

A multilocus approach to harvestman (Arachnida: Opiliones) phylogeny with emphasis on biogeography and the systematics of Laniatores

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Abstract

The internal phylogeny of the arachnid order Opiliones is investigated by including molecular data from five molecular markers for ca. 140 species totalling 43 families of Opiliones. The phylogenetic analyses consisted of a direct optimization (DO) approach using POY v. 4 and sophisticated tree search algorithms as well as a static alignment analysed under maximum likelihood. The four Opiliones suborders were well-supported clades, but subordinal relationships did not receive support in the DO analysis, with the exception of the monophyly of Palpatores (= Eupnoi + Dyspnoi). Maximum-likelihood analysis strongly supported the traditional relationship of Phalangida and Palpatores: (Cyphophthalmi ((Eupnoi + Dyspnoi) Laniatores)). Relationships within each suborder are well resolved and largely congruent between direct optimization and maximum-likelihood approaches. Age estimates for the main Opiliones lineages suggest a Carboniferous diversification of Cyphophthalmi, while its sister group, Phalangida, diversified in the Early Devonian. Diversification of all suborders predates the Triassic, and most major lineages predate the Cretaceous. The following taxonomic changes are proposed. Dyspnoi: *Hesperonemastoma* is transferred to Sabaconidae. Insidiatores: Sclerobunidae stat. nov. is erected as a family for *Zuma acuta*.

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Of the 11 extant arachnid orders, Opiliones (harvestmen or daddy-long-legs) constitutes the third largest in species diversity (Coddington et al., 2004). The group has received considerable attention in recent literature, which has led in part to international collaboration culminating in the production of a volume summarizing the current knowledge of its biology (Pinto-da-Rocha et al., 2007). The deep phylogenetic history of Opiliones, i.e. relationships among the four suborders Cyphophthalmi, Eupnoi, Dyspnoi, and Laniatores, has been investigated in detail,

focusing on such disparate character systems as genitalic organs (e.g. Martens, 1976; Martens et al., 1981), general morphology (Shultz, 1998; Giribet et al., 1999, 2002), and molecular sequence data (Giribet et al., 1999, 2002; Shultz and Regier, 2001). Interest in harvestmen phylogeny has its roots in understanding their biology, taxonomy, and the curious biogeographical patterns characteristic of many of its groups. Due to their low vagility (Giribet and Kury, 2007), some opilionid distributions show strikingly conserved patterns that match plate tectonics to a degree rarely observed in other globally distributed organisms (Juberthie and Massoud, 1976; Boyer et al., 2007b; Boyer and Giribet, 2007; Clouse and Giribet, 2007).

Phylogenetic studies of certain Opiliones groups have also flourished. The suborder Cyphophthalmi (see

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Fig. 1. Life habitus of Cyphophthalmi species: (a) *Huitaca* sp. nov. (Neogoveidae); (b) *Rakaia stewartiensis* (Pettalidae); (c) *Suzukielus sauteri* (Sironidae); (d) *Stylocellus* cf. *modestus* (Stylocellidae). Photos by G. Giribet.

Fig. 1) has been thoroughly investigated using morphology (Giribet and Boyer, 2002; Giribet, 2003; de Bivort and Giribet, 2004; Boyer and Giribet, 2007) and/or molecules (Boyer et al., 2005, 2007b; Schwendinger and Giribet, 2005; Boyer and Giribet, 2007, 2009; Clouse and Giribet, 2007; Murienne and Giribet, 2009; Murienne et al., 2009; Sharma and Giribet, 2009a). Numerical phylogenetic studies of the other suborders are scarcer. Studies of Eupnoi groups (Fig. 2) are restricted to morphological analyses of some Southern Hemisphere Phalangioidea (Hunt and Cokendolpher, 1991). A series of cladistics papers based on morphological data appeared in the mid 1980s focusing on subclades of Dyspnoi (Shear and Gruber, 1983; Shear, 1986) (Fig. 3). Although no comprehensive analysis has been amassed for this suborder, several dyspnooid genera have been represented in analyses by Giribet, Shultz and their collaborators (Shultz, 1998; Shultz and Regier, 2001; Giribet et al., 2002; Giribet and Dunlop, 2005), indirectly evidencing the need for family-level taxonomic revision (see Gruber in Pinto-da-Rocha and Giribet, 2007, p. 136–159).

The suborder Laniatores (Fig. 4), comprising 26 families (as recognized in Pinto-da-Rocha et al., 2007),

dwarfs the diversity of the other suborders (19 families for the other suborders; six Cyphophthalmi, six Eupnoi, seven Dyspnoi). Paradoxically, Laniatores is the least understood suborder with respect to phylogenetic relationships. Modern attempts to establish a phylogenetic system of Laniatores include the unpublished thesis work of Kury (1993), followed by a series of studies, including other unpublished theses, concentrating on some of the laniatorean subclades (Kury, 1994a,b, 1997a,b; Pinto-da-Rocha, 1997, 2002; Pinto-da-Rocha and Kury, 2003a; Pérez González, 2006; Orrico and Kury, 2009). The Southeast Asian family Sandokanidae (formerly Oncopodidae) has received both morphological (Schwendinger and Martens, 2002) and molecular (Sharma and Giribet, 2009b) treatment. Consequently, current hypotheses of laniatorean relationships are grossly unresolved and in urgent need of evaluation (see, for example, Giribet and Kury, 2007).

The rapid development of molecular techniques during the past two decades, in conjunction with globally coordinated collection efforts, confers an unprecedented opportunity to undertake a comprehensive phylogenetic analysis of harvestmen using a large amount of molecular data. For this purpose, we have



Fig. 2. Life habitus of Eupnoi examples: (a) *Caddo agilis* (Caddidae, Caddoidea); (b) *Phalangium opilio* (Phalangiidae, Phalangoidea); (c) *Pantopsalis* sp. (Monoscutidae, Phalangoidea), male with mites; (d) *Megalopsalis* sp. (Monoscutidae, Phalangoidea), female. Photos by Joe Warfel (a) and G. Giribet (b–d).

collected harvestman species for all but four families (missing taxa: Ogoveidae [Cyphophthalmi], Neopilionidae [Eupnoi] Briggsidae [formerly Pentanychidae] and Guasiniidae [Laniatores]), totalling 41 of the families recognized by Pinto-da-Rocha et al. (2007). Taxa sampled include 135 opilionid species plus outgroups—the largest published analysis included 35 species in 18 families (Giribet et al., 2002)—more than doubling the number of represented families and quadrupling the number of species. The previous analysis was particularly limited in the sampling of Cyphophthalmi (four species versus 43 in the present analysis) and Laniatores (11 species versus 64). Although the present dataset builds upon previous work, here we expand 28S rRNA from the 350-bp D3 expansion fragment (Giribet et al., 2002) to a 2.5-kb fragment for many taxa and to nearly complete 28S rRNA (ca. 3.5 kb) for a significant subset. Furthermore, we add a nuclear protein-coding gene (histone H3) and two mitochondrial markers, 16S rRNA and the protein-encoding cytochrome *c* oxidase subunit I (henceforth COI). We also incorporate a maximum-likelihood framework analysis to the previous strategy of parsimony direct optimization. The new dataset and analyses, by virtue of including opilionid

representatives from all major landmasses, allow us to explore biogeographical hypotheses, some of which have never been tested before with a comparable dataset. Finally, we provide age estimates for the origin and diversification of major opilionid lineages.

Materials and methods

Sampling of tissues and markers

Fresh opilionid tissues were collected in all major continental landmasses except Antarctica following standard protocols for tissue preservation for DNA work. Some variability in the mode of preservation exists due to differing fieldwork standards of colleagues who contributed specimens. Consequently, all freshly collected specimens were transferred to clean 96% EtOH, and specimens were kept at -80°C for long-term storage. The list of specimens, including voucher number and GenBank accession codes, is found in Table 1. Collection details are provided in Appendix 1.

Molecular methods follow standard protocols and primers described in previous publications (e.g. Giribet

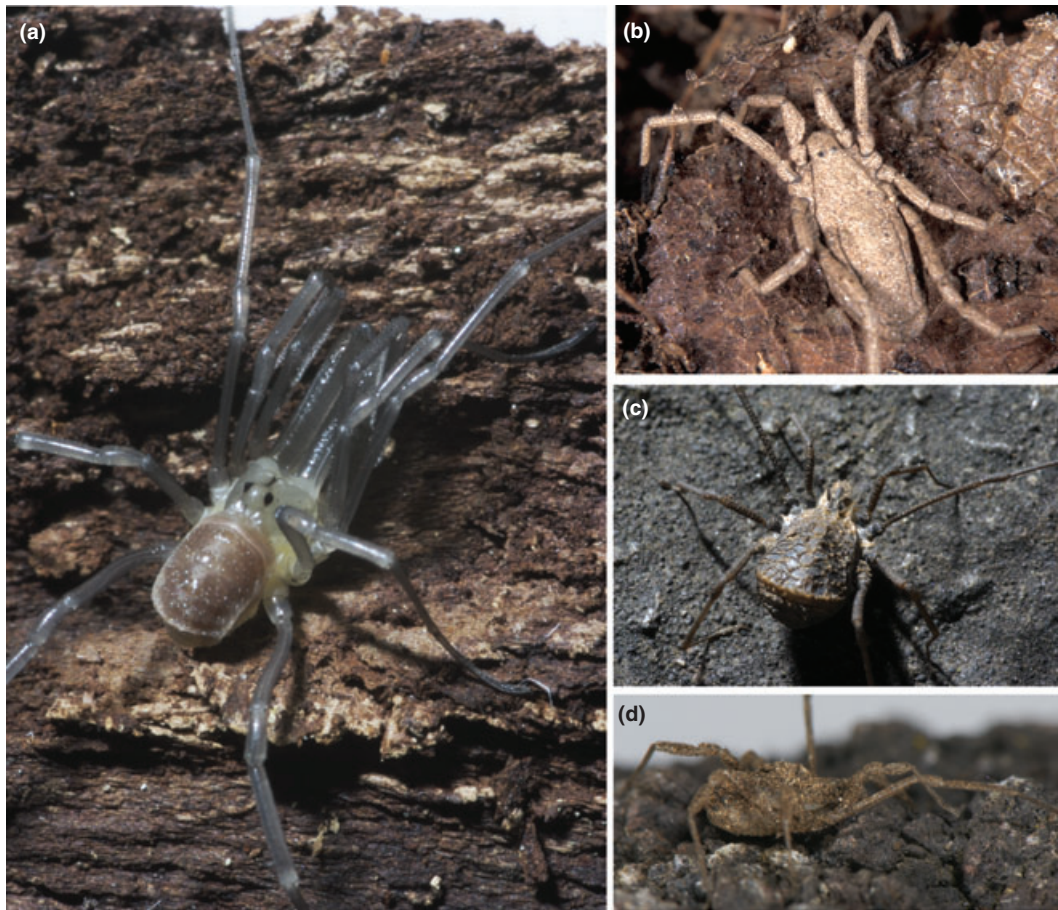


Fig. 3. Life habitus of Dyspnoi representatives: (a) *Taracus* sp. (Sabaconidae, Ischyropsalidoidea); (b) *Trogulus* sp. (Trogulidae, Troguloidea); (c) *Ortholasma* sp. (Nemastomatidae, Troguloidea); (d) *Anelasmoecephalus* sp. (Trogulidae, Troguloidea). Photos by G. Giribet.

et al., 2002; Schwendinger and Giribet, 2005; Edgecombe and Giribet, 2006). Five molecular loci were chosen for this study. Ribosomal sequence data of complete 18S rRNA and an approximately 2.2-kb fragment of 28S rRNA were selected to resolve deeper nodes in the trees, whereas the nuclear protein-coding gene histone H3, the mitochondrial ribosomal gene 16S rRNA, and the mitochondrial protein-coding gene COI were included to resolve more recent evolutionary events. The complete 18S rRNA locus was amplified in three overlapping fragments using the following primer pairs: 1F–4R, 3F–18sbi, and 18Sa2.0–9R. The 28S rRNA fragment was amplified in three overlapping fragments delimited by the primer pairs 28S rd1a–28Sb, 28Sa–28S rd5b, and 28S rd4.8a–28S rd7b1, except for the addition of two additional primer pairs for 28S rRNA found downstream of primer 28S rd7b1. The additional two primer pairs are 28S F2012 (5′-CCAAGGTKARYAGCCTCTRG-3′)–28S R2762 (5′-CCGCCCAGCCAAACTCCCC-3′) and 28S F2762 (5′-GGGGAGTTTGGCTGGGGC GG-3′)–28S OR2r (5′-TTCTGACTTAGAGGCGTT-CAG-3′). 16S rRNA was amplified with primer pair

16Sar–16Sb, H3 with H3aF–H3aR, and COI with LCO1490–HCOoutout.

Phylogenetic analyses

Phylogenetic analyses were based on dynamic homology using parsimony and static homology under the likelihood optimality criterion. The dynamic homology parsimony analyses were conducted using the computer software POY 4.0.0 rc 2885 (Varón et al., 2007) on a 200 processor cluster consisting of 8-core Dell Blades and using four processors from each Blade. POY 4 implements the direct optimization method (single-step phylogenetics; Wheeler, 1996). Tree searches were conducted using an opening indel cost of 3, indel extension cost of 1, and nucleotide substitution cost of 2 (De Laet, 2005), using the command “transform (tcm:(2, 1), gap_opening:2)”. Additional analyses under equal weights were also performed to test the stability of the key relationships discussed below. An initial Wagner tree was built and swapped using spr and tbr. After this initial tree was obtained, the command “transform



Fig. 4. Life habitus of Laniatores examples: (a) *Nuncia* sp. (Triaenonychidae, Triaenonychoidea); (b) *Scotolemon* sp. (Phalangodidae, Phalangodoidea); (c) *Gnomulus latoperculum* (Sandokanidae, Sandokanoidea); (d) *Stygnomma* sp. (Stygnommatidae, Samooidea); (e) *Chilon robustus* (Assamidae, Assamioidea); (f) *Acutisoma longipes* (Gonyleptidae, Gonyleptoidea). Photos by G. Giribet (a–c, e, f) and A. Pérez González (d).

(auto_sequence_partition)” was used to assign partitions automatically with the goal of speeding up searches (Giribet, 2001, 2007). The use of this command has been shown to empirically decrease execution time by *ca.* two orders of magnitude during tree building using the Wagner algorithm and during branch swapping (G. Giribet and A. Varón, unpublished data presented at Hennig Meeting 2007). After applying “auto_sequence_partition”, 100 Wagner addition trees were built, followed by spr and tbr branch swapping, and followed by a round of tree fusing and sectorial

searches (Goloboff, 1999, 2002). Support values were estimated by 100 jackknife replicates (Farris et al., 1996) using dynamic homology for resampling.

For the static homology analyses using maximum likelihood the sequences of each gene were aligned separately using the FFT-NS-i (slow; iterative refinement method) setting of the online multiple alignment program MAFFT ver. 6 (Kato et al., 2002; <http://align.bmr.kyushu-u.ac.jp/mafft/online/server/>). Ambiguous alignment positions were identified and removed with GBlocks ver. 0.91b (Castresana, 2000), using 50%

Table 1

List of species (and taxonomy) with MCZ voucher numbers and GenBank accession numbers for the amplified fragments. 18S rRNA, although amplified in three fragments, is indicated as a single sequence; 28S rRNA is divided into the four amplicons (Asterisks indicate sequences that have been updated in GenBank.)

Taxonomy	Species	MCZ Voucher	18S rRNA	28Sa	28Sb	28Sc	28Sd	16S	COI	H3
Pycnogonida	<i>Anoplodactylus eroticus/erectus</i>		AY859551	AY859550	AY859550	AY859550	AY859550	GQ912837	GQ912859	DQ390198
Pycnogonida	<i>Colossendeis</i> sp./ <i>stramentii/megalomix</i>		AF005440	AY210809	AY210809	AY210809	AY210809	DQ390027	AF259659	DQ390187
Xiphosura	<i>Limulus polyphemus</i>		U91490	AF212167	AF212167	AF212167	AF212167	U09397	AF216203	AF370813
Pseudoscorpiones	<i>Catocheiridius termitophilus</i>		AY859559	AY859558	AY859558	AY859558	AY859558			
Scorpiones	<i>Pandinus imperator</i>		AY210831	AY210830	AY210830	AY210830	AY210830	AF370856		
Solifugae	<i>Eremobates</i> sp.		AY859573	AY859572	AY859572	AY859572	AY859572			
Amblypygi	<i>Paraphrynos</i> sp.		AF005445	AY859594	AY859594	AY859594	AY859594			
Araneae	<i>Aphonopelma hentzi</i>		X13457	AY210803	AY210803	AY210803	AY210803			
	<i>Misumenops asperatus/nepenthicola</i>		AY210445	AY210461	AY210461	AY210461	AY210461			EF419123
Uropygi	<i>Mastigoproctus giganteus</i>		AF005446	AY859587	AY859587	AY859587	AY859587	AY156567	AF370830	
Neogoveidae	<i>Huitaca</i> sp.	DNA101407	DQ518090	DQ825596	DQ825596	DQ825596	DQ825596	DQ518050	DQ518129	DQ518167
Neogoveidae	<i>Metagova</i> sp.	DNA101410	DQ518091	DQ825597	DQ825597	DQ825597	DQ825597	EU673586*	GQ912860	DQ518168
Neogoveidae	<i>Metasiro americanus</i>	DNA101552	DQ825542	DQ825595	DQ825595	DQ825595	DQ825595	DQ825616	DQ825645	DQ825513
Neogoveidae	<i>Neogova</i> sp.	DNA101408	DQ825543	DQ825598	DQ825598	DQ825598	DQ825598	DQ825618	DQ825648	DQ825514
Neogoveidae	<i>Paragovia sironoides</i>	DNA101059	DQ518092	DQ825606	DQ825606	DQ825606	DQ825606	DQ518051	DQ518131	DQ518169
Neogoveidae	<i>Paragovia</i> sp.	DNA101052	DQ825546	DQ825601	DQ825601	DQ825601	DQ825601	DQ825620	DQ825648	DQ825515
Neogoveidae	<i>Paragovia</i> sp.	DNA101057	DQ825547	DQ825602	DQ825602	DQ825602	DQ825602	DQ825621	DQ825648	DQ825516
Neogoveidae	<i>Paragovia</i> sp.	DNA100462	AY639493	DQ825603	DQ825603	DQ825603	DQ825603		AY639458	AY639458
Pettalidae	<i>Aoraki denticulata denticulata</i>	DNA100961	DQ518001	DQ518040	DQ518040	DQ518040	DQ518040	DQ518069	DQ518126	DQ518158
Pettalidae	<i>Aoraki inerma inerma</i>	DNA100967	DQ518003	DQ518041	DQ518041	DQ518041	DQ518041			DQ518159
Pettalidae	<i>Austropurcellia forsteri</i>	DNA100945	DQ517983	DQ518022	DQ518022	DQ518022	DQ518022	DQ518064	DQ518110	DQ518146
Pettalidae	<i>Austropurcellia scoparia</i>	DNA100946	DQ517982	DQ518021	DQ518021	DQ518021	DQ518021	DQ518065	DQ518108	DQ518146
Pettalidae	<i>Chiloeogova oedipus</i>	DNA100413	DQ517969	DQ825571	DQ825571	DQ825571	DQ825571	EU673566	EU673661	EU673672
Pettalidae	<i>Karripurcellia harveyi</i>	DNA101303	DQ517980	DQ825578	DQ825578	DQ825578	DQ825578	DQ518062	DQ518106	DQ518143
Pettalidae	<i>Neopurcellia salmoni</i>	DNA100939	DQ517998	DQ518037	DQ518037	DQ518037	DQ518037	DQ518066	DQ825638	EU673694
Pettalidae	<i>Parapurcellia silvicola</i>	DNA100385	EU673590	DQ825574	DQ825574	DQ825574	DQ825574	AY639553	AY639582	EU673674
Pettalidae	<i>Pettalus</i> sp.	DNA101282	DQ825537	DQ825576	DQ825576	DQ825576	DQ825576	DQ825613	DQ825636	EU673676
Pettalidae	<i>Pettalus</i> sp.	DNA101288	DQ517979	DQ518015	DQ518015	DQ518015	DQ518015	DQ518061	DQ518105	DQ518142
Pettalidae	<i>Purcellia illustrans</i>	DNA100387	DQ517971	DQ825573	DQ825573	DQ825573	DQ825573	AY639554	EU673665	EU673673
Pettalidae	<i>Rakaitia antipodiana</i>	DNA100957	DQ517988	DQ518031	DQ518031	DQ518031	DQ518031	DQ518072	DQ518115	DQ518151
Pettalidae	<i>Rakaitia florensis</i>	DNA101295	DQ517986	DQ518025	DQ518025	DQ518025	DQ518025	DQ518083	DQ518113	DQ518149
Pettalidae	<i>Rakaitia stewartiensis</i>	DNA100944	DQ517994	DQ518028	DQ518028	DQ518028	DQ518028	DQ518080	DQ518117	DQ518149
Sironidae	<i>Cyphophthalmus duricorius</i>	DNA100487	AY639461	DQ513120	DQ513120	DQ513120	DQ513120	AY639526	AY639556	GQ912901
Sironidae	<i>Cyphophthalmus giorgievici</i>	DNA100498	AY639464	DQ825587	DQ825587	DQ825587	DQ825587	AY639530	AY639560	AY639560
Sironidae	<i>Cyphophthalmus</i> sp.	DNA101342	AY918870	DQ513117	DQ513117	DQ513117	DQ513117	AY639530	AY918878	AY918880
Sironidae	<i>Paramiopsalis ramulosus</i>	DNA100459	AY639489	DQ513121	DQ513121	DQ513121	DQ513121	AY639550	DQ825641	AY918880
Sironidae	<i>Parasiro colfaiiti</i>	DNA101383	AY918872	DQ513122	DQ513122	DQ513122	DQ513122	AY918877	DQ825642	GQ912900
Sironidae	<i>Siro acaroides</i>	DNA100488	AY639490	DQ513128	DQ513128	DQ513128	DQ513128	AY639551	DQ825643	DQ825643
Sironidae	<i>Siro exilis</i>	DNA100489	AY639491	DQ513124	DQ513124	DQ513124	DQ513124	AY639551	AY639579	AY639579
Sironidae	<i>Siro kamitakensis</i>	DNA101613	DQ513148	DQ513134	DQ513134	DQ513134	DQ513134			

Table 1
(Continued)

Taxonomy	Species	MCZ Voucher	18S rRNA	28Sa	28Sb	28Sc	28Sd	16S	COI	H3
Sironidae	<i>Siro rubens</i>	DNA100457	AY428818	AY859602	AY859602	AY859602	AY859602		DQ513111	
Sironidae	<i>Siro valdeorum</i>	DNA100461	AY639492	DQ513123	DQ513123	DQ513123	DQ513123	AY639552	AY639580	AY639457
Sironidae	<i>Suzukiellus sauteri</i>	DNA101543	DQ513138	DQ513116	DQ513116	DQ513116	DQ513116	DQ518086	DQ513108	DQ518166
Stylocellidae	<i>Fangensis cavernarum</i>	DNA101460	DQ133714	GQ912755	GQ912755	GQ912755	GQ912755		DQ133740	DQ518132
Stylocellidae	<i>Fangensis insulanus</i>	DNA100388	DQ133710	DQ825551	DQ825551	DQ825551	DQ825551		DQ133737	
Stylocellidae	<i>Fangensis spelaeus</i>	DNA100669	DQ133712	DQ825554	DQ825554	DQ825554	DQ825554		AY639583	AY639460
Stylocellidae	<i>Stylocellus lydekkeri</i>	DNA101064	DQ133717	DQ825564	DQ133729	DQ133729	DQ133729		DQ825626	
Stylocellidae	<i>Stylocellus</i> sp.	DNA101469	DQ825522	DQ825564	DQ825564	DQ825564	DQ825564		DQ825632	
Stylocellidae	<i>Stylocellus</i> sp.	DNA101468	DQ885524	DQ825561	DQ825561	DQ825561	DQ825561			
Stylocellidae	<i>Stylocellus</i> sp.	DNA101500	DQ825534							
Troglosironidae	<i>Troglosiro aelleni</i>	DNA100345	AY639497*	DQ825580	DQ825580	DQ825580	DQ825580	AY639555	AY639584	DQ518164
Troglosironidae	<i>Troglosiro longifossa</i>	DNA100867	DQ518089	DQ825582	DQ825582	DQ825582	DQ825582	DQ518084	DQ825639	DQ518165
Caddidae	<i>Caddo agilis</i>	DNA100308	U91487		U91502					
Monoscutidae	<i>Megalopsalis</i> sp.	DNA100783	EF108573	EF108576	EF108576	EF108576	EF108576	EF108582	EF108587	EF108593
Monoscutidae	<i>Pantopsalis</i> sp.	DNA100702	GQ912707	GQ912756	GQ912756	GQ912756	GQ912756	GQ912838	GQ912861	GQ912902
Phalangidae	<i>Odiellus trogluoides</i>	DNA100310	X81441*		U91500					
Phalangidae	<i>Opilio parietinus</i>	DNA100304	AF124938	AF124966	AF124966	AF124966	AF124966	AF370858*	AF370832	
Phalangidae	<i>Rhampsinium opilio</i>	DNA100307	AF124937	AF124965	AF124965	AF124965	AF124965			
Phalangidae	<i>Rhampsinium</i> sp.	DNA100710	GQ912708	GQ912757	GQ912757	GQ912757	GQ912757	GQ912839	GQ912862	GQ912903
Protolophidae	<i>Protolophus singularis</i>	DNA101033	EF028095	EF028096	EF028096	EF028096	EF028096	EF108581	EF108586	EF108592
Selerosomatidae	<i>Astrobumus grallator</i>	DNA100311	AF124939	AF124967	AF124967	AF124967	AF124967		AF370833	
Selerosomatidae	<i>Dalquestia formosa</i>	DNA100322	AF124936	AF124964	AF124964	AF124964	AF124964			
Selerosomatidae	<i>Eurybunus</i> n.sp.	DNA100532	GQ912709	GQ912759	GQ912759	GQ912759	GQ912759			
Selerosomatidae	<i>Hadr-obunus maculosus</i>	DNA100314	AF124941	AF124969	AF124969	AF124969	AF124969			
Selerosomatidae	<i>Homalenotus</i> sp.	DNA100709	GQ912710	GQ912761	GQ912761	GQ912761	GQ912761			GQ912904
Selerosomatidae	<i>Marthana</i> sp.	DNA100613	GQ912711	GQ912762	GQ912762	GQ912762	GQ912762	GQ912840	GQ912863	GQ912905
Selerosomatidae	<i>Letobunum</i> sp.	DNA100309	AF124940	AF124968	AF124968	AF124968	AF124968		GQ912864	
Selerosomatidae	<i>Nelima sivatica</i>	DNA100323	U92486	U91501	U91501	U91501	U91501			
Selerosomatidae	<i>Prionostemma</i> sp.	DNA100315	GQ912712	GQ912763	GQ912763	GQ912763	GQ912763			
Ceratolasmatidae	<i>Ceratolasma tricantha</i>	DNA100329	AF124943	GQ912764	GQ912764	GQ912764	GQ912764	EF108583	GQ912865	EF108594
Sabaconidae	<i>Hesperonemastoma modestum</i>	DNA100312	AF124942	GQ912765	GQ912765	GQ912765	GQ912765	EF108583	EF108588	
Ishyropsalididae	<i>Ishyropsalis luteipes</i>	DNA100705	U37000*	GQ912767	GQ912767	GQ912767	GQ912767	GQ912841	GQ912866	GQ912906
Sabaconidae	<i>Sabacon cavicolens</i>	DNA100305	AF124944	GQ912768	GQ912768	GQ912768	GQ912768	GQ912842		
Sabaconidae	<i>Sabacon</i> sp.	DNA100711	GQ912713	GQ912769	GQ912769	GQ912769	GQ912769		GQ912866	
Sabaconidae	<i>Taracus</i> sp.	DNA100320	AF124945	GQ912770	GQ912770	GQ912770	GQ912770	GQ912843	GQ912867	
Dicranolasmatidae	<i>Dicranolasma soerenseni</i>	No voucher	U37001	U91498	U91498	U91498	U91498			
Nemastomatidae	<i>Dendrolasma parvulum</i>	DNA100318	EF108574	EF108578	EF108578	EF108578	EF108578	EF108584	EF108589	
Nemastomatidae	<i>Nemastoma bimaculatum</i>	No voucher	AF124947	AF124974	AF124974	AF124974	AF124974			
Nemastomatidae	<i>Nemastoma hankiewiczii</i>	DNA100717	GQ912714	GQ912772	GQ912772	GQ912772	GQ912772	GQ912845	GQ912868	
Nemastomatidae	<i>Nemastomella dubia</i>	DNA100706	U37002*	GQ912774	GQ912774	GQ912774	GQ912774	GQ912844	GQ912869	
Nemastomatidae	<i>Ortholasma</i> sp.	DNA100343	AF124946	GQ912775	GQ912775	GQ912775	GQ912775	GQ912846	GQ912870	
Nipponopsalididae	<i>Nipponopsalis abei</i>	DNA100306	AF124948	GQ912777	GQ912777	GQ912777	GQ912777			
Trogulidae	<i>Anclasmoecephalus cambridgei</i>	DNA101043	GQ912715	GQ912778	GQ912778	GQ912778	GQ912778	GQ912847	GQ912871	
Trogulidae	<i>Trogulus nepaeiformis</i>	DNA100325	AF124949	GQ912779	GQ912779	GQ912779	GQ912779	GQ912848	GQ912872	

Table 1
(Continued)

Taxonomy	Species	MCZ Voucher	18S rRNA	28Sa	28Sb	28Sc	28Sd	16S	COI	H3
Cladonychiidae	<i>Erebomaster flavescens</i>	DNA101444	GQ912716		GQ912781	GQ912781				
Cladonychiidae	<i>Holoscotolemon jaqueti</i>	DNA101037	GQ912717	GQ912782	GQ912783	GQ912783			GQ912873	GQ912907
Cladonychiidae	<i>Theromaster brunneus</i>	DNA100701	GQ912718		GQ912784	GQ912784	GQ912784			GQ912908
Travuniidae	<i>Hadzicana clavigera</i>	DNA101459	FJ796479			GQ912785	GQ912785		FJ796491	FJ796498
Travunioidae inc. sedis	<i>Trojanella serbica</i>	DNA100911	GQ912719		GQ912786	GQ912786			GQ912874	GQ912909
Sclerobunidae	<i>Zuma acuta</i>	No voucher	AF124951		AF124978					
Synthetonychiidae	<i>Synthetonychia</i> sp. 100866	DNA100796/100866	GQ912720		GQ912787			GQ912849	GQ912875	GQ912910
Trienonychiidae	<i>Equitius doniae</i>	DNA100607	U37003	GQ912788	GQ912788	GQ912788	GQ912789		EF108590	EF108595
Trienonychiidae	<i>Fumontana deprehendor</i>	DNA100700	GQ912721		GQ912790		GQ912791			GQ912911
Trienonychiidae	<i>Larifuga cf. capensis</i>	DNA100727	GQ912722	GQ912792	GQ912792	GQ912792			GQ912876	GQ912912
Trienonychiidae	<i>Rostromontia cf. capensis</i>	DNA100724	GQ912723	GQ912793	GQ912793	GQ912793				GQ912913
Trienonychiidae	<i>Trienobunus</i> sp.	DNA100328	AF124950	GQ912794	GQ912794	GQ912794			GQ912877	GQ912914
Trienonychiidae	<i>Trienobunus cf. armstrongi</i>	DNA100617	GQ912724		GQ912795	GQ912795			GQ912878	GQ912915
Sandokanidae	<i>Gnomulus dalat</i>	DNA101101	FJ796482	FJ796486	FJ796486	FJ796486		FJ796474	FJ796495	FJ796504
Sandokanidae	<i>Gnomulus latopericulum</i>	DNA102028						FJ475850	GQ912879	
Sandokanidae	<i>Gnomulus</i> sp. (<i>rostratus</i> group)	DNA101102	FJ796483	FJ796487	FJ796487	FJ796487		FJ796473		FJ796503
Sandokanidae	<i>Marienshiellus</i> sp.	No voucher	FJ796485					FJ796476	FJ796497	
Sandokanidae	<i>Sandokan malayanus</i>	DNA100321	EF108575	GQ912796	GQ912796	GQ912796	GQ912796	EF108585	EF108591	EF108596
Sandokanidae	<i>Sandokan truncatus</i>	DNA101099	FJ796484	FJ796488	FJ796488	FJ796488	GQ912797	FJ796475	FJ796496	FJ796505
Phalangodidae	<i>Bishopella laciniosa</i>	DNA100324	AF124952		GQ912798	GQ912798		GQ912850	GQ912880	
Phalangodidae	<i>Scotolemon lespesi</i>	DNA100326	U37005*	GQ912799	GQ912799	GQ912799		GQ912851		GQ912916
Phalangodidae	<i>Scotolemon</i> sp.	DNA101042	GQ912725	GQ912800	GQ912800	GQ912800		GQ912852	GQ912881	GQ912917
Pyramidopidae	<i>Maioreus randoi</i>	No voucher	U37004		U91505					
Pyramidopidae	<i>Conomma oedipus</i>	DNA101051	GQ912726	GQ912801	GQ912801	GQ912801	GQ912801	GQ912853	GQ912882	GQ912918
Assamiidae	<i>Chilon robustus</i>	DNA100766	GQ912727	GQ912802	GQ912802	GQ912802	GQ912803			GQ912919
Assamiidae	<i>Pakrongius</i> sp.	DNA101416	GQ912728	GQ912804	GQ912804	GQ912804		GQ912854		
Assamiidae	<i>Seuthes inermis</i>	DNA100765	GQ912729	GQ912805	GQ912805	GQ912805				GQ912920
Epedanidae	<i>Trionyxellinae</i> sp.	DNA101430	GQ912730	GQ912806	GQ912806	GQ912806			GQ912883	GQ912921
Agoristenidae	<i>Pseudoepedanus doiensis</i>	DNA101438	GQ912731	GQ912807	GQ912807	GQ912807				GQ912922
Cosmetidae	<i>Avina albionata</i>	DNA100333	GQ912732	GQ912808	GQ912808	GQ912808			GQ912884	
Cosmetidae	<i>Gnortula granulata</i>	DNA100332	FJ796480				GQ912809		FJ796492	FJ796499
Cosmetidae	<i>Gnidia holnbergi</i>	DNA100398	U37006*	U91507	U91507	U91507				GQ912923
Cosmetidae	<i>Metalibitia paraguayensis</i>	DNA100476	GQ912733	GQ912810	GQ912810	GQ912810	GQ912810			GQ912924
Cranaiidae	<i>Heterocraneus</i> sp.	DNA101443	GQ912734	GQ912811	GQ912811	GQ912811	GQ912811		GQ912885	GQ912925
Cranaiidae	<i>Santinezia serratotibialis</i>	DNA100426	GQ912735	GQ912812	GQ912812	GQ912812	GQ912813		GQ912886	GQ912926
Gonyleptidae	<i>Glysterus</i> sp.	DNA101422	FJ796481	GQ912814	GQ912814	GQ912814		FJ796472	FJ796493	FJ796502
Gonyleptidae	<i>Goniosoma longipes</i>	DNA101112	GQ912736	GQ912815	GQ912815	GQ912815				
Gonyleptidae	<i>Megapachylus grandis</i>	DNA101113	GQ912737	GQ912816	GQ912816	GQ912816				
Gonyleptidae	<i>Pachylodes thorellii</i>	DNA100424	U37007*	U91508	U91508	U91508			GQ912887	
Manaosbiidae	<i>Rhopalocraneus albilineatus</i>	DNA100334	GQ912738	GQ912817	GQ912817	GQ912817			GQ912888	
Manaosbiidae	<i>Zygopachylus n.sp.</i>	DNA101425	GQ912739	GQ912818	GQ912818	GQ912818	GQ912818	GQ912854	GQ912889	
Stygnidae	<i>Stygnoplus clavotibialis</i>	DNA100331	GQ912740	GQ912819	GQ912819	GQ912819				GQ912927
Stygnopsidae	<i>Hoplobunus</i> sp.	DNA100327	AF124953	GQ912820	GQ912820	GQ912820	GQ912821	GQ912856	GQ912890	GQ912928
Stygnopsidae	<i>Karos cf. rugosus</i>	DNA101419	GQ912741	GQ912822	GQ912822	GQ912822			GQ912891	GQ912929

Table 1
(Continued)

Taxonomy	Species	MCZ Voucher	18S rRNA	28Sa	28Sb	28Sc	28Sd	16S	COI	H3
Biantidae	<i>Metabiantes longipalpis</i>	DNA100769	GQ912742		GQ912823					GQ912930
Biantidae	<i>Metabiantes</i> sp.	DNA100703	GQ912743		GQ912824				GQ912892	GQ912931
Biantidae	<i>Metabiantes</i> sp.	DNA100704	GQ912744		GQ912825					GQ912932
Escadabiidae	<i>Baculigerus</i> n.sp.	DNA100640	GQ912745		GQ912826			GQ912857		GQ912933
<i>Incertae sedis</i>	<i>Costabrinna</i> sp.	DNA101428	GQ912746							
Kimuliidae	<i>Minuella</i> sp.	DNA101388	GQ912747		GQ912827					GQ912934
Podocetidae	<i>Dongmoa</i> sp.	DNA101100	FJ796477	FJ796489	FJ796489			FJ796471		FJ796500
Samoidae	<i>Pellobunus insularis</i>	DNA101421	GQ912748		GQ912829				GQ912894	GQ912935
	<i>Samoidae</i> sp. juv.	DNA101116/7	GQ912749		GQ912830					GQ912936
Stygnomatidae	<i>Stygnomma</i> n.sp.	DNA101415			GQ912831				GQ912895	GQ912937
Stygnomatidae	<i>Stygnomma</i> sp.	DNA101423	GQ912752						GQ912896	GQ912938
Fissiphallidae	<i>Fissiphallius chicoi</i>	DNA101551	GQ912750		GQ912832					GQ912939
Icaleptidae	<i>Icaleptidae</i> gen. nov., sp. nov.	DNA101420	GQ912751		GQ912833		GQ912833			GQ912940
Zalmoxiidae	<i>Ethobunus zalmoxiiformis</i>	DNA101424	FJ796478	FJ796490	FJ796490				FJ796494	FJ796501
Zalmoxiidae	<i>Pirassunungoleptes calcaratus</i>	DNA101114	GQ912753	GQ912834	GQ912835				GQ912897	GQ912941
Zalmoxiidae	<i>Zalmoxis</i> sp.	DNA100914	GQ912754		GQ912836			GQ912858	GQ912898	GQ912942

as the minimum number of sequences for a conserved position (differs between genes) and as the minimum number of sequences for a blank position (differs between genes), four as the maximum number of contiguous non-conserved positions, five as the minimum length of a block, with half allowed gap positions and using a similarity matrix. After removing ambiguous sites the combined alignment consisted of 6398 nucleotides.

Maximum-likelihood tree searches of the combined and partitioned datasets were conducted with RAxML ver. 7.0.3 (Stamatakis, 2006) on the CIPRES cluster (San Diego Supercomputation Center), using the GTR model of sequence evolution with corrections for a discrete gamma distribution and a proportion of invariant sites (GTR + I + Γ), as selected in Modeltest v.3.7 (Posada, 2005; Posada & Crandall, 1998) under the Akaike information criterion (Posada & Buckley, 2004). Support values were estimated based on 1000 bootstrap replicates (Felsenstein, 1985) using the fast bootstrap method implemented in RAxML (Stamatakis et al., 2008).

Age estimate analyses

The ages of several clades were estimated on the maximum-likelihood tree using a standard likelihood method with a relaxed clock (Sanderson, 1997). This method based on branch length and rates autocorrelations avoids the assumption of a strict molecular clock. Divergence times were estimated using the penalized likelihood method (Sanderson, 2002) with the TN algorithm and a logarithmic penalty function, as recommended by the cross-validation procedure implemented in r8s 1.71 (Sanderson, 2003). This method requires at least one node of fixed age within the tree. We fixed the root of the Opiliones tree to 420 Ma based on the age of fossil scorpions (e.g. Dunlop et al., 2008) and the putative sister-group relationship of Scorpiones to Opiliones (Shultz, 2007) or the putative monophyly of Dromopoda (Shultz, 1990; Wheeler and Hayashi, 1998; Giribet et al., 2002). We also fixed the age of Eupnoi to 400 Ma due to the presence of a member of the clade in the Rhynie cherts of Scotland (Dunlop et al., 2003, 2004 (for 2003)). We used a series of minimum age constraints: 340 Ma for Sclerosomatidae based on the Lower Carboniferous East Kirkton *Brigantibunum listoni* (Dunlop and Anderson, 2005); 300 Ma for Dyspnoi based on the Upper Carboniferous Coal Measures specimens (Dunlop, 2007); and 120 and 165 Ma for the cyphophthalmid families Pettalidae and Neogoveidae, respectively, based on the breakup of the landmasses that they inhabit (see Boyer et al., 2007b). Although use of vicariant events in dating phylogenies is a dubious practice, we note that their inclusion has no effect on clade age estimates (the origins

of both Pettalidae and Neogoveidae pre-date their respective constraints in this and other analyses). By contrast, the age of the root has perceptible ramifications throughout the phylogeny.

Age estimates are interpreted as tentative due to the many assumptions that go into such estimates. However, the discovery of key fossil opilionid lineages within the last decade (listed above) offers a unique opportunity to explore the pace of cladogenesis in a group of arachnids, which are plagued by limitations in the fossil record. Moreover, the implementation of error bars and degree of taxonomic sampling are meant to convey a sense of relative spans—not exact ages—of the divergence times obtained in this analysis.

Results and discussion

Although the subordinal relationships of Opiliones have received considerable attention (Martens, 1976, 1980, 1986; Martens et al., 1981; Giribet, 1997; Shultz, 1998; Giribet et al., 1999, 2002; Shultz and Regier, 2001; Giribet and Dunlop, 2005), phylogenetic studies within each of the suborders are few in number, with the notable exception of Cyphophthalmi. Due to the taxon sampling of the present analyses we were able to examine in detail the internal relationships of each of the four suborders. Subordinal relationships were not resolved with high support in the parsimony direct optimization analysis and remained unstable to parameter set variation (i.e. sensitivity analysis). By contrast, they were resolved with high bootstrap support in the maximum-likelihood analysis.

Outgroups and deep Opiliones relationships

Despite the large sampling effort in the number of both taxa and characters, a first evident result is a lack of support for deep splits in the parsimony direct optimization analysis, although support is higher in the maximum-likelihood tree. Outgroup relationships are unstable and receive little support in both methods. When rooted with pycnogonids, the pseudoscorpion *Calocheiridius* appears as the sister to all the other species in the parsimony analysis (Fig. 5), while the xiphosuran *Limulus polyphemus* appears sister to other arachnids in the maximum-likelihood analysis. Tetrapulmonata and Araneae are the only outgroup relationships to receive high jackknife frequencies in the direct optimization analysis (85 and 98%, respectively) and 100% bootstrap support in the maximum-likelihood analysis. Scorpiones (direct optimization) or Solifugae (maximum likelihood) appear as the sister group to Opiliones, forming the clade Stomothecata (Shultz, 2007) in the direct optimization analysis (Fig. 5), although again, support is below 51% for any of the relationships.

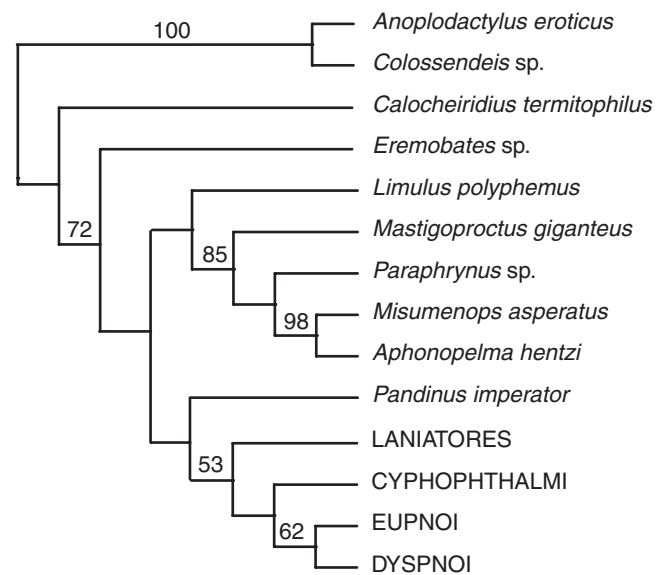


Fig. 5. Relationships among the main opilionid lineages and outgroups for the direct optimization analysis. This tree, obtained under parameter set 3221, had a cost of 60 827 steps. The clades Cyphophthalmi, Palpatores (Eupnoi + Dyspnoi), and Laniatores are expanded in Figs 6–8. Numbers at nodes indicate jackknife frequencies.

Monophyly of Opiliones receives 53% jackknife support in the direct optimization analysis, and 100% bootstrap support in the maximum-likelihood analysis, the latter yielding conventional relationships in the deepest parts of the tree. Of the four suborders three are well supported by the data in the direct optimization analysis [97% jackknife frequency (JF) for Cyphophthalmi, 98% for Laniatores, 100% for Dyspnoi], as shown in previous analyses of Opiliones relationships, molecular, morphological, or combined. Monophyly of Eupnoi is more unstable and received 64% JF. Monophyly of Eupnoi is furthermore questionable when analysing partial sequences of an Acropsopilioninae from New Zealand. The issue of Eupnoi monophyly, as well as that of Caddidae, will require further study, and due to the limited sampling will not be discussed here in further detail. Maximum-likelihood analysis corroborates the monophyly of all suborders; Cyphophthalmi, Dyspnoi and Laniatores each received 100% bootstrap support (BS), and Eupnoi received 98%.

Although relationships among the four Opiliones suborders are not resolved in the parsimony direct optimization analysis, maximum-likelihood analysis finds strong support for the monophyly of Phalangida (98% BS) and Palpatores (92% BS). Most of the direct optimization analyses, including multiple parameter sets, yield monophyly of Palpatores, placing Eupnoi and Dyspnoi as each other's sister taxa (62% JF). Monophyly of Palpatores, reflecting the traditional taxonomy of Opiliones, has been advocated by Shultz

(1998) and Shultz and Regier (2001) but disputed by Giribet et al. (1999, 2002). In the direct optimization analysis Palpatores appears as the sister group to Cyphophthalmi—a relationship contradicted by most modern analyses of Opiliones relationships (Giribet et al., 1999, 2002; Shultz and Regier, 2001) and by the maximum-likelihood tree—albeit without significant support. Laniatores therefore appears as the sister group to the other Opiliones in the direct optimization analysis, again, without significant support (Fig. 5), or as the sister group of Eupnoi + Dyspnoi (=Palpatores), forming the clade Phalangida in the maximum-likelihood analysis. From these results we conclude that support for Palpatores is higher than that for the alternative clade DyspnoLaniatores, and that maximum likelihood is better at recovering the traditional deep splits of Opiliones phylogeny than the direct optimization parsimony analysis for the data presently available.

Cyphophthalmi

Relationships among the Cyphophthalmi families have been a recent focus using morphology or molecules (Giribet and Boyer, 2002; de Bivort and Giribet, 2004; Boyer and Giribet, 2007; Boyer et al., 2007b; Clouse and Giribet, 2007). Monophyly of the families Pettalidae, Troglósironidae, Neogoveidae, and Stylocellidae are well supported in most of these previous analyses, as they are in this study (Figs 6 and 9). The family Sironidae is not monophyletic in the direct optimization analyses, and we find that the membership in Sironidae by the Japanese genus *Suzukielus* and the western European genus *Parasiro* is often refuted. Sironidae appears monophyletic in the maximum-likelihood analysis, although with BS below 50% for the membership of both the problematic genera *Parasiro* and *Suzukielus*. The position of Sironidae is consistent between the two analytical treatments; Sironidae form a clade with the Tropical cyphophthalmid families Neogoveidae + Troglósironidae under both direct optimization and maximum likelihood, but under neither method does this relationship receive significant resampling support. The clade composed of species from New Caledonia (Troglósironidae), the Afrotropical region, and the Neotropics (Neogoveidae) receives a JF value of 92% under direct optimization, and 100% BS under maximum likelihood bootstrap analysis, recapitulating this interesting relationship (Boyer et al., 2007b). A sister-group relationship of Pettalidae to the remaining Cyphophthalmi receives 64% JF under direct optimization for the clade including all non-pettalid species, and 97% bootstrap support in the maximum-likelihood tree, considerably better supported than in previous analyses (e.g. Giribet and Boyer, 2002; Boyer et al., 2007b). The position of Ogoveidae remains untested, as no material was available for molecular study at the time of analysis.

Relationships within Pettalidae have been investigated in greater detail in other studies (Giribet, 2003; Boyer and Giribet, 2007, 2009; Boyer et al., 2007b). Monophyly for all genera represented by more than one species is well supported, and the South African genus *Parapurcellia* appears as sister to all other species, as found in earlier studies (Boyer and Giribet, 2007). Support values for other generic relationships are marginal.

Within Stylocellidae, under direct optimization the genus *Stylocellus* appears paraphyletic whereas *Fangensis* is monophyletic. Both genera are monophyletic in the maximum-likelihood analysis (*Fangensis*, 96% BS; *Stylocellus*, 73% BS). Neogoveids are characterized by three clades, the North American *Metasiro*, the South American genera (*Huitaca*, *Metagovea*, *Neogovea*) and the tropical west African species, as in all previous studies (Boyer et al., 2007b). Nevertheless, whether the South American clade is sister to the African clade or to the North American *Metasiro* cannot be discriminated with certainty with this data set.

Finally, sironids are represented by three lineages, which do not form a clade under direct optimization: *Parasiro*, *Suzukielus* and a clade containing the remaining European and North American genera. This latter clade receives low jackknife (< 50%) and bootstrap (71%) support, but appears stable to analytical changes, and includes a group of *Paramiopsalis* + *Cyphophthalmus* and a clade uniting the species of *Siro*, both from Europe and from the USA. These results are also obtained in most analyses of sironid relationships using denser taxon sampling (Boyer et al., 2007b; Murienne and Giribet, 2009). The fact that *Parasiro* and *Suzukielus* may form independent lineages from sironids has been also proposed in previous analyses of discrete morphological characters (Giribet and Boyer, 2002; de Bivort and Giribet, 2004), but analysis of continuous morphological characters sometimes supports monophyly of Sironidae (B. de Bivort, R. Clouse, G. Giribet, unpublished data), reflecting the maximum-likelihood topology. A relationship of *Odontosiro* to *Parasiro*, as suggested by morphology, remains uninvestigated molecularly, as is the relationship of *Iberosiro* to *Paramiopsalis* (de Bivort and Giribet, 2004), and therefore they continue to be assessed based on morphological data alone. *Suzukielus* constitutes a monotypic genus inhabiting Honshu (Japan) where it can be locally abundant (Giribet et al., 2006) and shows a unique combination of sironid and pettalid characters. *Parasiro*, a genus containing three described species, is restricted to the western Mediterranean, including the north-east Iberian Peninsula, French Pyrenees, Corsica, Sardinia, and the Italian Peninsula (Juberthie, 1958; de Bivort and Giribet, 2004; Murienne and Giribet, 2009). The species of *Parasiro* lack typical sironid apomorphies, such as the presence of anal glands, and are apomorphic for several

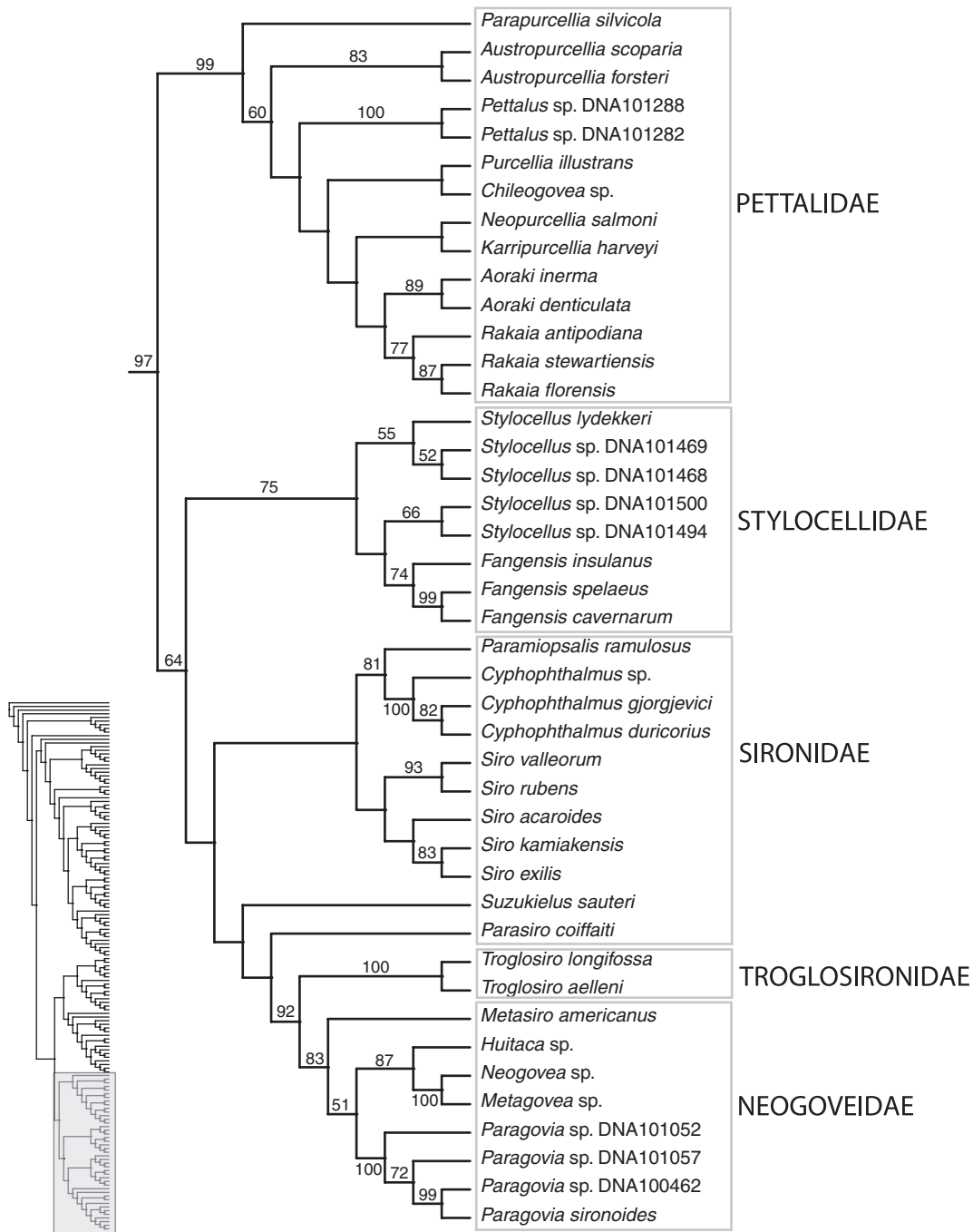


Fig. 6. Cyphophthalmi subtree (continues from Fig. 5) showing the relationships among the main cyphophthalmid lineages based on the direct optimization analysis. Numbers at nodes indicate jackknife frequencies.

key characters, such as the presence of opisthosomal sternites 8 and 9 fused medially, or the absence of a sensory structure at the tip of the ovipositor.

Given the topologies obtained in this study, a sister-group relationship of pettalids to the remaining families seems to be a robust outcome. After the discovery of anal gland pores in the males of several lineages of

Stylocellidae (Rambla, 1994; Schwendinger et al., 2004; Schwendinger and Giribet, 2005) in addition to Pettalidae and Sironidae, and having homologized the anal glands with the opisthosomal sternal glands of Neogoveidae and Troglosironidae (Sharma and Giribet, 2005), we now can reconstruct the presence of some sort of opisthosomal sexually dimorphic glands in the common

ancestor of all cyphophthalmids. Likewise, it is now believed that the presence of eyes among the members of Cyphophthalmi is more widespread than previously thought, as it is now known from most Stylocellidae and Pettalidae (Sharma and Giribet, 2006; Boyer and Giribet, 2007).

Eupnoi

Compared with previous analyses of Eupnoi relationships (Giribet et al., 2002), we have added two members of the family Monoscutidae, as well as further diversity within Phalangiidae and especially Sclerosomatidae, to include additional tropical and Southern Hemisphere species, with the aim of placing the genera *Eurybunus* and *Dalquestia* with more certainty. Attempts to include further caddid diversity were frustrated by the lack of other species or by difficulties and inconsistencies in amplifying and sequencing a single available specimen of *Acropsopilio neozelandiae* from New Zealand (Acropsopilioninae).

Both direct optimization and maximum-likelihood analyses supported a sister-group relationship of Caddidae (represented by the single species *Caddo agilis*) to the remainder Eupnoi (75% JF; 97% BS) (Shultz and Regier, 2001; Giribet et al., 2002). *Eurybunus* is thereafter placed as the sister group to all other species, which form a well-supported clade (80% JF; 99% BS), justifying a familial status of this genus. Cokendolpher (1984) included *Eurybunus* in the family Sclerosomatidae under the “*Metopilio* group” of uncertain subfamilial affinity (Cokendolpher and Lee, 1993: 16) together with the genera *Dalquestia*, *Diguetinus*, *Globipes*, and *Metopilio* from Mexico and the south-west USA, but our data do not support a relationship to *Dalquestia*. *Dalquestia* was already found to be an independent phalangioid in the analyses of Giribet et al. (2002), and again there may be phylogenetic justification for a familial status including this group of species from the south-west USA and north-west Mexico.

Phalangiidae and Monoscutidae are each monophyletic (JF of 67 and 99%, and BS of 100 and 100%, respectively). Sclerosomatidae, as traditionally defined, is not monophyletic, as none of the members of the “*Metopilio* group” group with the other sclerosomatids and the protolophid *Protolophus singularis* nests within Sclerosomatidae in the direct optimization analyses (monophyly of Sclerosomatidae, including *Protolophus*, receives 86% JF), or as its sister group (100% BS) in the maximum-likelihood analysis. While a relationship of Protolophidae to Sclerosomatidae has been recognized on the basis of the simple straight penis and opisthosomal ornamentation (Cokendolpher in Pinto-da-Rocha and Giribet, 2007, p. 128), it has been traditionally recognized as a separate family (Cokendolpher and Lee, 1993; Pinto-da-Rocha et al., 2007).

Sclerosomatidae constitutes the largest Eupnoi family, and includes the subfamilies Gagrellinae, Gyinae (not included in this study), Leiobuninae, and Sclerosomatinae. Of the subfamilies represented here by multiple individuals (Gagrellinae: *Marthana* and *Prionostemma*; Leiobuninae: *Leiobunum*, *Hadrobunus*, and *Nelima*; Sclerosomatinae: *Astrobunus* and *Homalenotus*) neither is monophyletic and it seems that the group is in urgent need of revision. However, taxon sampling within the family is too small to make statements about the phylogenetic relationships and composition of its subfamilies, as discussed by Tourinho (in Pinto-da-Rocha and Giribet, 2007, p. 130).

The members of Phalangiidae include representatives of three subfamilies, Opilioninae (*Opilio*), Oligolophinae (*Odiellus*), and Phalangiinae (*Phalangium* and *Rhampsinitus*), with Phalangiinae receiving 85% JF and 100% BS. The relationship of Phalangiidae to the other phalangioid families remains largely unanswered, except for the exclusion of *Eurybunus*.

Morphological analyses of the phalangioid groups are extremely scarce (Hunt and Cokendolpher, 1991). The traditional view is that the entapophysate forms—the Northern Hemisphere families containing species with an entapophysis in the spiracles—constitute a clade while the Southern Hemisphere non-entapophysate species (spiracles covered by a grate of spines) constitute a grade (Hunt and Cokendolpher, 1991) as the grate may constitute a plesiomorphy (Hunt, 1990; Hunt and Cokendolpher, 1991) (see Cokendolpher and Taylor in Pinto-da-Rocha and Giribet, 2007, pp. 120–121). Our analyses include only two New Zealand specimens of Monoscutidae and therefore do not allow testing for the monophyly of the non-entapophysate forms. Including Australian members of Monoscutidae as well as species within Neopilionidae, currently unsampled, will allow for a better understanding of the phalangioid relationships. Nevertheless, our analyses are conclusive in placing Protolophidae sister to or within Sclerosomatidae, in excluding the genus *Eurybunus* from Sclerosomatidae, and to a lesser degree in placing *Dalquestia* outside the three main phalangioid families.

Dyspnoi

Compared with Eupnoi, Dyspnoi is a smaller group, but has received more phylogenetic attention (Shear and Gruber, 1983; Shear, 1986; Giribet and Dunlop, 2005; Schönhofer and Martens, 2008). Our study expands on the sampling from previous studies (Shultz and Regier, 2001; Giribet et al., 2002), and the new results support many of the previous findings. The two clades Ischyropsalidoidea and Troguloidea are well supported in both sets of analyses (Figs 7 and 9). Within Ischyropsalidoidea, Ceratolasmatidae appears polyphyletic, with *Ceratolasma* as sister to Ischyropsalididae (67% JF;

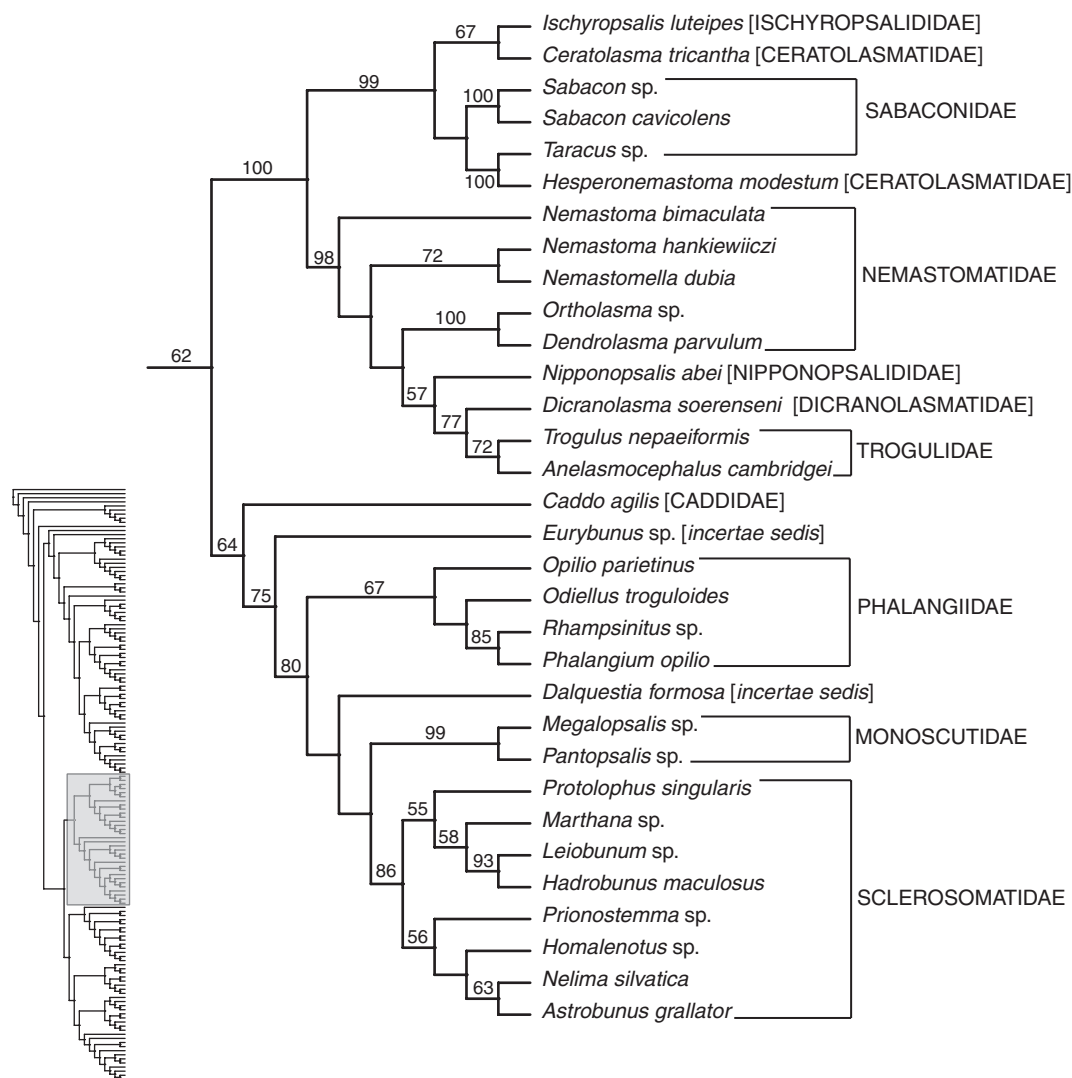


Fig. 7. Subtree illustrating the relationships among the Palpatores lineages Eupnoi and Dyspnoi (continues from Fig. 5). Numbers at nodes indicate jackknife frequencies.

100% BS), while *Hesperonemastoma* appears strongly supported as the sister group to the sabaconid *Taracus* (100% JF; 100% BS). A relationship of *Hesperonemastoma* to *Taracus* and to Sabaconidae is found, at least in part, in the studies of Shultz and Regier (2001) and Giribet et al. (2002).

Ceratolasma has been traditionally considered close to *Ischyropsalis* (Gruber, 1969, 1978) until it was included in the polyphyletic family Ceratolasmatidae (Shear, 1986), a hypothesis undermined by subsequent phylogenetic analyses of Dyspnoi that found a sister-group relationship of *Ceratolasma* + *Ischyropsalis*, with *Hesperonemastoma* related to Sabaconidae (Shultz and Regier, 2001; Giribet et al., 2002), relationships that are further supported in the current analyses. Gruber (in Pinto-da-Rocha and Giribet, 2007: 136–142) divided the members of Ceratolasmatidae into three groups, one

including the genera *Ceratolasma* + *Acuclavella*, related to Ischyropsalididae, another including *Hesperonemastoma*, and a third group including the genus *Crosbycus*, which was considered sister to *Hesperonemastoma* by Shear (1986). The phylogenetic position of these latter genera has been in flux. Although Gruber (1978) proposed a relationship that would subsequently be included in Sabaconidae (Shear, 1986), he questioned this relationship in the same article, stating that “*Hesperonemastoma* is quite aberrant in, for example, scutum type and midgut anatomy (Dumitrescu 1975, Shear, 1975); it may belong in yet another separate family with *Crosbycus* and an undescribed genus from Washington state [sic.] (Shear, in litt.)” (Gruber, 1978, p. 106).

In the absence of molecular data for *Crosbycus* and *Acuclavella* but due to the strong phylogenetic evidence provided here and elsewhere we propose a single

emendation to the current taxonomy, which refers to the inclusion of *Hesperonemastoma* in the family Sabaconidae (see below). *Acuclavella* is probably related to *Ceratolasma* and therefore belongs within Ceratolasmatidae, while the position of *Crosbycus* remains unresolved.

The new analyses include further sampling within Troglulidae and Nemastomatidae and show support for the monophyly of Trogluloidea (98% JF; 100% BS) as well as moderate support for the sister-group relationship of Dicranolasmatidae to Troglulidae (77% JF; 61% BS). While *Nipponopsalis* appears as the sister group to the latter clade in the direct optimization analysis, jackknife support is low (57%), and it is sister to all other trogluloids in the maximum-likelihood analysis, although without significant support. Finally, Nemastomatidae appears paraphyletic with respect to the other trogluloid families in the direct optimization analysis, but is monophyletic (90% BS) in the maximum-likelihood analysis. Nemastomatidae represents the largest and most diverse group of trogluloid harvestmen, and therefore its (unsupported) paraphyly in the direct optimization parsimony analysis could be due to sparse taxon sampling, as exemplified by the absence of numerous genera from the Caucasus (see an example of diversity in Martens, 2006). Support for the two Ortholasmatinae species *Ortholasma* sp. and *Dendrolasma parvulum* is strong (100% JF; 100% BS), confirming the monophyly of this Amphipacific subfamily (both species from North America and Japan were sampled).

The results obtained here illustrate the need for taxonomic emendation of the families of Ischyropsalidoidea. *Hesperonemastoma* is transferred to Sabaconidae, but other emendations will require the inclusion of members of the genera *Crosbycus* and *Acuclavella*. The taxonomy of Trogluloidea better reflects the evolutionary relationships of the superfamily but does not solve the relative position of *Nipponopsalis* with respect to the other families.

Sabaconidae—emended diagnosis. Sclerotization of body dorsal surface variable: from poorly sclerotized, with small opisthosomal scutum tenue and maximal development a scutum laminatum (*Sabacon* and *Taracus*) to well sclerotized, forming a scutum magnum (opisthosomal scutum and carapace not separated by a suture, in *Hesperonemastoma*). Metapeltidial sensory cones absent (*Hesperonemastoma*), or present as one (*Taracus*) or two (*Sabacon*). Ocularium near frontal edge of carapace; set far back from frontal border in *Taracus*. Chelicerae variable, from slender (*Sabacon*, *Hesperonemastoma*) to strongly elongated (*Taracus*). Moveable finger of chela with many narrow diaphanous teeth (few coarse teeth in most-proximal third in *Taracus*). Glandular (“epigamic”) organs in second segment absent (present in *Sabacon*). Pedipalpus moderately long to elongate,

slender, tibia and tarsus strongly inflated (only slightly inflated in *Hesperonemastoma*) with narrowed bases (“stalked”) and densely covered with glandular plumose “bottlebrush” setae. Tarsus folded back against medial side of tibia in *Sabacon*. Sexual dimorphism: body sclerotization, especially opisthosomal scutum, generally better developed in males (in *Sabacon* and *Taracus*); some species have minor differences in shape and development of genital operculum, chelicerae, and pedipalps. Penis shaft variable, from stout to slender; one pinnate penial muscle extending along most of truncus with short distal tendon (muscle very short, concentrated on base, with long tendon in *Sabacon*). Glans conical with variable setation and with long and tapering point. Ovipositor length variable, from short to elongate, unsegmented with two apical lobes, soft cuticle. Setation variable.

Laniatores

Of the four suborders of Opiliones, the largely tropical Laniatores encompasses almost two-thirds (over 4000 species) of described opilionid diversity, but has received far less than commensurate phylogenetic study, with a few exceptions of analyses often restricted to species groups or related genera from South America (e.g. Kury, 1993; Pérez González, 2006), Southeast Asia (Sharma and Giribet, 2009b), or Australia (Hunt, 1996).

Subsequent to numerous changes in systematics proposed by many authors over 170 years (reviewed by Kury in Pinto-da-Rocha et al., 2007; Giribet and Kury, 2007), Laniatores is presently divided into two tenuous infraorders, Insidiatores and Grassatores (Kury, 2003). The former is probably a paraphyletic entity, while the latter harbours most of the order’s striking examples of morphological, behavioural, and ecological diversity. Approximately half of all known Opiliones species (and 22 of the 45 families) are within Grassatores (Kury in Pinto-da-Rocha et al., 2007, pp. 159–168).

Previous attempts to address the phylogenetic relationships of Laniatores using molecular data were deficient in many ways. For example, Giribet et al. (2002) included 11 laniatorean species (three Insidiatores, eight Grassatores) comprising the families Triaenonychidae (+ Sclerobunidae), Sandokanidae (as Oncopodidae), Phalangodidae (+ *Maiorerus*), Cosmetidae, Gonyleptidae, and Stygnopsidae, and therefore omitted a large proportion of the familiar diversity and entire lineages such as Zalmoxoidea/Samooidea. Likewise, Shultz and Regier (2001) restricted their analyses to another set of 11 species (two Insidiatores, nine Grassatores) in the families Triaenonychidae (+ Sclerobunidae), Gonyleptidae, and Phalangodidae (+ Epedanidae). While our Cyphophthalmi dataset was obtained from previous work in Giribet’s laboratory

(Boyer et al., 2007b), and sampling within Eupnoi and Dyspnoi was only slightly improved with respect to previous datasets (Giribet et al., 2002), our sampling efforts were aimed towards adding resolution to the Laniatores tree and to test the informal phylogenies that have been proposed recently (Giribet and Kury, 2007). With these goals in mind, we were able to analyse 60 representative species, including all laniatorean families except Briggsidae (formerly Pentanychidae) and Guasiniidae. The degree of taxonomic sampling therefore allows us to test for higher groupings within Laniatores and to suggest some important taxonomic emendations. Given the species density of our data set, we also aimed to include a much larger molecular dataset than used previously (Giribet et al., 2002) mainly by increasing the 28S rRNA dataset to include almost 2.8 kb of data. The use of complete or nearly complete 28S rRNA has resulted in increased resolution in several studies of arthropod relationships (Mallatt and Giribet, 2006). The addition of the two mitochondrial markers and histone H3 was expected to contribute to improving relationships within Laniatores.

Nevertheless, and as in the previous suborders, some deep relationships remain unstable and/or poorly supported, but resolution for families and some interfamilial relationships are well supported. Grassatores appear monophyletic in most analyses (Figs 8 and 9). Insidiatores relationships are more unstable and often appear to be paraphyletic, as suggested previously (Giribet and Kury, 2007). These two groups are discussed in the following sections.

Insidiatores. Loman (1903) first recognized the morphological gap between Travunioidea (erstwhile combined in a single family, Triaenonychidae) and the other Laniatores and proposed a new suborder for this family—Insidiatores—but this change was mostly ignored by subsequent authors, until it was resurrected informally in an unpublished thesis (Kury, 1993). Currently it is hypothesized that the Northern Hemisphere “travunioids” and the Southern Hemisphere ones belong to distinct clades (Giribet and Kury, 2007). Our results largely corroborate this fundamental division. We consistently recovered a clade including the members of the Northern Hemisphere Travuniidae (*Hadziana*), Cladonychiidae (*Holoscotolemon*, *Theromaster*, *Erebomaster*; not forming a clade), *Trojanella*, and the “trienonychid” *Zuma acuta*, for which we adopt the family name Sclerobunidae stat. nov. This name has first been used as Sclerobuninae by Dumitrescu (1976) and never used by subsequent authors, only to be reused almost 30 years later (Kury, 2003) to include three North American genera. Briggsidae, which probably belongs within this clade, was not included in the analyses. This group of families constitutes the superfamily Travunioidea (*sensu* Giribet

and Kury, 2007). However, the internal relationships of the superfamily remain to be investigated in detail, especially after the study of more diversity, including briggsids.

Another clade consistently recovered encompasses the true triaenonychids from the Southern Hemisphere, including sampled species from South Africa and Australia, but it should most certainly include others from southern South America and New Zealand, which are not sampled here. This clade has been referred to as Triaenonychoidea (*sensu* Giribet and Kury, 2007). In the parsimony direct optimization analysis (Fig. 8), Triaenonychoidea (represented by the single family Triaenonychidae) and Travunioidea are sister clades, whereas the New Zealand family Synthetonychiidae and the North American species *Fumontana deprehendor* (traditionally in Triaenonychidae) appear as earlier lineages. In contrast, the maximum-likelihood analysis places *Fumontana* as sister to the other triaenonychids (72% BS), and Triaenonychoidea is sister to Travunioidea (95% BS). *Fumontana* is a monotypic genus, once thought to be one of the rarest Opiliones in North America (Shear, 1977) and has been considered a true member of the otherwise Southern Hemisphere Triaenonychidae (Giribet and Kury, 2007; Thomas and Hedin, 2008). Our analyses do not provide unambiguous support for the membership of *Fumontana* within Triaenonychoidea, and this should be tested further in a broader analysis of Insidiatores phylogeny. Synthetonychiidae was proposed as an endemic family to New Zealand (Forster, 1954) and its validity is supported by both analyses, which place it as sister to all other Laniatores. Synthetonychiidae is therefore another palaeoendemic relict of New Zealand, in the same category as the tuatara, adding more contradictory evidence to the total submersion hypothesis of New Zealand (e.g. Trewick et al., 2007).

Grassatores. Grassatores was proposed in an unpublished thesis (Kury, 1993) and subsequently formalized in his catalogue of Laniatores of the New World (Kury, 2003) to include all the non-Insidiatores Laniatores. The group is monophyletic under most analyses and includes the clades Sandokanoidea (formerly Oncopodoidea) and Gonyleptoidea proposed by previous authors (Martens, 1976; Martens et al., 1981; Giribet et al., 1999, 2002). However, this apparent result was due to the sparse sampling in these analyses, which neglected most of the Grassatores diversity. In the current analyses, Grassatores relationships are far more complex. Taxon sampling includes members of all families with the exception of Guasiniidae (González-Sponga, 1997).

Our analyses support monophyly of Grassatores (95% BS in maximum-likelihood analyses) and a basal split between true Phalangodidae (including the Holarctic genera *Bishopella* from the USA and *Scotolemon*

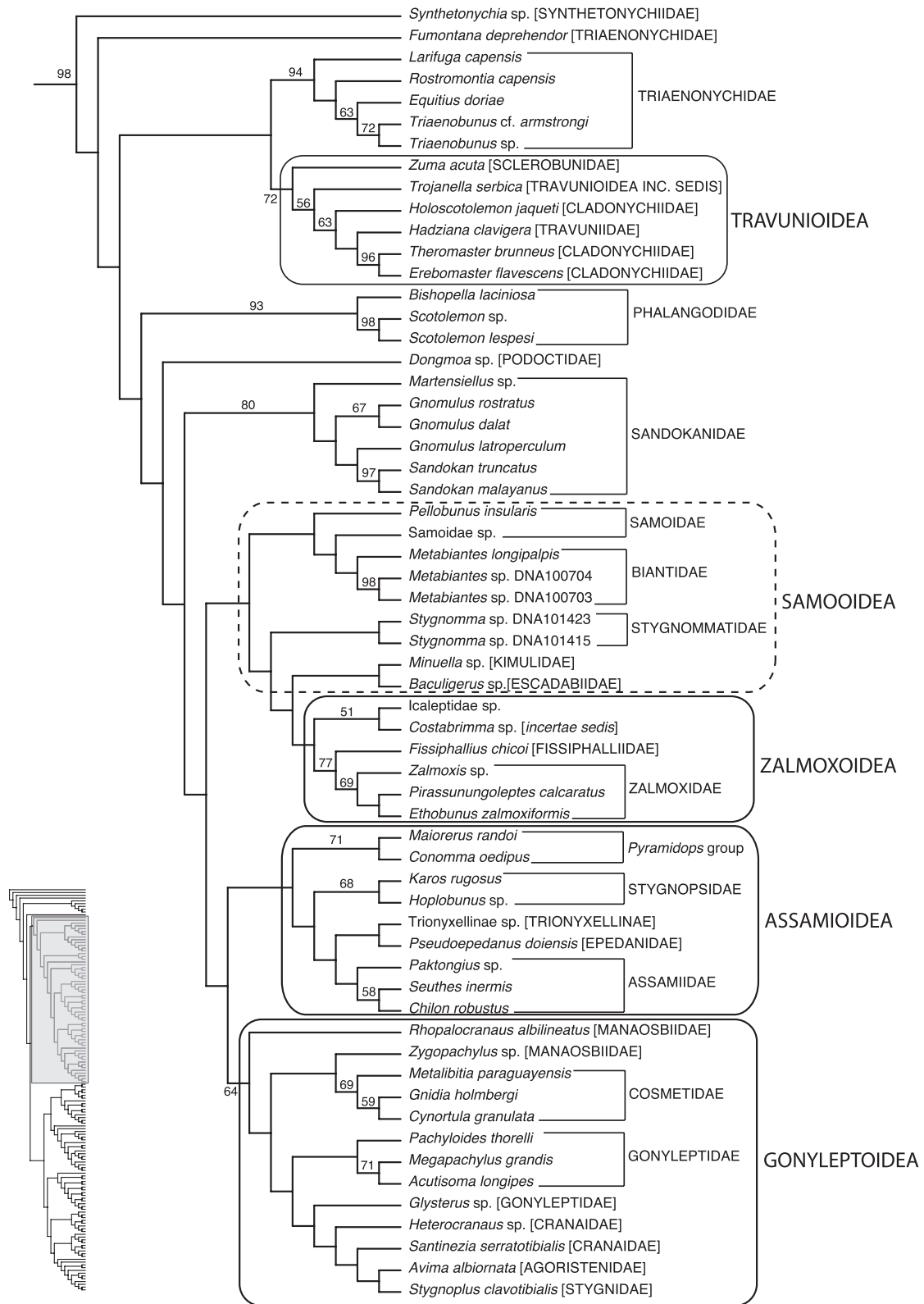


Fig. 8. Laniatores subtree (continues from Fig. 5). The main Laniatores superfamilies are indicated inside the solid boxes. Samoidea is paraphyletic, as indicated by a dashed box. Numbers at nodes indicate jackknife frequencies.

from Europe, but excluding the African “phalangodids” (*Conomma* and *Maiorerus*) and the remaining families, the latter clade receiving 97% BS in the maximum-likelihood analyses. The sister clade of Phalangodidae splits into Sandokanidae + Podoctidae and the remaining families in the maximum-likelihood analysis, but places Podoctidae as sister group to Sandokanidae + other families (included in Samooidea, Zalmoxoidea, Assamioidea, and Gonyleptoidea) in the direct optimization analysis. Neither analysis provides significant support for this basal split.

The African “phalangodids” *Conomma* (from Equatorial Guinea) and *Maiorerus* (from the Canary Islands), which are well supported as the clade that we call *Pyramidops* group—recognized as a new family by Staręga (1992) and informally used as the family Pyramidopidae in several online resources—are unstable and their sister group is unresolved. The epedanid *Pseudoepedanus* appears as the sister group to Trionyxellidae in the direct optimization analysis (Fig. 8) but it forms a clade with the *Pyramidops* group in the maximum-likelihood tree (Fig. 9). Both placements are poorly supported, but tend to place the *Pyramidops* group in phylogenetic proximity to Assamioidea.

A relationship of Kimulidae + Escadabiidae (although with low JF support; 60% BS) was first reported by Kury (1993) and subsequently by Pérez González and Kury (in Pinto-da-Rocha et al., 2007, p. 209), although they included both families in a monophyletic Samooidea.

Gonyleptoidea—The analyses recovered three main clades of gonyleptoid families. A clade including the families Manaosbiidae (polyphyletic), Cosmetidae, and Gonyleptidae (paraphyletic with respect to Cranidae, Agoristenidae, and Stygnidae) receives JF of 64% and BS of 93%, higher than any other clade comprising multiple families. This clade is similar to *Gonyleptoidea sensu* Giribet and Kury (2007) and corresponds to *Gonyleptoidea*, as currently recognized by Kury’s online classification of Opiliones (<http://www.museunacion-al.ufrj.br/mndi/Aracnologia/opiliones.html>, accessed 13 November 2008). Paraphyly of Gonyleptidae and Cranidae and polyphyly of Manaosbiidae will require further investigation with a better representation of their subfamilies, especially of Gonyleptidae, which includes more than 800 species. In the case of Manaosbiidae, *Rhopalocranaus* corresponds to a typical manaosbiid, but *Zygopachylus* lacks some typical manaosbiid synapomorphies such as the swollen proximal tarsomeres of the basitarsus I of the male. *Gonyleptoidea* is the largest superfamily of Opiliones, with ca. 2500 species in the New World, mostly in South America and the Antilles. The group includes a large number of subfamilies whose relationships remain to be better understood. The internal phylogeny of the group has been divided into families with a tarsal process and those without the process, but this division does not correspond to the

relationships found here. Nonetheless, support for most clades is low and other than the monophyly of the superfamily, little can be concluded from the current taxon sampling.

Assamioidea—A clade comprising the families Assamiidae, Epedanidae, Stygnopsidae, and the *Pyramidops* group—the superfamily Assamioidea, according to Kury’s online classification—is found as the sister group to *Gonyleptoidea* in the parsimony direct optimization analysis. This clade includes an assemblage of Old World families plus Stygnopsidae, mostly from North America and Central America, but is entirely missing from South America. In our analyses Assamiidae is represented by species from Equatorial Guinea and Thailand, which do not form a clade with the Trionyxellinae from Sri Lanka. Trionyxellinae (along with three other subfamilies) was considered a separate family by Mello-Leitão (1949), and our analysis supports this view, contrary to other authors, e.g. Staręga (1992) who suggested merging it again into Assamiidae. Maximum-likelihood analysis places Trionyxellinae as sister to the typical Assamiidae, albeit with negligible support (Fig. 9). The rest of the clade is formed by *Pseudoepedanus* (Epedanidae) from Thailand, the North American stygnopsids (*Karos* and *Hoplobunus*), and two African genera, *Maiorerus* and *Conomma*. The latter two genera have been included in Phalangodidae (Rambla, 1993), but they do not cluster with the true phalangodids (see Results and discussion above). For them we use the name *Pyramidops* group. Maximum likelihood fails to recover Assamioidea and instead places the North American stygnopsids (*Karos* and *Hoplobunus*) sister to the gonyleptoids, leaving the other assamioids as a grade co-mingled with samoooid lineages. Given that the group is not consistently supported across the analyses, we leave this group as a matter for future investigation.

Zalmoxoidea—Fissiphalliidae, Icaleptidae, and Zalmoxidae form the clade *Zalmoxoidea sensu* Giribet and Kury (2007) (67% BS), which also includes the family Guasiniidae, not sampled here. Fissiphalliidae is well supported as the sister group to Zalmoxidae (77% JF; 98% BS), as previously recognized (Kury and Pérez G., 2002). Whereas Fissiphalliidae has a restricted Neotropical distribution, Zalmoxidae has a very broad distribution, with species in the Neotropics as well as Afrotropical, Australasian, and Indo-Malay territories. Our sampling of zalmoxids included three species, one from Costa Rica, one from Brazil, and one from New Caledonia. Despite wide geographical range, the family is supported as monophyletic. Icaleptidae is found sister to a species of the genus *Costabrimma*, from Costa Rica, and of uncertain affinities (51% JF; 80% BS). This species pair is sister to the clade composed of Zalmoxidae + Fissiphalliidae. A relationship between these three families (Fissiphalliidae, Icaleptidae, and Zalmoxidae) has been suggested previously (Kury and Pérez G.,

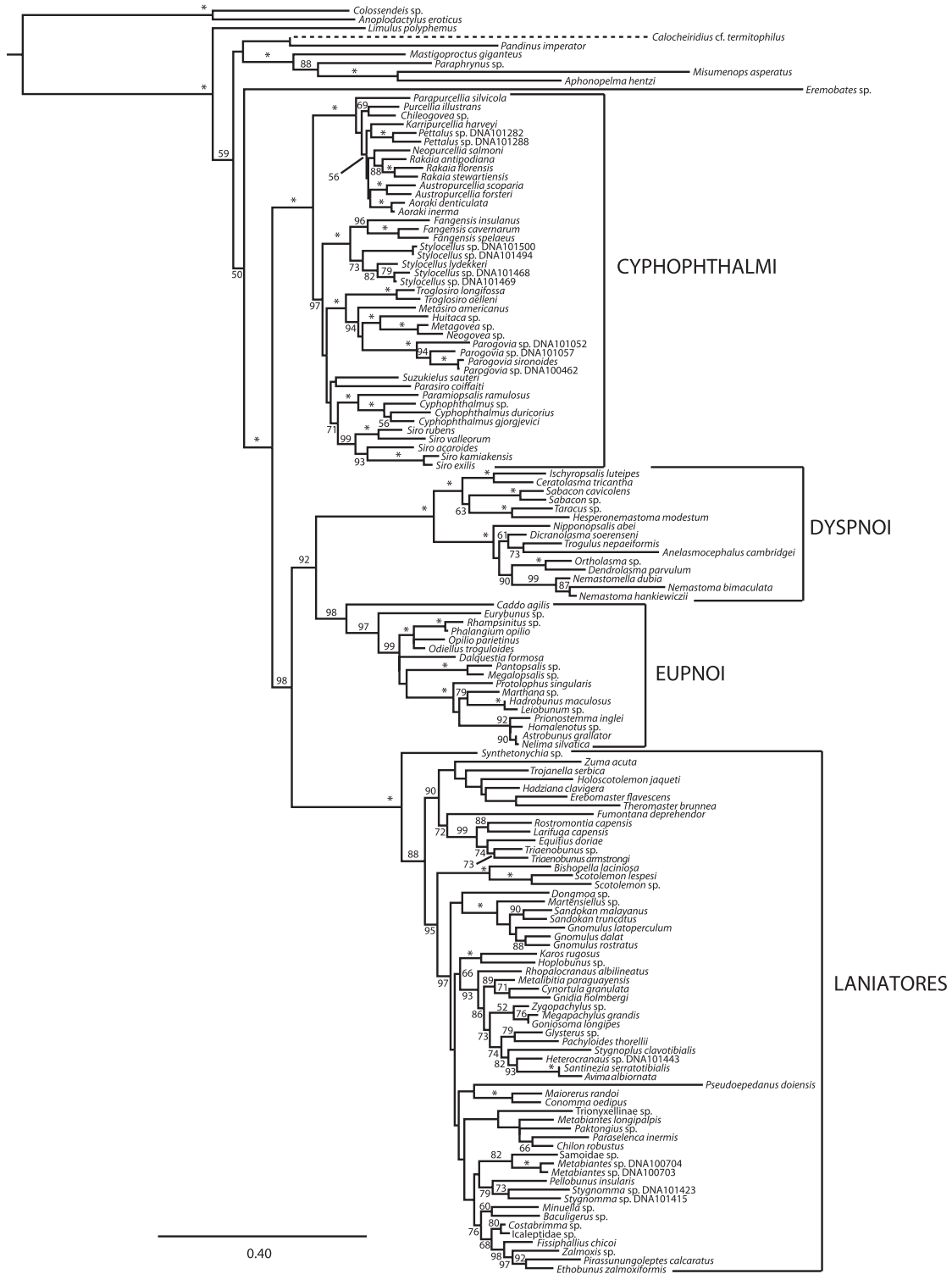


Fig. 9. Tree based on the static alignment analysed under maximum likelihood with GTR + Γ + I (–lnL = 102,390.92). Numbers at nodes indicate bootstrap support; asterisks indicate 100% bootstrap resampling frequency. Dashed line indicates truncated branch (*Calocheiridius cf. termitophilus*; original branch length 2.5416).

2002; Pinto-da-Rocha and Kury, 2003b). The restricted Neotropical nature of Fissiphalliidae, Guasiniidae, and

Icaleptidae contrasts with the much broader Zalmoxiidae. Whether the former represent relicts or the latter a

recent expansion remains to be studied (P. Sharma and G. Giribet, work in progress).

Samooidea—*Zalmoxoidea* appears nested within a group of families often recognized as forming part of *Samooidea* (*Biantidae*, *Escadabiidae*, *Kimulidae*, *Samoidae*, *Stygnommatidae*) in both sets of analyses (Figs 8 and 9), although resampling support for the *Samooidea*–*Zalmoxoidea* clade is low. *Podoctidae* does not form part of this clade, and instead appears much more basal in the tree, forming a grade with *Sandokanidae* (Fig. 8) or as sister to *Sandokanidae* (Fig. 9). *Samooidea* (*sensu* Giribet and Kury, 2007; see also Kury's online classification) is therefore polyphyletic. If *Podoctidae* is removed, *Samooidea* is still paraphyletic with respect to *Zalmoxoidea*. Likewise, *Samoidae*, represented only by species from Equatorial Guinea (*Samoidae* sp.) and Costa Rica (*Pellobunus insularis*), is not monophyletic. Pérez González and Kury (in Pinto-da-Rocha et al., 2007, p. 226) already noted that typical samoids are restricted to Polynesia, Melanesia, Australia, Mexico, the West Indies, and Venezuela and cast doubt upon the samoid kinship of the Indonesian and African species. Broader sampling of the samooidean groups is imperative for resolving phylogenetic relationships in this group. *Biantidae*, represented by species from Tropical West Africa (Equatorial Guinea) and southern Africa (Swaziland), forms a clade, but most of its diversity, concentrated in the Indian subcontinent and the Neotropics, was not represented in our study. Maximum-likelihood analysis casts some doubt on the monophyly of *Biantidae*, but neither result is supported. *Stygnommatidae* is represented by Neotropical species, i.e. *Stygnommatidae sensu stricto* by Pérez González (in Pinto-da-Rocha et al., 2007, p. 229–232). In the parsimony direct optimization analysis (Fig. 8), the family *Stygnommatidae* is sister to a clade formed by *Kimulidae* + *Escadabiidae* + *Zalmoxoidea* (with low support) whereas maximum-likelihood analysis places *Stygnommatidae* sister to a typical *Samoidae*, *Pellobunus*, as defined by Pérez González and Kury (in Pinto-da-Rocha et al., 2007, p. 224–226) with strong bootstrap support (79%). The hypothesis that the North American (Mexican) and the Indo-Malayan species do not belong to *Stygnommatidae* (Pérez González, 2006; Pérez González in Pinto-da-Rocha et al., 2007) remains to be tested with molecular data. The Neotropical endemics *Escadabiidae* and *Kimulidae* form a clade, which is sister to *Zalmoxoidea* (76% BS), reinforcing the hypothesis that zalmoxids have a Neotropical origin, despite representation in the Indo-pacific region.

In conclusion, the *Grassatores* phylogenetic scheme is similar to the one proposed mostly informally by A. Kury, based on morphology alone (Kury, 1992, 1993). Despite some instability and low nodal support, the trees derived from the two analyses are largely congru-

ent. Three families appear at the base of the tree, *Phalangodidae*, *Podoctidae*, and *Sandokanidae*, although they do not form a clade. In some instances *Phalangodidae*, *Podoctidae*, and *Sandokanidae* form a grade at the base of *Grassatores*. A relationship of *Podoctidae* to *Sandokanidae*, both distributed mostly along Wallacea and Sundaland, has been suggested in a recent molecular analysis of sandokanid relationships using eight molecular markers (Sharma and Giribet, 2009b). If the relationship of these two families is further supported, and their relationship to the Holarctic *Phalangodidae* is confirmed, these three families may constitute Kury's "Phalangodoidea", but the current analyses suggest that it is a grade. The relationship of "Samooidea" and *Zalmoxoidea* seems plausible, as is the sister-group relationship of *Assamioidea* and *Gonyleptoidea*. This may well constitute the basis for an improved classification of *Laniatores*.

Age estimates

Given the dense taxon sampling and the reliable fossil record for *Opiliones* and some of their proposed sister groups, we estimated ages for the origin of the *Opiliones* clades, fixing the age of *Eupnoi* to 400 Ma and that of the root to 420 Ma (see Methods). Fixing the root at 500 Ma, prior to the emergence of land arthropods but after the Cambrian explosion, did not have a noticeable impact in the clade estimates. Age estimates are largely imprecise, but they are intended to convey the relative span of particular divergences, not hard ages. This estimation aimed to compare the relative origins of each group. Having fixed these ages, the major clades of *Opiliones* were estimated as follows: *Cyphophthalmi* at 345 Ma, *Phalangida* at 415 Ma, *Palpatores* at 409 Ma, *Laniatores* at 305 Ma, *Insidiatores* at 257 Ma, *Grassatores* at 262 Ma, and *Dyspnoi* at 293 Ma (Fig. 10; see standard deviation of ages in Table 2). These ages are largely consistent with the *Opiliones* fossil record, specifically fossil taxa not included as calibration points.

Some salient points of the dating of the *Opiliones* tree are the inferences about the diversification of most of the modern groups. The crown-group *Cyphophthalmi* seems to have diversified almost 70 Ma after its sister group *Phalangida*, an unexpected outcome. The age of *Cyphophthalmi* was already inferred to be between 174 and 296 Ma by Boyer et al. (2007b), and their young age may be explained by the use of certain biogeographical constraints as fixed stages, which should have been designated minimum ages, had other fixed age estimates been available. Nevertheless, the common ancestor of modern *Cyphophthalmi* seems comparatively young given the existence of ingroup *Phalangida* in the Rhynie cherts of Scotland in the Early Devonian [Dunlop et al., 2004 (for 2003)].

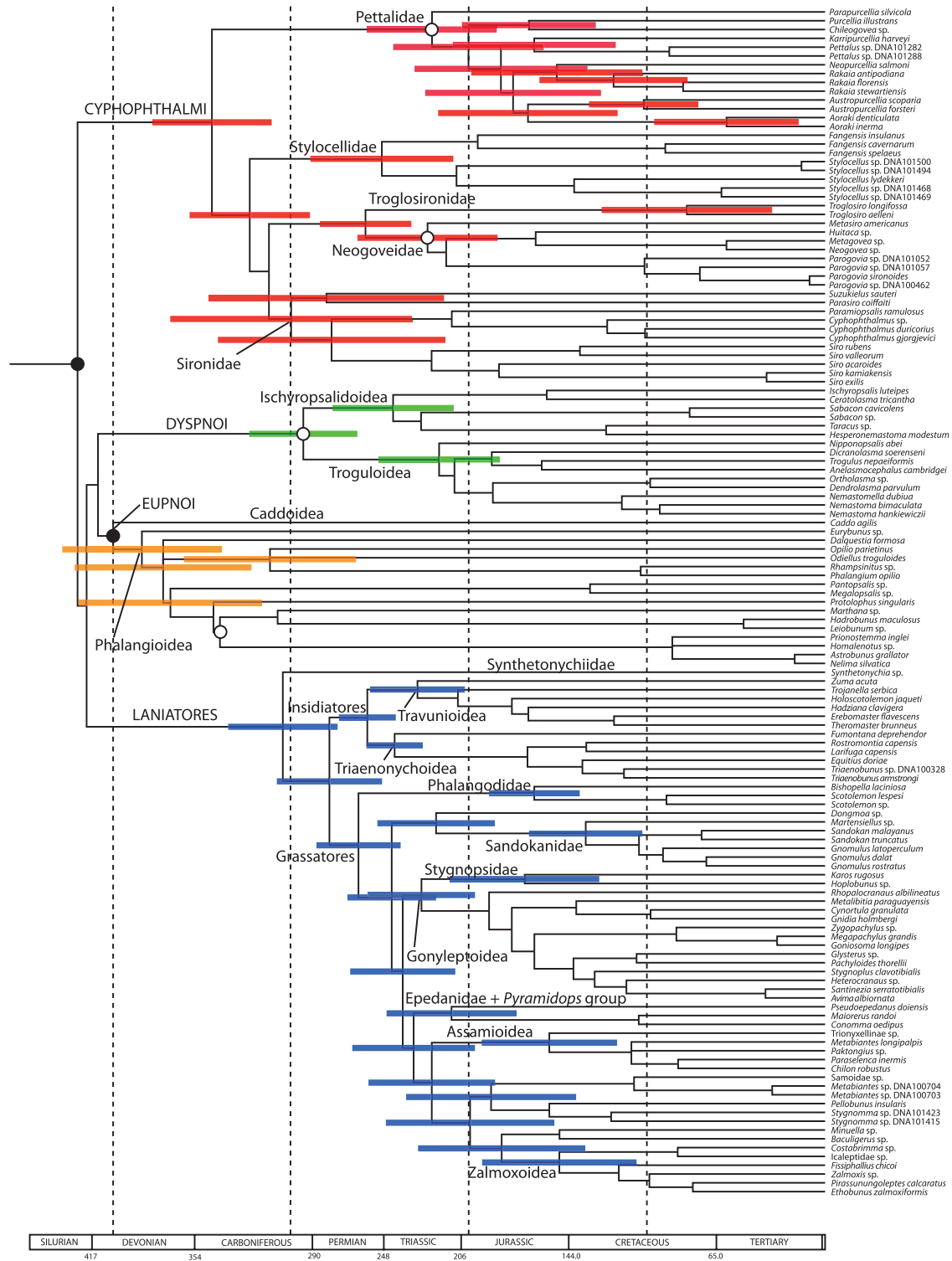


Fig. 10. Evolutionary timetree of Opiliones inferred from maximum-likelihood analysis. Coloured bars indicate 95% confidence intervals for estimations of divergence times. Closed circles indicate fixed nodes; open circles indicate nodes constrained as minimum ages.

The diversification of the cypophthalmid families spans a large fraction of geological time, with most families diversifying in the Triassic (Pettalidae, Neogoveidae, Stylocellidae;

although the sampled stylocellids are missing some of the basal lineages; R. Clouse and G. Giribet, unpublished results) and the Carboniferous

Table 2
Age estimates and standard deviations for selected opilionid clades

Node	Age (Ma)	SD
Cyphophthalmi	344.51	17.91
Pettalidae	220.85	19.4
Pettalidae excluding <i>Parapurcellia</i>	200.36	22.57
<i>Purcellia</i> + <i>Chileogovea</i>	166.46	20.08
<i>Karripurcellia</i> + <i>Pettalus</i>	163.41	24.44
<i>Karripurcellia</i> + <i>Pettaluss</i> + Aus/NZ	182.12	25.94
<i>Austropurcellia</i> + <i>Aoraki</i>	166.94	26.91
<i>Neopurcellia</i> + <i>Rakaia</i>	150.74	25.67
Aus/NZ	175.31	26.36
<i>Rakaia</i>	118.85	22.213
<i>Aoraki</i>	55.42	21.66
<i>Austropurcellia</i>	101.93	16.39
Stylocellidae	249.04	21.43
Troglosironidae	77.65	25.56
Neogoveidae	223.35	21.02
Sironidae	299.99	36.25
<i>Suzukielus</i> + <i>Parasiro</i>	280.17	35.27
Sironidae excluding <i>Suzukielus</i> and <i>Parasiro</i>	277.27	34.11
Trogl + Neo + Siro + Stylo	323.35	18.04
Trogl + Neo + Siro	312.46	n/a (not in all trees)
Trogl + Neo	258.22	13.73
Palpatores	408.52	14.76
Dyspnoi	293.16	16.21
Ischyropsalidoidea	242.71	18.19
Troguloidea	216.86	18.21
Eupnoi	400	n/a (fixed)
Phalangioida	383.85	23.9
Eupnoi excluding Caddidae and <i>Eurybunus</i>	371.92	26.52
Eupnoi excluding Caddidae, <i>Eurybunus</i> , Phalangiidae	367.82	27.43
Phalangiidae	311.89	25.79
Laniatores	304.68	16.41
Travunioidea	229.03	14.21
Triaenonychoidea	241.87	8.45
Insidiatores (excluding Synthetonychiidae)	257.2	8.53
Phalangodidae	163.38	13.61
Sandokanidae	134.49	16.99
Sandokanidae + Podoctidae	218.58	17.62
Gonyleptoidea	226.77	16.04
Epedanidae + <i>Pyramidops</i> group	209.9	19.5
Assamiidae + <i>Metabiantes</i> 1.	154.95	20.3
Samooidea	187.67	25.49
Zalmoxoidea	149.31	23.15
Zalmoxoidea + Kimulida + Escadabiidae	181.71	25.08
Zal. + Kim. + Esc. + Samoidae	199.46	25.26
Zal. + Kim. + Esc. + Sam. + Assamidae	220.98	18.99
Zal. + Kim. + Esc. + Sam. + Assam. + Epe. + Pyr.	231.19	18.39
ZKESAE + Gonyleptoidea	237.3	15.76
ZKESAE + Gon. + Sandok. + Podoc.	243.5	13.28
Grassatores	262.17	12.62
Stygnopsidae	168.82	22.4

Permian (Sironidae). A single family, Troglosironidae, undergoes diversification in the Late Cretaceous/Early Tertiary, but this oddity is probably related to the relictual nature of this Neocaledonian family (Sharma and Giribet, 2009a) and the sparse taxon sampling of the family in this study. The age of each family, which is estimated independently of vicariant events, is consistent with the postulated vicariance biogeography. It is

interesting to note that the divergence of the New Zealand Cyphophthalmi ranges between 55 (SD 21%) Ma for two species of *Aoraki*, 119 Ma for *Rakaia*, and 150 Ma for a monophyletic group that includes the New Zealand genera *Neopurcellia* + *Rakaia*. This strongly contrasts with the idea that the New Zealand Cyphophthalmi are recent arrivals to the islands (McGlone, 2005; Trewick et al., 2007; Goldberg et al., 2008; Landis

et al., 2008) and corroborates the notion that very old lineages do indeed inhabit New Zealand. Likewise, and with only two species sampled, diversification of the New Caledonian endemic genus *Troglosiro* could have happened *ca.* 77 Ma, much pre-dating the total submersion episode that would have ended by 37 Ma (Grandcolas et al., 2008).

Diversification of Dyspnoi probably occurred during the Carboniferous/Permian and that of its two clades, Ischyropsalidoidea and Trogluloidea, took place during the Triassic. The group remains restricted to the Northern Hemisphere and therefore it probably never dispersed to the southern part of Pangaea. Diversification of most laniatorid lineages also occurred during the Triassic, although the main splits occurred earlier, during the Late Carboniferous and Permian, leading to the modern New Zealand endemic Synthetonychiidae, and to the Northern Hemisphere Travunioidea and mostly Southern Hemisphere Triaenonychoidea (with the exception of *Fumontana*). Interestingly, the main clade of Triaenonychoidea (from the Southern Hemisphere, excluding *Fumontana*) seems to have diversified in the Jurassic, after the separation of Gondwana from Laurasia. Diversification of Gonyleptoidea also occurred between the Middle and Late Jurassic, suggesting a modern colonization of the Caribbean region and North America.

Biogeographical implications

Opiliones is an old group of Palaeozoic origin [Dunlop et al., 2003, 2004 (for 2003), Dunlop (2007)] comprised of some groups characterized by extremely low vagility, i.e. limited dispersal ability (Giribet and Kury, 2007). Although certain synanthropic Eupnoi have dispersed extensively, most Cyphophthalmi, Dyspnoi, and Laniatores have limited distribution ranges. The low vagility of Opiliones has been clearly shown in two species of Cyphophthalmi and one Laniatores, which show deep genetic divergences and population structure for geographically close populations (Boyer et al., 2007a; Thomas and Hedin, 2008; Sharma and Giribet, 2009a). Species-level biogeographical patterns have also been illustrated in analyses of different groups of Opiliones (Pinto-da-Rocha et al., 2005; Boyer and Giribet, 2007, 2009; Boyer et al., 2007b; Clouse and Giribet, 2007; Murienne et al., 2009; Sharma and Giribet, 2009a). These qualities of Opiliones are indicative of the utility of this model for studies of historical biogeography.

Cyphophthalmi have already been utilized in an array of phylogenetic and biogeographical studies (Schwendinger and Giribet, 2005; Boyer and Giribet, 2007, 2009; Boyer et al., 2007b; Clouse and Giribet, 2007; Murienne and Giribet, 2009; Murienne et al., 2009; Sharma and Giribet, 2009a), largely due to the fidelity of constituent

families to ancient landmasses: Pettalidae to temperate Gondwana, Neogoveidae to tropical Gondwana, Sironidae to Laurasia, and Stylocellidae to Southeast Asia (Benavides and Giribet, 2007; Boyer and Giribet, 2007; Boyer et al., 2007b; Clouse and Giribet, 2007; Giribet et al., 2007). Two other families are much narrower in their distributions, with Ogoveidae restricted to Cameroon, Gabon, and Equatorial Guinea in tropical west Africa (Giribet and Prieto, 2003) but of uncertain relationships, and Troglisironidae restricted to New Caledonia (Sharma and Giribet, 2009a,c), but with a clear relationship to the tropical Gondwanan Neogoveidae. This relationship of New Caledonia to tropical Gondwana is also found in Zalmoxoidea (see below).

Certain sister clades show complementary distributions. Within Insidiatores there is a clear distinction between the Northern Hemisphere Travunioidea and the Southern Hemisphere Triaenonychoidea, excluding *Fumontana*, which has been postulated to be related to the Southern Hemisphere families (Giribet and Kury, 2007). Within Grassatores many families are restricted to specific landmasses, although others are admittedly widespread (Giribet and Kury, 2007). An interesting pair is constituted by the superfamilies Assamioidea and Gonyleptoidea, the former distributed in the Old World and North America, whereas the latter is mostly found in South America, Mesoamerica, and the Antilles, with almost no overlap except for Cosmetidae, which has been able to colonize North America, and Stygnopsidae, which extends into Guatemala and El Salvador.

Zalmoxoidea is an interesting clade that includes three exclusively Neotropical families and the pan-tropical Zalmoxidae. The placement of Zalmoxoidea as sister to two other exclusively Neotropical families, Escadabiidae and Kimulidae, in our analyses suggests that Zalmoxidae may have undergone a large range expansion, and it is currently used as a model for oceanic dispersal in the South Pacific (P. Sharma, work in progress). The alternative reconstruction requires postulating that five previously broadly distributed families all became relicts in the Neotropics, a scenario that is not very parsimonious. Furthermore, the distributions of superfamilies “Samooidea” and Zalmoxoidea are mostly complementary, with many samooiid families constituting Old World/Holarctic groups, whereas the clade comprising Zalmoxoidea, Escadabiidae, and Kimulidae (and including perhaps the Neotropical stygnommatids) constitutes a Neotropical radiation. These hypotheses have not been rigorously explored and are contingent upon improved understanding of Neotropical diversity, some of it still undescribed, within Samoidea and Biantidae (A. Pérez González, unpublished data).

It seems there are few cases of Opiliones genera that span major continental landmasses. Salient examples available in the literature are some supposed

trans-Tasman genera, such as the pettalids *Neopurcellia* and *Rakaia*, which have been recently redefined as a result of a phylogenetic analysis clearly showing that both of these genera are restricted to New Zealand, whereas their Australian counterparts belong with the Queensland genus *Austropurcellia* (Boyer and Giribet, 2007). Other examples exist, most notably within Triaenonychidae, with the genera *Nuncia* distributed in New Zealand, Australia, and South America, and *Ceratomontia* found in South America and South Africa (Forster, 1954; Maury, 1990; Mendes and Kury, 2008). While the phylogeny of *Nuncia* is currently under investigation (S. Vélez and G. Giribet, work in progress), the relationships among *Ceratomontia* species of both continental landmasses have been recently investigated, showing monophyly of the South American species, whereas the South African *Ceratomontia* are paraphyletic and include other South African genera (Mendes and Kury, 2008). These results indicate, as in the case of the pettalids, that phylogeny is in agreement with biogeographical hypotheses but not necessarily with taxonomy. Other better supported transoceanic genera occur within Dyspnoi: e.g. *Crosbycus* occurs in eastern North America and eastern Asia; *Sabacon* is widespread in Eurasia and North America. Finally, the Eupnoi family Caddidae does not only have transoceanic genera such as *Austropsopilio*, *Acropsopilio*, and *Hesperopilio* (Shear, 1975; Shultz and Cekalovic, 2003, 2006) but also a case of two transoceanic species, *Caddo agilis* and *C. pepperella*, which can be found in eastern North America and eastern Asia (Shear, 1975; Shultz and Regier, 2009).

The ancient age, species richness, global distribution, and limitations in vagility characteristic of Opiliones predisposes this order to unparalleled utility in the study of historical biogeography. However, taxonomic revision is imperative in order to perform accurate phylogenetic analyses and test biogeographical hypotheses. The advent of dense taxonomic sampling in conjunction with phylogenetic analysis throughout Opiliones may provide better insight into the evolutionary history of this peculiar group of arachnids.

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References

- Benavides, L.R., Giribet, G., 2007. An illustrated catalogue to the South American species of the cyphophthalmid family Neogoveidae (Arthropoda, Opiliones, Cyphophthalmi) with a report on 37 new species. *Zootaxa* 1509, 1–15.
- de Bivort, B.L., Giribet, G., 2004. A new genus of cyphophthalmid from the Iberian Peninsula with a phylogenetic analysis of the Sironidae (Arachnida : Opiliones : Cyphophthalmi) and a SEM database of external morphology. *Invertebr. Syst.* 18, 7–52.
- 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). *J. Biogeogr.* 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. *Mol. Phylogenet. Evol.* 36, 554–567.
- Boyer, S.L., Baker, J.M., Giribet, G., 2007a. Deep genetic divergences in *Aoraki denticulata* (Arachnida, Opiliones, Cyphophthalmi): a widespread 'mite harvestman' defies DNA taxonomy. *Mol. Ecol.* 16, 4999–5016.
- Boyer, S.L., Clouse, R.M., Benavides, L.R., Sharma, P., Schwendinger, P.J., Karunarathna, I., Giribet, G., 2007b. Biogeography of the world: a case study from cyphophthalmid Opiliones, a globally distributed group of arachnids. *J. Biogeogr.* 34, 2070–2085.
- Castresana, J., 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol. Biol. Evol.* 17, 540–552.
- Clouse, R.M., Giribet, G., 2007. Across Lydekker's Line — first report of mite harvestmen (Opiliones : Cyphophthalmi : Stylocellidae) from New Guinea. *Invertebr. Syst.* 21, 207–227.
- Coddington, J.A., Giribet, G., Harvey, M.S., Prendini, L., Walter, D.E., 2004. Arachnida. In: Cracraft, J., Donoghue, M.J. (Eds.), *Assembling the Tree of Life*. Oxford University Press, New York, pp. 296–318.
- Cokendolpher, J.C., 1984. A new genus of North American harvestmen (Arachnida: Opiliones: Palpatores). In: Horner, N.V. (Ed.) *Festschrift for Walter W. Dalquest in Honor of His Sixty-sixth Birthday*. Midwestern State University, Wichita Falls, pp. 27–43.
- Cokendolpher, J.C., Lee, V.F., 1993. *Catalogue of the Cyphopalpatores and Bibliography of the Harvestmen (Arachnida, Opiliones) of Greenland, Canada, U.S.A., and Mexico*. Vintage Press, Lubbock, Texas.
- De Laet, J.E., 2005. Parsimony and the problem of inapplicables in sequence data. In: Albert, V.A. (Ed.) *Parsimony, Phylogeny, and Genomics*. Oxford University Press, Oxford, pp. 81–116.
- Dumitrescu, D., 1976. Recherches morphologiques sur l'appareil digestif (intestin moyen) des Gonyleptomorphi (Arachnida, Opilionida). *Travaux du Muséum d'Histoire Naturelle Grigore Antipa* 17, 17–30.
- Dunlop, J.A., 2007. Paleontology. In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.), *Harvestmen: The Biology of Opiliones*. Harvard University Press, Cambridge, MA, pp. 247–265.
- Dunlop, J.A., Anderson, L.I., 2005. A fossil harvestman (Arachnida, Opiliones) from the Mississippian of East Kirkton, Scotland. *J. Arachnol.* 33, 482–489.
- Dunlop, J.A., Anderson, L.I., Kerp, H., Hass, H., 2003. Preserved organs of Devonian harvestmen. *Nature* 425, 916.
- Dunlop, J.A., Anderson, L.I., Kerp, H., Hass, H., 2004 (for 2003). A harvestman (Arachnida: Opiliones) from the Early Devonian Rhynie cherts, Aberdeenshire, Scotland. *Trans. R. Soc. Edinb. Earth Sci.* 94, 341–354.
- Dunlop, J.A., Tetlie, O.E., Prendini, L., 2008. Reinterpretation of the Silurian scorpion *Proscorpius osborni* (Whitfield): Integrating data from Palaeozoic and Recent scorpions. *Palaeontology* 51, 303–320.
- Edgecombe, G.D., Giribet, G., 2006. A century later—a total evidence re-evaluation of the phylogeny of scutigeromorph centipedes (Myriapoda : Chilopoda). *Invertebr. Syst.* 20, 503–525.
- Farris, J.S., Albert, V.A., Källersjö, M., Lipscomb, D., Kluge, A.G., 1996. Parsimony jackknifing outperforms neighbor-joining. *Cladistics* 12, 99–124.
- Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39, 783–791.
- Forster, R.R., 1954. The New Zealand harvestmen (Sub-order Laniatores). *Canterbury Mus. Bull.* 2, 1–329.
- Giribet, G., 1997. Filogenia molecular de Artrópodos basada en la secuencia de genes ribosomales. PhD thesis. Departament de Biologia Animal, Universitat de Barcelona, Barcelona.
- Giribet, G., 2001. Exploring the behavior of POY, a program for direct optimization of molecular data. *Cladistics* 17, S60–S70.
- Giribet, G., 2003. *Karripurcellia*, a new pettalid genus (Arachnida : Opiliones : Cyphophthalmi) from Western Australia, with a cladistic analysis of the family Pettalidae. *Invertebr. Syst.* 17, 387–406.
- Giribet, G., 2007. Efficient tree searches with available algorithms. *Evol. Bioinform.* 3, 1–16.
- Giribet, G., Boyer, S.L., 2002. A cladistic analysis of the cyphophthalmid genera (Opiliones, Cyphophthalmi). *J. Arachnol.* 30, 110–128.
- Giribet, G., Dunlop, J.A., 2005. First identifiable Mesozoic harvestman (Opiliones: Dyspnoi) from Cretaceous Burmese amber. *Proc. R. Soc. B* 272, 1007–1013.
- Giribet, G., Kury, A.B., 2007. *Phylogeny and Biogeography*. In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.), *Harvestmen: The Biology of Opiliones*. Harvard University Press, Cambridge, MA, pp. 62–87.
- Giribet, G., Prieto, C.E., 2003. A new Afrotropical *Ogovea* (Opiliones, Cyphophthalmi) from Cameroon, with a discussion on the taxonomic characters in the family Ogoveidae. *Zootaxa* 329, 1–18.
- Giribet, G., Rambla, M., Carranza, S., Bagnuà, J., Riutort, M., Ribera, C., 1999. Phylogeny of the arachnid order Opiliones (Arthropoda) inferred from a combined approach of complete 18S and partial 28S ribosomal DNA sequences and morphology. *Mol. Phylogenet. Evol.* 11, 296–307.
- 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., Tsurusaki, N., Boyer, S.L., 2006. Confirmation of the type locality and the distributional range of *Suzukielus sauteri* (Opiliones, Cyphophthalmi) in Japan. *Acta Arachnologica* 55, 87–90.
- 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. *Zool. J. Linn. Soc.* 151, 663–670.
- Goldberg, J., Trewick, S.A., Paterson, A.M., 2008. Evolution of New Zealand's terrestrial fauna: a review of molecular evidence. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 363, 3319–3334.
- Goloboff, P.A., 1999. Analyzing large data sets in reasonable times: solutions for composite optima. *Cladistics* 15, 415–428.
- Goloboff, P.A., 2002. Techniques for analyzing large data sets. In: DeSalle, R., Giribet, G., Wheeler, W. (Eds.), *Techniques in Molecular Systematics and Evolution*. Birkhäuser Verlag, Basel, pp. 70–79.
- González-Sponga, M.A., 1997. Arácnidos de Venezuela: una nueva familia, dos nuevos géneros y dos nuevas especies de Opiliones Laniatores. *Acta Biol. Venez.* 17, 51–58.
- Grandcolas, P., Muriene, J., Robillard, T., DeSutter-Grandcolas, L., Jourdan, H., Guilbert, E., 2008. New Caledonia: a very old Darwinian island? *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 363, 3309–3317.
- Gruber, J., 1969. Weberknechte der Familien Sironidae und Trogludidae aus der Türkei (Opiliones, Arachnida). *Revue de la Faculté des Sciences de l'Université d'Istanbul* 34, 75–88.
- Gruber, J., 1978. Redescription of *Ceratolasma tricantha* Goodnight and Goodnight, with notes on the family Ischyropsalidae (Opiliones, Palpatores). *J. Arachnol.* 6, 105–124.
- Hunt, G.S., 1990. Taxonomic value of spiracle microstructure in the Megalopsalidae (Opiliones, Phalangioidea). *Acta Zoologica Fennica* 190, 187–194.
- Hunt, G.S., 1996. A preliminary phylogenetic analysis of Australian Triaenonychidae (Arachnida: Opiliones). *Rev. Suisse Zool.*, vol. hors série I, pp. 295–308. In: Mahner, V. (Ed.), *Proceedings of the XIIIth International Congress of Arachnology*, Geneva, 3–8 September 1995.
- Hunt, G.S., Cokendolpher, J.C., 1991. Ballarrinae, a new subfamily of harvestmen from the southern hemisphere (Arachnida, Opiliones, Neopilionidae). *Rec. Aust. Mus.* 43, 131–169.
- Juberthie, C., 1958. Révision du genre *Parasiro* (Opilions, Sironidae) et descriptions de *Parasiro minor* n. sp. *Bulletin du Muséum*, 2e série 30, 159–166.

- Juberthie, C., Massoud, Z., 1976. Biogéographie, taxonomie et morphologie ultrastructurale des opilions cyphophthalmes. *Rev. Écol. Biol. Sol* 13, 219–231.
- 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 Res.* 30, 3059–3066.
- Kury, A.B., 1992. The false Cranainae of the Brazilian Atlantic forest (Opiliones, Gonyleptidae). *Trop Zool* 5, 279–291.
- Kury, A.B., 1993. Análise filogenética de Gonyleptoidea (Arachnida, Opiliones, Laniatores). PhD thesis. Universidade de São Paulo, São Paulo.
- Kury, A.B., 1994a. Early lineages of Gonyleptidae (Arachnida Opiliones Laniatores). *Trop Zool* 7, 343–353.
- Kury, A.B., 1994b. The genus *Yania* and other presumed Tricommatidae from South American highlands (Opiliones, Cranainae, Prostygminae). *Revue Arachnologique* 10, 137–145.
- Kury, A.B., 1997a. A new subfamily of Agoristenidae, with comments on suprageneric relationships of the family (Arachnida, Opiliones, Laniatores). *Trop Zool* 10, 333–346.
- Kury, A.B., 1997b. The genera *Saramacia* Roewer and *Syncranaus* Roewer, with notes on the status of the Manaosbiidae (Opiliones, Laniatores). *Boletim do Museu Nacional, N.S. Zoologia, Rio de Janeiro* 374, 1–22.
- Kury, A.B., 2003. Annotated catalogue of the Laniatores of the New World (Arachnida, Opiliones). *Revista Ibérica de Aracnología Volumen especial monográfico 1*, 1–337.
- Kury, A.B., Pérez, G.A., 2002. A new family of Laniatores from northwestern South America (Arachnida, Opiliones). *Revista Ibérica de Aracnología* 6, 3–11.
- Landis, C.A., Campbell, H.J., Begg, J.G., Mildenhall, D.C., Paterson, A.M., Trewick, S.A., 2008. The Waipounamu Erosion Surface: questioning the antiquity of the New Zealand land surface and terrestrial fauna and flora. *Geol. Mag.* 145, 173–197.
- Loman, J.C.C., 1903. On the classification of Opiliones. *Tijd. ned. dierk. Vereen* 8, 62–66.
- Mallatt, J., Giribet, G., 2006. Further use of nearly complete 28S and 18S rRNA genes to classify Ecdysozoa: 37 more arthropods and a kinorhynch. *Mol. Phylogenet. Evol.* 40, 772–794.
- Martens, J., 1976. Genitalmorphologie, System und Phylogenie der Weberknechte (Arachnida, Opiliones). *Entomologica Germanica, Zeitschrift für das Gesamtgebiet der wissenschaftlichen Entomologie* 3, 51–68.
- Martens, J., 1980. Versuch eines Phylogenetischen Systems der Opiliones. In: Gruber, J. (Ed.), *Proceedings 8th International Congress of Arachnology, Vienna. Verhandlungen. Verlag H. Egerman, Vienna, Austria*. pp. 355–360.
- Martens, J., 1986. Die Groggliederung der Opiliones und die Evolution der Ordnung (Arachnida). *Actas X Congr. Int. Arachnol., Jaca* 1, 289–310.
- Martens, J., 2006. Harvestmen from the Caucasus (Arachnida, Opiliones, Nemastomatidae). *Senckenbergiana biologica* 86, 145–210.
- Martens, J., Hoheisel, U., Götze, M., 1981. Vergleichende Anatomie der Legeröhren der Opiliones als Beitrag zur Phylogenie der Ordnung (Arachnida). *Zoologische Jahrbücher, Abteilung für Anatomie und Ontogenie der Tiere* 105, 13–76.
- Maury, E.A., 1990. Triaenonychidae sudamericanos. VI. Tres nuevas especies del género *Nuncia* Loman 1902 (Opiliones, Laniatores). *Bol. Soc. Biol. Concepción, Chile* 61, 103–119.
- McGlone, M.S., 2005. Goodbye Gondwana. *J. Biogeogr.* 32, 739–740.
- de Mello-Leitão, C.F., 1949. Famílias, subfamília, espécies e gêneros novos de opilões e notas de sinonímia. *Boletim do Museu Nacional Nova Série, Zoologia, Rio de Janeiro* 94, 1–33.
- Mendes, A.C., Kury, A.B., 2008. Intercontinental Triaenonychidae—the case of *Ceratontia* (Opiliones, Insidiatores). *J. Arachnol.* 36, 273–279.
- Murienne, J., Giribet, G., 2009. The Iberian Peninsula: ancient history of a hot spot of mite harvestmen (Arachnida: Opiliones: Cyphophthalmi: Sironidae) diversity. *Zool. J. Linn. Soc.* 156, 785–800.
- Murienne, J., Karaman, I., Giribet, G., 2009. Explosive evolution of an ancient group of Cyphophthalmi (Arachnida: Opiliones) in the Balkan Peninsula. *J. Biogeogr.*, doi: 10.1111/j.1365-2699.2009.02180.x.
- Orrico, V.G.D., Kury, A.B., 2009. A cladistic analysis of the Stygnicraninae Roewer, 1913 (Arachnida, Opiliones, Cranainae)—where do longipalp cranoids belong? *Zool. J. Linn. Soc.* 157, 470–494.
- Pérez González, A., 2006. Revisão sistemática e análise filogenética de Stygnommatidae (Arachnida: Opiliones: Laniatores). PhD thesis. Museu Nacional, Universidade Federal de Rio de Janeiro, Rio de Janeiro.
- Pinto-da-Rocha, R., 1997. Systematic review of the Neotropical family Stygnidae (Opiliones, Laniatores, Gonyleptoidea). *Arquivos de Zoologia* 33, 163–342.
- Pinto-da-Rocha, R., 2002. Systematic review and cladistic analysis of the Caelopyginae (Opiliones, Gonyleptidae). *Arquivos de Zoologia, São Paulo* 36, 357–464.
- Pinto-da-Rocha, R., Giribet, G., 2007. Taxonomy. In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.), *Harvestmen: The Biology of Opiliones*. Harvard University Press, Cambridge, MA, pp. 88–246.
- Pinto-da-Rocha, R., Kury, A.B., 2003a. Phylogenetic analysis of *Santinezia* with description of five new species (Opiliones, Laniatores, Cranainae). *J. Arachnol.* 31, 173–208.
- Pinto-da-Rocha, R., Kury, A.B., 2003b. Third species of Guasiniidae (Opiliones, Laniatores) with comments on familial relationships. *J. Arachnol.* 31, 394–399.
- Pinto-da-Rocha, R., da Silva, M.B., Bragagnolo, C., 2005. Faunistic similarity and historic biogeography of the harvestmen of southern and southeastern Atlantic rain forest of Brazil. *J. Arachnol.* 33, 290–299.
- Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.) 2007. *Harvestmen: The Biology of Opiliones*. Harvard University Press, Cambridge, MA.
- Posada, D., 2005. Modeltest 3.7. Program and documentation available at darwin.uvigo.es. Vigo.
- Posada, D., Buckley, T., 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Syst. Biol.* 53, 793–808.
- Posada, D., Crandall, K.A., 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14, 817–818.
- Rambla, M., 1993. *Maiorerus randoi* n. gen., n. sp., the first Laniatorid from a Canary Island cave (Opiliones, Phalangodidae). *Mémoires de Biospéologie* 20, 177–182.
- Rambla, M., 1994. Un nouveau Cyphophthalme du sud-est asiatique, *Fangensis leclerci* n. gen. n. sp. (Opiliones, Sironidae). *Mémoires de Biospéologie* 21, 109–114.
- Sanderson, M.J., 1997. A nonparametric approach to estimating divergence times in the absence of rate constancy. *Mol. Biol. Evol.* 14, 1218–1231.
- Sanderson, M.J., 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Mol. Biol. Evol.* 19, 101–109.
- 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.
- Schönhofer, A.L., Martens, J., 2008. Revision of the genus *Trogulus* Latreille: the *Trogulus coriziformis* species-group of the western Mediterranean (Opiliones: Trogludidae). *Invertebr. Syst.* 22, 523–554.
- Shultz, J.W., Regier, J.C., 1998. *Caddo agilis* and *C. pepperella* (Opiliones, Caddidae) diverged phylogenetically before acquiring their disjunct, sympatric distributions in Japan and North America. *J. Arachnol.* 37, 328–240.

- Schwendinger, P.J., Giribet, G., 2005. The systematics of the south-east Asian genus *Fangensis* Rambla (Opiliones: Cyphophthalmi: Stylocellidae). *Invertebr. Syst.* 19, 297–323.
- Schwendinger, P.J., Martens, J., 2002. Penis morphology in Oncopodidae (Opiliones, Laniatores): Evolutionary trends and relationships. *J. Arachnol.* 30, 425–434.
- Schwendinger, P.J., Giribet, G., Steiner, H., 2004. A remarkable new cave-dwelling *Stylocellus* (Opiliones, Cyphophthalmi) from Peninsular Malaysia, with a discussion on taxonomic characters in the family Stylocellidae. *J. Nat. Hist.* 38, 1421–1435.
- Sharma, P., Giribet, G., 2005. A new *Troglosiro* species (Opiliones, Cyphophthalmi, Troglosironidae) from New Caledonia. *Zootaxa* 1053, 47–60.
- Sharma, P., Giribet, G., 2006. A new *Pettalus* species (Opiliones, Cyphophthalmi, Pettalidae) from Sri Lanka with a discussion on the evolution of eyes in Cyphophthalmi. *J. Arachnol.* 34, 331–341.
- Sharma, P., Giribet, G., 2009a. A relict in New Caledonia: Phylogenetic relationships of the family Troglosironidae (Opiliones: Cyphophthalmi). *Cladistics* 25, 279–294.
- Sharma, P., Giribet, G., 2009b. Sandokanid phylogeny based on eight molecular markers—the evolution of a southeast Asian endemic family of Laniatores (Arachnida, Opiliones). *Mol. Phylogenet. Evol.* 52, 432–447.
- Sharma, P.P., Giribet, G., 2009c. The family Troglosironidae (Opiliones: Cyphophthalmi) of New Caledonia. *Zoologia Neocaledonia* 7. Biodiversity Studies in New Caledonia, Paris, pp. 83–123.
- Shear, W.A., 1975. The opilionid family Caddidae in North America, with notes on species from other regions (Opiliones, Palpatores, Caddoidea). *J. Arachnol.* 2, 65–88.
- Shear, W.A., 1977. *Fumontana deprehendor* n. gen., n. sp., the first triaenonychid opilionid from eastern North America (Opiliones: Laniatores: Triaenonychidae). *J. Arachnol.* 3, 177–183.
- Shear, W.A., 1986. A cladistic analysis of the opilionid superfamily Ischyropsalidoidea, with descriptions of the new family Ceratolasmatidae, the new genus *Acuclavella*, and four new species. *Am. Mus. Novit* 2844, 1–29.
- Shear, W.A., Gruber, J., 1983. The opilionid subfamily Ortholasmatinae (Opiliones, Troguloidea, Nemastomatidae). *Am. Mus. Novit* 2757, 1–65.
- Shultz, J.W., 1990. Evolutionary morphology and phylogeny of Arachnida. *Cladistics* 6, 1–38.
- Shultz, J.W., 1998. Phylogeny of Opiliones (Arachnida): an assessment of the “Cyphopalpatores” concept. *J. Arachnol.* 26, 257–272.
- Shultz, J.W., 2007. A phylogenetic analysis of the arachnid orders based on morphological characters. *Zool. J. Linn. Soc.* 150, 221–265.
- Shultz, J.W., Cekalovic, T., 2003. First species of *Austropsopilio* (Opiliones, Caddoidea, Caddidae) from South America. *J. Arachnol.* 31, 20–27.
- Shultz, J.W., Cekalovic, T., 2006. First species of *Hesperopilio* (Opiliones, Caddoidea, Caddidae) from South America. *J. Arachnol.* 34, 46–50.
- Shultz, J.W., Regier, J.C., 2001. Phylogenetic analysis of Phalangida (Arachnida, Opiliones) using two nuclear protein-encoding genes supports monophyly of Palpatores. *J. Arachnol.* 29, 189–200.
- Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22, 2688–2690.
- Stamatakis, A., Hoover, P., Rougemont, J., 2008. A rapid bootstrap algorithm for the RAxML Web servers. *Syst. Biol.* 57, 758–771.
- Starega, W., 1992. An annotated check-list of Afrotropical harvestmen, excluding the Phalangidae (Opiliones). *Ann. Natal Mus.* 33, 271–336.
- Thomas, S.M., Hedin, M., 2008. Multigenic phylogeographic divergence in the paleoendemic southern Appalachian opilionid *Fumontana deprehendor* Shear (Opiliones, Laniatores, Triaenonychidae). *Mol. Phylogenet. Evol.* 46, 645–658.
- Treweek, S.A., Paterson, A.M., Campbell, H.J., 2007. Hello New Zealand. *J. Biogeogr.* 34, 1–6.
- Varón, A., Vinh, L.S., Bomash, I., Wheeler, W.C., 2007. POY 4.0 Beta 1983 American Museum of Natural History. <http://research.amnh.org/scicomp/projects/poy.php>, New York.
- Wheeler, W.C., 1996. Optimization alignment: the end of multiple sequence alignment in phylogenetics? *Cladistics* 12, 1–9.
- Wheeler, W.C., Hayashi, C.Y., 1998. The phylogeny of extant chelicerate orders. *Cladistics* 14, 173–192.

Appendix 1

List of specimens used in the molecular study with collecting data and MCZ accession voucher number. Collection data for species in square brackets are available in Giribet et al. (2002).

Cyphophthalmi

- Aoraki denticulata denticulata* (Forster, 1948) (MCZ DNA100961)
St. Arnaud (41°48'29"S, 172°50'01"E, 618 m), Brunner Peninsula, Lake Rotoiti, Bay of Plenty, North Island (New Zealand), leg. S.L. Boyer, C. D'Haese, G. Giribet, 30.i.2003
- Aoraki inerma inerma* (Forster, 1948) (MCZ DNA100967)
Lake Waikareiti (38°43'44"S, 177°09'46"E, 851 m), Te Urewera National Park, North Island (New Zealand), leg. S.L. Boyer, C. D'Haese, G. Giribet, 21.i.2003
- Austropurcellia scoparia* Juberthie, 1988 (MCZ DNA100946)
Mt. Lewis State Forest (16°35'40"S, 145°16'45"E, 1000 m), Queensland (Australia), leg. C. D'Haese, G. Giribet, 17.ii.2003
- Austropurcellia forsteri* (Juberthie, 2000) (MCZ DNA100945)
Emmagen Creek (16°03'41"S, 145°27'43"E, 22 m), Daintree National Park, Cape Tribulation section, Queensland (Australia), leg. C. D'Haese, G. Giribet, 18.ii.2003
- Chileogovea* sp. (MCZ DNA100413)
Puyehue (41°30'30"S, 72°37'00"W, 350 m), Osorno Province (Chile), leg. F. Álvarez, J. Coddington, J. Miller, 21.xii.2000, mixed forest with *Fitzroya cupressoides*
- Cyphophthalmus duricorius* Joseph, 1868 (for 1869) (MCZ DNA100847)
Podsko Karjeva jama, Zg. Besnica (Slovenia), leg. M. Comatti, 7.x.2001
- Cyphophthalmus gjorgjevici* (Hadzi, 1933) (MCZ DNA100498)
Between Matka and Skopje (Macedonia), leg. I. Karaman
- Cyphophthalmus* sp. (MCZ DNA101342)
Kljuch village, Distr. Petrich, Mt Belasica (Bulgaria), leg. P. Mitov, 22.v.2004
- Fangensis cavernarum* Schwendinger and Giribet, 2005 (MCZ DNA101460)
Tham Nam Phrathat (14°23'53"N, 99°04'52"E, 570 m), Si Sawat District, Kanchanaburi Province (Thailand), leg. P.J. Schwendinger, 17.vii.2004 (MHNG TH-04/01)
- Fangensis insulanus* Schwendinger and Giribet, 2005 (MCZ DNA100388)
Ko Siray (7°53'06"N, 98°26'13"E, 30 m), Phuket Province (Thailand), leg. P.J. Schwendinger, 12.viii.2000
- Fangensis spelaeus* Schwendinger and Giribet, 2005 (MCZ DNA100669)
Tham Kaeng Lawa (14°17'59"N, 98°58'59"E, 150 m), Sai Yok District, Kanchanaburi Province (Thailand), leg. P.J. Schwendinger, 30.iv.2003 (MHNG TH-03/01)
- Huitaca* sp. (MCZ DNA101407)

Road from Arcabuco to Moniquirá, Departamento de Boyacá (Colombia), 5°46'46"N, 073°27'13"W, 2,559 m, leg. L.R. Benavides, D. Campos, E. Flórez, G. Giribet, 30.xi.2004

Karripurcellia harveyi Giribet, 2003 (MCZ DNA101303)

Bicentennial Tree (34°29'42"S, 115°58'31"E, 138 m), Warren National Park, Western Australia (Australia), leg. G. Giribet, S. Huff, M.K. Nishiguchi, 10.vii.2004

Metagovea sp. (MCZ DNA101410)

Reserva Natura Río Nambí (1°17'06"N, 78°04'25"W, 1448 m), Municipio de Barbacoas, Corregimiento de Altaquer, Departamento de Nariño (Colombia), leg. L.R. Benavides, L. Cabrera, G. Castillo, C. Flórez, G. Giribet, M. Romo, V. Solarte, 10-11.xi.2004

Metasiro americanus (Davis, 1933) (MCZ DNA101532)

Weeping Ridge Trail (30°33'53"N, 84°57'05"W, 38 m), Torreya State Park, Florida (USA), leg. S.L. Boyer, R.M. Clouse, G. Giribet, 12.iii.2005

Neogovea sp. (MCZ DNA101408)

Track to Calderón (4°02'41"S, 69°59'23"W, 87 m), off km 22 N of Leticia, Departamento del Amazonas (Colombia), leg. L.R. Benavides, G. Giribet, R. Mesa, 6.xi.2004

Neopurcellia salmoni Forster, 1948 (MCZ DNA100939)

Haast Pass (44°06'28"S, 169°21'19"E, 580 m), South Island (New Zealand), leg. S.L. Boyer, C. D'Haese, G.D. Edgecombe, G. Giribet, 8.ii.2003

Paragovia sironoides Hansen, 1921 (MCZ DNA101059)

Rebola (3°43'32"N, 8°50'17"E, 299 m), Baney District, Bioko (Equatorial Guinea), leg. G. Giribet, C. Prieto, 10.viii.2003

Paragovia sp. (MCZ DNA100462)

Edyabe, Reserva Natural de Río Campo (2°19'59"N, 9°48'11"E), Región Continental (Equatorial Guinea), leg. J. Lapuente, 6.viii.2001

Paragovia sp. (MCZ DNA101052)

Parque Nacional de Monte Alén (1°39'29"N, 10°18'41"E, 784 m), Niefang District, Región Continental (Equatorial Guinea), leg. G. Giribet, C. Prieto, 31.vii.2003

Paragovia sp. (MCZ DNA101057)

Bongoro, Reserva Natural de Río Campo (2°07'52"N, 9°52'18"E, 20 m), Bata District, Región Continental (Equatorial Guinea), leg. C. Prieto, 8.viii.2003

Paramiopsalis ramulosus Juberthie, 1962 (MCZ DNA100459)

Valle del Río Barragán (42°18'54"N, 8°29'12"W, 262 m), Moscoso, Pontevedra, Galicia (Spain), leg. G. Giribet, M.K. Nishiguchi, 21.vii.2001

Parapurcellia silvicola (Lawrence, 1939) (MCZ DNA100385)

Nkandhla Forest (28°44'39"S, 31°08'15"E, 1065 m), Kwazulu-Natal (South Africa), leg. G. Giribet, L. Prendini, 4.iv.2001

Parasiro coiffaiti Juberthie, 1956 (MCZ DNA101383)

Font del Vidre (42°09'09"N, 1°55'49"E, 814 m), Berga, Barcelona, Catalunya (Spain), leg. S. Carranza, G. Giribet, 2.vi.2004

Pettalus sp. (MCZ DNA101282)

Knuckles Range (7°23'05"N, 80°49'01"E, 1455 m), Central Province (Sri Lanka), leg. S.L. Boyer, G. Giribet, I. Karunarathna, P. Sharma, 16.vi.2004

Pettalus sp. (MCZ DNA101288)

Batamandige (6°33'19"N, 80°22'13"E, 309 m) (Sri Lanka), leg. S.L. Boyer, G. Giribet, I. Karunarathna, P. Sharma, 21.vi.2004

Purcellia illustrans Hansen & Sørensen, 1904 (MCZ DNA100387)

Skeleton Gorge (33°58'58"S, 18°25'28"E, 214 m), Kirstenbosch National Botanical Garden, Table Mountain, Cape Town (South Africa), leg. G. Giribet, L. Prendini, 9.iv.2001

Rakaia antipodiana Hirst, 1925 (MCZ DNA100957)

Mount Algidus (43°15'05"S, 171°22'03"E, 529 m), Rakaia Gorge, South Island (New Zealand), leg. S.L. Boyer, C. D'Haese, G. Giribet, 4.ii.2003

Rakaia florensis (Forster, 1948) (MCZ DNA101295)

Pigeon Saddle (40°49'57"S, 172°58'08"E, 309 m), Abel Tasman National Park, Golden Bay, South Island (New Zealand), leg. S.L. Boyer, G. Giribet, 3.vii.2004

Rakaia stewartiensis Forster, 1948 (MCZ DNA100944)

Fern Gully (46°53'35"S, 168°06'14"E, 36 m), Stewart Island (New Zealand), leg. S.L. Boyer, C. D'Haese, G. Giribet, 11.ii.2003

Siro acaroides (Ewing, 1923) (MCZ DNA100488)

15 km W of Philomath, Woods Creek Rd., Benton Co., Oregon (USA), leg. A. Moldenke, 22.vii.1996

Siro exilis Hoffman, 1963 (MCZ DNA100489)

Swallow Falls State Park, Garrett Co., Maryland (USA), leg. J.W. Shultz, vii.2000

Siro kamiakensis (Newell, 1943) (MCZ DNA101611)

East of Hayden Lake on Hayden Lake Road (47°44'47"N, 116°42'07"W, 728 m), Coeur d'Alene National Forest, Kootenai Co., Idaho (USA), leg. S.L. Boyer, R.M. Clouse, G. Giribet, 17.vi.2005

Siro rubens Latreille, 1804 (MCZ DNA100457)

Mont Aigoual (44°05'00"N, 3°34'53"E, 826 m), P.N. des Cévennes, Massif Central (France), leg. G. Giribet, 26.vii.2001

Siro valleurum Chemini, 1990 (MCZ DNA100461)

c/o Baite Sedernello, Colzate, Bergamo, Lombardia (Italy), leg. Ferrario, Pantini, Pellizzoli, M. Valle, 2.viii.2001

Stylocellus lydekkeri Clouse and Giribet, 2007 (MCZ DNA101064)

Wondiwoi Mountains, 300–980 m alt., Wasior, Wandammen Bay, Manokwari Province, Irian Jaya (Indonesia), leg. A. Riedel, 3.i.2001

Stylocellus sp. (MCZ DNA101468)

Gua Mardua, Pengadan, East Kalimantan, Borneo (Indonesia), leg. L. Deharveng, Bedos, 19.viii.2004

Stylocellus sp. (MCZ DNA101469)

Tabah Penanjung Nature Reserve, Bengkulu Province, Sumatra (Indonesia), leg. P.J. Schwendinger, 27.ii.2000 (MHNG SPM002162-75)

Stylocellus sp. (MCZ DNA101494)

Khao Kai Jae Waterfall (9°55'05"N, 98°56'34"E, 80 m), Suan District, Chumphon Province (Thailand), leg. P.J. Schwendinger, 17.vii.2002 (MHNG TH-02/08, SPM004389-94)

Stylocellus sp. (MCZ DNA101500)

Ko Siray (7°53'07"N, 98°26'14"E, 30 m), E of Phuket City, Phuket Province (Thailand), leg. P.J. Schwendinger, 22.vii.2002

Suzukiehus sauteri (Roewer, 1916) (MCZ DNA101543)

Airin Camping Ground (35°38'03"N, 139°14'28"E, 294 m), Mt. Takao, Tokyo Prefecture, Honshu (Japan), leg. S.L. Boyer, G. Giribet, Y. Minagoshi, N. Tsurusaki, 13.iv.2005

Troglosiro aelleni Juberthie, 1979 (MCZ DNA100345)

Aoupinie (21°11'S, 165°19'E, 850 m) (New Caledonia), leg. G.B. Monteith, 20.xi.2000 (QM Berlesate 1035)

Troglosiro longifossa Sharma and Giribet, 2005 (MCZ DNA100867)

Gîte Kanua (22°21'S, 166°58'E, 20 m), Port Boisé (New Caledonia), leg. G.B. Monteith,

21 xi 2001 (Queensland Museum Berlesate 1043, from sieved rainforest litter)

Eupnoi

[*Astrobunus grallator* Simon, 1879 (MCZ DNA100311) (Spain)]

[*Caddo agilis* Banks, 1892 (MCZ DNA100308) (USA)]

[*Dalquestia formosa* (Banks, 1910) (MCZ DNA100322) (USA)]

[*Hadrobunus maculosus* (Wood, 1868) (MCZ DNA100314) (USA)]

Homalenotus sp. (MCZ DNA100709)

Mont Aigoual (44°06'05"N, 3°31'32"E, 1250 m), P.N. des Cévennes, Massif Central (France), leg. G. Giribet, 26.vii.2001

Eurybunus n. sp. (MCZ DNA100532)

20 mi S of Malibu Beach, Baja California (Mexico), leg. M.K. Nishiguchi, 15.v.2001

[*Leiobunum* sp. (MCZ DNA100309) (USA)]

Marthana sp. (MCZ DNA100613)

Doi Inthanon National Park, Ampuhr Jom Thong, Chiang Mai (Thailand), leg. D. Lohman, 2002

Megalopsalis sp. (MCZ DNA100783)
 Waiatu Caves, Southland, South Island (New Zealand), leg. C.J. Vink, 5.x.2002
 [*Nelima silvatica* (Simon, 1879) (MCZ DNA100323) (Spain)]
 [*Odiellus troglodoides* (Roewer, 1923) (MCZ DNA100310) (Spain)]
 [*Opilio parietinus* (DeGeer, 1778) (MCZ DNA100304) (Canada)]
Pantopsalis sp. (MCZ DNA100702)
 Otari-Wilton's Bush, Wellington, North Island (New Zealand), leg. J. Derraik, 16.i.2002
 [*Phalangium opilio* Linnaeus, 1758 (MCZ DNA100307)]
Prionostemma sp. (MCZ DNA100315)
 Mount St Benedict (10°39'49"N, 61°23'56"W), Trinidad (Trinidad and Tobago), leg. L. Prendini, 28.vi.1999
Protolophus singularis Banks, 1893 (MCZ DNA101033)
 Upper Pine Creek (32.83327°N, 116.54624°W), E base of Guatay Mountain, San Diego Co., CA (USA), leg. D. Palmer & D. Stokes, 3.vii.2003
Rhampsinitus sp. (MCZ DNA100710)
 Newlands Forest, Table Mountain, Cape Province (South Africa), leg. G. Giribet & L. Prendini, 9.iv.2001

Dyspnoi

Anelasmacephalus cambridgei (Westwood, 1874) (MCZ DNA101043)
 Font de la Doble Ona, Bagà, Serralada del Cadí, Barcelona, Catalunya (Spain), leg. M.A. Arnedo & E. de Mas, 18.iv.2003
 [*Ceratolasma tricantha* Goodnight & Goodnight, 1942 (MCZ DNA100329) (USA)]
Dendrolasma parvulum (Suzuki, 1963) (MCZ DNA100318)
 Kyobashira Pass, Tokushima Prefecture, Shikoku (Japan), leg. N. Tsurusaki, 21.v.1999
 [*Dicranolasma soereni* Thorell, 1876 (No voucher)]
 [*Hesperonemastoma modestum* (Banks, 1894) (MCZ DNA100312) (USA)]
 [*Ischyropsalis luteipes* Simon, 1872 (MCZ DNA100705) (Spain)]
 [*Nemastoma bimaculatum* (Fabricius, 1775) (no voucher) (France)]
Nemastoma hankiewiczii Kulczynski, 1909 (MCZ DNA100717)
 Valle del Río Barragán (42°19'04"N, 8°29'16"W, 209 m), Moscoso, Pontevedra, Galicia (Spain), leg. G. Giribet & M.K. Nishiguchi, 21.vii.2001
 [*Nemastomella dubia* (Mello-Leitão, 1936) (MCZ DNA100706) (Spain)]
 [*Nipponopsalis abei* (Sato & Suzuki, 1939) (MCZ DNA100306) (Japan)]
 [*Ortholasma* sp. (MCZ DNA100343) (USA)]
 [*Sabacon cavicolens* (Packard, 1884) (MCZ DNA100305) (USA)]
Sabacon sp. (MCZ DNA100711)
 Mont Aigoual (44°05'18"N, 3°34'54"W, 617 m), P.N. des Cévennes, Massif Central (France), leg. G. Giribet, 26.vii.2001
 [*Taracus* sp. (MCZ DNA100320) (USA)]
 [*Trogulus nepaeiformis* (Scopoli, 1763) (MCZ DNA100325) (Spain)]

Laniatores

Acutisoma longipes Roewer, 1913 (MCZ DNA101112)
 Cachoeira do Saltão (22°23'35"S, 47°53'11"W, 785 m), Brotas, Estado de São Paulo (Brazil), leg. G. Giribet, 12.xii.2003
Avima albiornata (Goodnight & Goodnight, 1947) (MCZ DNA100333)
 Mount St Benedict (10°39'49"N, 61°23'56"W), Trinidad (Trinidad and Tobago), leg. L. Prendini, 28.vi.1999
Baculigerus sp. (MCZ DNA100640)

Folhiço, Parque Ecológico de Cocó, Fortaleza (Brazil), leg. A.B. Kury, I. Kury & M. Kury, 29.vi.2002
 [*Bishopella laciniosa* (Crosby & Bishop, 1924) (MCZ DNA100324) (USA)]
Chilon robustus Sørensen, 1896 (MCZ DNA100766)
 Bicurga (1°22'49"N, 10°28'00"E), Región Continental (Equatorial Guinea), leg. C. Prieto, 7.vii.2001
Conomma oedipus Roewer, 1949 (MCZ DNA101051)
 Musola (3°25'53"N, 8°37'08"E), Bioko (Equatorial Guinea), leg. M. Fero, G. Giribet & C. Prieto, 9.viii.2003
Costabrimma sp. (MCZ DNA101428)
 Río Mile creek, 1 km N Gandoca, Provincia de Limón (Costa Rica), leg. D.S. Chandler, 9.ii.2002
Cynortula granulata Roewer, 1912 (MCZ DNA100332)
 Mount St Benedict (10°39'49"N, 61°23'56"W), Trinidad (Trinidad and Tobago), leg. L. Prendini, 28.vi.1999
Dongmoa sp. (MCZ DNA101100)
 25 km NW of Tan Phu (11°25'22"N, 107°25'42"E, 130 m), Cat Tien NP, Dong Nai Province (Vietnam), leg. P.J. Schwendinger, 26.viii.2003
 [*Equitius doriae* Simon, 1880 (MCZ DNA100607) (Australia)]
Erebomaster flavescens flavescens Cope, 1872 (MCZ DNA101444)
 Patton Cave, HNF ca. 12 mi SE Bloomington, Deam Wilderness Area, Monroe Co., Indiana (USA), leg. J. Lewis, S. Rafail & H. Huffman, 9.ix.2000
Ethobunus zalmoxiformis (Roewer, 1949) (MCZ DNA101424)
 Volcán Poás National Park, Alajuela Province (Costa Rica), leg. A. Pérez González, 25.x.2004
Fissiphallius chicoi Tourinho & Pérez González, 2006 (MCZ DNA101551)
 São José community (1.197°S, 51.783°W), Gurupá municipality, Pará State (Brazil), leg. C. A. Rheims & F. Rego, 18.x.2003
Fumontana deprehendor Shear, 1977 (MCZ DNA100700)
 Hwy 19W, E of Spivey Gap, ~3 mi W of Sioux, along big Creek (36°02.054'N, 82°24.257'W), Yancey Co., North Carolina (USA), leg. M. Hedin & M. Lowder, 21.viii.2001
Glysterus sp. (MCZ DNA101422)
 Siquirres, Provincia de Limón (Costa Rica), leg. A. Pérez González & C. Viquez, 27.x.2004
 [*Gnidia holmbergi* (Sørensen, 1884) (MCZ DNA100398) (Argentina)]
Gnomulus dalat Schwendinger & Martens, 2006 (MCZ DNA101101) (MHNG SV-03/20)
 Datania Waterfall (11°54'02"N, 108°26'54"E, 1300 m), ca. 5 km S of Da Lat, Lam Dong Province (Vietnam), leg. P.J. Schwendinger, 5.ix.2003
Gnomulus latoperculum Schwendinger and Martens, 2002 (MCZ DNA102028)
 Gunung Ambang, near Modinding (0°45'32"N, 123°25'23"E, 1461 m), NE of Kotamobagu, North Sulawesi (Indonesia), leg. R. Clouse, G. Giribet & C. Rahmadi, 19.vi.2006
Gnomulus sp. (rostratus group) (MCZ DNA101102) (MHNG TH-03/15)
 Ko Siray (7°53'06"N, 98°26'13"E, 30 m), Phuket Province (Thailand), leg. P.J. Schwendinger, 23.v.2003
Hadziana clavigera (Simon, 1879) (MCZ DNA101459)
 Ibarra (241 m), Tolosa, Guipuzcoa, Euskadi (Spain), leg. C. Prieto, 4.xii.2004
Heterocranaus sp. (MCZ DNA101443)
 Laguna Grande de Mojanda (0°07'59"N, 78°16'30"W, 3750 m), Otavalo, Imbarura Prov. (Ecuador), leg. R. Vila, 28.iii.2003
Holoscotolemon jaqueti (Corti, 1905) (MCZ DNA101037)
 Zlatibor Mountains, Sargan (Yugoslavia), leg. I. Karaman, vii.2003
 [*Hoplobunus* sp. (MCZ DNA100327) (USA)]
 Icaleptidae n. gen., n. sp. (MCZ DNA101420)
 Reserva Natura Río Nambí (1°17'06"N, 78°04'25"W, 1448 m), Municipio de Barbacoas, Corregimiento de Altaquer, Departamento

- de Nariño (Colombia), leg. L. Benavides, L. Cabrera, G. Castillo, C. Flórez, G. Giribet, M. Romo, V. Solarte, 10-11.xi.2004
- Karos* cf. *rugosus* Goodnight & Goodnight, 1971 (MCZ DNA101419)
- Grutas de Atoyac (18°55.373'N, 46°45.885'W, 300 m), Veracruz (Mexico), leg. O. Francke, E. González, J. Ponce & L. Prendini, vii.2002
- Larifuga* cf. *capensis* Lawrence, 1931 (MCZ DNA100727; ex MZC 34182)
- Newlands Forest, Table Mountain, Cape Province (South Africa), leg. G. Giribet & L. Prendini, 9.iv.2001
- [*Maiorerus randoi* Rambla, 1993 (No voucher) (Spain)]
- Martensillus* sp. (MNH, Geneva)
- Mulu National Park, 100 km SEE of Miri (4°00'N, 114°49'E, 200 m), Sarawak, Borneo (Malaysia), leg. A. Schulz (WINKLER-extraction sample AS/03-7)
- Megapachylus grandis* Roewer, 1913 (MCZ DNA101113)
- Cachoeira do Saltão (22°23'35"S, 47°53'11"W, 785 m), Brotas, Estado de São Paulo (Brazil), leg. G. Giribet, 12.xii.2003
- Metabiantes longipalpis* Kauri, 1985 (MCZ DNA100769)
- Vabe (1°08'07"N, 9°46'09"E), Región Continental (Equatorial Guinea), leg. C. Prieto, 31.vii.2001
- Metabiantes* sp. (MCZ DNA100703)
- Sarah campsite (old archaeological site) (26°11'44"S, 31°59'24"E, 188 m), Mlawula Nature Reserve (Swaziland), leg. R. Boycott, G. Giribet & L. Prendini, 2.iv.2001
- Metabiantes* sp. (MCZ DNA100704)
- Sarah campsite (old archaeological site) (26°11'44"S, 31°59'24"E, 188 m), Mlawula Nature Reserve (Swaziland), leg. R. Boycott, G. Giribet & L. Prendini, 2.iv.2001
- Metalibitia paraguayensis* (Sørensen, 1884) (MCZ DNA100476)
- Road to Tafí del Valle (27°05'01"S, 65°39'56"W, 487 m), Tucumán (Argentina), leg. G. Giribet, 16.ix.2001
- Minuella* sp. (MCZ DNA101388)
- Colonia Tovar (Venezuela), leg. A. Pérez González, A. Giupponi & O. Villarreal Manzanilla, xii. 2003
- [*Pachyloides thorellii* Holmberg, 1878 (MCZ DNA100424) (Argentina)]
- Paktongius* sp. (MCZ DNA101416)
- Kanchanaburi (14°34.449'N, 98°50.921'E), Thong Pha Phum District, Mae Kong Watershed Reserve Station (Thailand), leg. D. Lohman, 29.xi.2003
- Pellobunus insularis* Banks, 1905 (MCZ DNA101421)
- Hone creek, Finca Alberto Moore, Provincia de Limón (Costa Rica), leg. C. Viquez, 12-15.iii.2004
- Pirassungoleptes calcaratus* H. Soares, 1966 (MCZ DNA101114)
- Cachoeira do Saltão (22°23'35"S, 47°53'11"W, 785 m), Brotas, Estado de São Paulo (Brazil), leg. L.E. Acosta, 12.xii.2003
- Pseudoepedanus doiensis* Suzuki, 1969 (MCZ DNA101438)
- Huay Khok Ma, Doi Suthep, Chiang Mai (Thailand), leg. P.J. Schwendinger, 19.xi.2003
- Rhopalocraneus albilineatus* Roewer, 1932 (MCZ DNA100334)
- Mount St Benedict (10°39'49"N, 61°23'56"W), Trinidad (Trinidad and Tobago), leg. L. Prendini, 28.vi.1999
- Rostromontia* cf. *capensis* Lawrence, 1931 (MCZ DNA100724)
- Newlands Forest, Table Mountain, Cape Province (South Africa), leg. G. Giribet & L. Prendini, 9.iv.2001
- Samoidae sp. (MCZ DNA101116) (juvenile specimen)
- Pico Basilé (3°40'15"N, 8°50'52"E, 1050 m), Bioko (Equatorial Guinea), leg. G. Giribet & C. Prieto, 11.viii.2003
- [*Sandokan malayanus* (MCZ DNA100321) (as *Oncopus* cf. *alticeps* Pocock, 1897 in Giribet et al., 2002) (Malaysia)]
- Sandokan truncatus* (Thorell, 1891) (MCZ DNA101099)
- Jungle Fall Valley (1°20'53"N, 103°46'35"E, 80 m), Bukit Timah Nature Reserve (Singapore), leg. P.J. Schwendinger, 3.viii.2003
- Santinezia serratotibialis* Roewer, 1932 (MCZ DNA100426)
- Mount St Benedict (10°39'49"N, 61°23'56"W), Trinidad (Trinidad and Tobago), leg. L. Prendini, 28.vi.1999
- [*Scotolemon lespesi* Lucas, 1860 (MCZ DNA100326) (Spain)]
- Scotolemon* sp. (MCZ DNA101042)
- Barranco de Atamaria (300 m from Casa Ceniza) (41°11'57"N, 2°14'42"E, 133 m), Murcia (Spain), leg. M.A. Arnedo, E. de Mas & Miñano, 9.v.2003
- Seuthes inermis* Santos & Prieto, 2009 (MCZ DNA100765)
- Monte Sabio (1°05'N, 9°42'E), Cogo, Bioko (Equatorial Guinea), leg. C. Prieto, 30.vii.2001
- Stygnomma* n. sp. (MCZ DNA101415)
- Track heading West of km 13.5 N of Leticia (4°07'13"S, 69°58'31"W, 84 m), Departamento de Amazonas (Colombia), leg. L.R. Benavides, G. Giribet & R. Mesa, 5.xi.2004
- Stygnomma* sp. (MCZ DNA101423)
- Siquirres, Provincia de Limón (Costa Rica), leg. A. Pérez González, 27.x.2004
- Stygnoplus clavotibialis* (Goodnight & Goodnight, 1947) (MCZ DNA100331)
- Mount St Benedict (10°39'49"N, 61°23'56"W), Trinidad (Trinidad and Tobago), leg. L. Prendini, 28.vi.1999
- Synthetonychia* sp. (MCZ DNA100796/100866)
- Near Horopito (39°22'S, 175°23'E), North Island (New Zealand), leg. Department of Conservation (ex. Pitfall trap site 1), 6.iv.2001
- Theromaster brunneus* (Banks, 1902) (MCZ DNA100701)
- 0.25 mi N Rich Mtn Lookout (35°55.875'N, 82°48.260'W), Madison Co., North Carolina (USA), leg. M. Hedin & M. Lowder, 21.viii.2001
- Triaenobunus* cf. *armstrongi* Forster, 1955. (MCZ DNA100617)
- Chaelundi Road, 1.9 km from Big Bull Road (30°08'51"S, 152°26'38"E, 1197 m), Mt. Hyland Nature Reserve, New South Wales (Australia), leg. G.D. Edgecombe & Y.-y. Zhen, 23.ii.2002
- [*Triaenobunus* sp. (MCZ DNA100328) (Australia)]
- Trionyxellinae sp. (MCZ DNA101430)
- Peradeniya (7°16'56"N, 80°35'58"E, 575 m), Central Province (Sri Lanka), leg. S. Boyer, G. Giribet, I. Karunaratna & P. Sharma, 17.vi.2004
- Trojanella serbica* Karaman, 2005 (MCZ DNA100911)
- Vladikina Ploca, Mt. Stara Planina, Serbia (Serbia and Montenegro), leg. I. Karaman, 2003
- Zalmoxis* sp. (MCZ DNA100914, ex. MCZ 52978)
- Ningua Res. Camp, Province Sud (New Caledonia), leg. G.B. Monteith, xi.2001
- [*Zuma acuta* Goodnight & Goodnight, 1942 (No voucher) (USA)]
- Zygopachylus* n. sp. (MCZ DNA101425)
- Hone creek, Finca Alberto Moore, Provincia de Limón (Costa Rica), leg. C. Viquez, 8-9.i.2004