fig. 5e) resembling that of animals from the temperate Gondwanan family Pettalidae (Fig. 5f,g) rather than Laurasian Sironidae (Fig. 5h), where sternites 8 and 9 and tergite IX fuse into a corona analis. In fact, *Suzukielus* has defied monophyly of the family in previous morphological cladistic analyses (Giribet & Boyer, 2002; De Bivort & Giribet, 2004).

Parasiro is not closely related to any other sironid species under any analysis. *Parasiro* currently includes three species on both sides of the Pyrenees, on the Italian Peninsula and on the islands of Corsica and Sardinia, and this genus has many differences with respect to the other sironids (De Bivort & Giribet, 2004) and may well require a new familial designation.

While some sironid species are doubtlessly the best-known cyphophthalmids, the family also includes a number of poorly known monotypic genera (*Iberosiro*, *Odontosiro*, *Paramiopsalis*, *Suzukielus*, *Tranteeva*) that may require further study.

Concluding remarks

By studying a group of organisms with not only an ancient origin, low vagility and restricted habitats but also a present global distribution, we have been able to test biogeographical hypotheses at a scale rarely attempted. Our results strongly support the presence of a circum-Antarctic clade of formerly temperate Gondwanan species, a clade restricted to tropical Gondwana and a Southeast Asian clade that originated from a series of early Gondwanan terranes that rifted off northwards from the Devonian to the Triassic and accreted to tropical Laurasia (Metcalfe & Irving, 1990). The latter clade subsequently diversified from north to south-east (Clouse & Giribet, 2007). The relationships among the Laurasian species remain more obscure, although this may reflect real taxonomic deficiencies, as had occurred previously with the genus *Metasiro*, once considered a sironid because of its North American distribution.

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