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First molecular phylogeny of the major clades of Pseudoscorpiones (Arthropoda: Chelicerata)

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ABSTRACT

The phylogenetic relationships of the major lineages of the arachnid order Pseudoscorpiones are investigated for the first time using molecular sequence data from two nuclear ribosomal genes and one mitochondrial protein-encoding gene. The data were analyzed using a dynamic homology approach with the new program POY v.4 under parsimony as the optimality criterion. The data show monophyly of Pseudoscorpiones as well as many of its superfamilies (Feaelloidea, Chthonioidea, Cheiridioidea and Sternophoroidea), but not for Neobisiodea or Garypoidea. Cheliferoidea was not monophyletic either due to the position of *Neochelanops*, which grouped with some garypoids. In all the analyses, Feaelloidea constituted the sister group to all other pseudoscorpions; Chthonioidea is the sister group to the remaining families, which constitute the group Iocheirata sensu Harvey—a clade including pseudoscorpions with venom glands within the pedipalpal fingers. This phylogenetic pattern suggests that venom glands evolved just once within this order of arachnids.

1. Introduction

The arachnid order Pseudoscorpiones is one of the oldest lineages of terrestrial organisms with the group represented in Middle Devonian shales from the Panther Mountain Formation of New York (Shear et al., 1989, 1991). The order is currently represented by 3385 named species which are placed in 439 genera and 25 families (Harvey, 2007, 2008) which contributes, however, only a small proportion of total named arachnid diversity due to the large numbers of named spiders and mites (Harvey, 2007), but rivals in numbers with the more familiar scorpions and harvestmen (Coddington et al., 2004).

Pseudoscorpions occupy virtually all terrestrial habitats on earth, but are most common in leaf litter, soil or under bark of trees and logs. They can be frequently found in caves, and many species occur in sea-shore littoral habitats. They are generally quite small with the body lengths of adults ranging from 0.5 to 5 mm; however, some species attain lengths surpassing 1 cm. Pseudoscorpions are free-living, but some unusual strategies are adopted by some taxa, of which the most notable is their tendency towards phoretic behavior where individuals attach themselves using their pedipalps to other organisms such as other arachnids, insects, mammals or birds, which may facilitate transport to different habitats (Vachon, 1940; Beier, 1948; Muchmore, 1971; Zeh and Zeh, 1992a,b; Judson, 2005). Reproduction occurs via the deposition of a spermatophore on the substrate by the male, which is then picked up by the female. Spermatophore deposition is usually performed by males in the absence of females, but active courtship is performed between pairs within the superfamily Cheliferoidea (Weygoldt, 1969). Many pseudoscorpions have venom glands within the pedipalpal fingers, thus representing only one of three arachnid orders with venomous capabilities, apart from spiders and scorpions. The venomous pseudoscorpion clade was identified and named by Harvey (1992).

The order Pseudoscorpiones is clearly monophyletic and supported by several apomorphies (Shultz, 2007) of which the only features unique to the group are the presence of silk glands discharging via the movable cheliceral finger, and the presence of a serrula exterior and serrula interior on the cheliceral fingers. Pseudoscorpiones have, for over a century, been regarded as the sister group to Solifugae which are included in the clade Haplocnemata (Börner, 1904; Weygoldt and Paulus, 1979; Shultz, 1990; Wheeler and Hayashi, 1998; Giribet et al., 2002; Shultz, 2007) or Apatellata (van der Hammen, 1986, 1989). Haplocnemata have been retrieved as sister group to Scorpiones forming the clade Novogenuata (Shultz, 1990; Wheeler and Hayashi, 1998; Giribet et al., 2002); as sister group to Opiliones (Giribet et al., 2002); as sister group to Scorpiones + Opiliones (=Stomothecata), forming

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the clade named Dromopoda (Shultz, 2007); or as sister group to Pantetrapulmonata (Shultz, 2007), to mention just the best-supported resolutions. An alternative hypothesis, in which Solifugae grouped with Acari rather than Pseudoscorpiones, was proposed by Alberti and Peretti (2002).

1.1. Classification and phylogenetic hypotheses

Relationships amongst pseudoscorpions have not been extensively studied, and nearly all classifications have been based upon traditional, non-numerical assessments of morphological features. The earliest classifications simply recognized a single family, Cheliferidae, and a variety of subfamilies, but there was little consensus amongst arachnologists regarding the internal composition. Early classifications (e.g., Simon 1879; Thorell, 1883; Daday 1888; Balzan, 1892; Hansen, 1893, 1894; With, 1906) were superseded by a novel classification proposed by Chamberlin (1929, 1930, 1931) using a wide array of specimens from all over the world. This classification (see Fig. 1) has had the most lasting impact on all subsequent classifications and signifies the modern era in the systematics of pseudoscorpions. Chamberlin proposed three suborders, Heterosphyronida, Diplosphyronida and Monosphyronida, with the latter two suborders forming the group Homosphyronida. Heterosphyronida contained the sole family Chthoniidae with three subfamilies Chthoniinae, Dithinae and Tridenchthoniinae (Chamberlin, 1929), but Dithinae was later raised to family-level (Chamberlin, 1931). Diplosphyronida contained two superfamilies, Neobisioidea for Neobisiidae, Syarinidae, Hyidae and Ideoroncidae, and Garypoidea for Garypidae, Menthidae and Olpiidae (Chamberlin, 1930, 1931). Monosphyronida contained Feaelloidea for Feaellidae and Pseudogarypidae, Cheiridioidea for Cheiridiidae, Pseudochiridiidae and Sternophoridae, and Cheliferoidea for Chernetidae, Myrmochernetidae, Atemnidae and Cheliferidae (Chamberlin, 1931).

Beier (1932a,b), in a masterful survey of the entire order based upon large numbers of specimens including type material of most previously described species that were lodged in European institutions, adopted Chamberlin's scheme, recognizing three suborders: Chthoniinea (equivalent to Heterosphyronida), Neobisiinea (equivalent to the Diplosphyronida) and Cheliferinea (equivalent to Monosphyronida). He also introduced two changes at the family-level: Hyidae was made a subfamily of Ideoroncidae; Pseudochiridiinae was made a subfamily of Cheiridiidae. Most significantly, he transferred Feaelloidea to Diplosphyronida, also including the family Synsphyronidae for the genus *Synsphyronus*. Chamberlin (1943) returned *Synsphyronus* to Garypidae, reaffirming its close resemblance to other typical garypids.

Although largely identical in composition, the subordinal names proposed by Chamberlin and Beier remained in use for many years, with American authors generally utilizing Chamberlin's names (e.g., Hoff, 1958), and European authors generally employing Beier's names.

Several modifications occurred in later decades: two new families, Vachoniidae (Chamberlin, 1947) and Gymnobisiidae (Beier, 1947) were described; Withiidae were separated from Cheliferidae (Weygoldt, 1970); Miratemnidae were separated from Atemnidae (Dumitresco and Orghidan, 1970); Bochicidae were separated from Ideoroncidae (Muchmore, 1982b); Myrmochernetidae were synonymized with Chernetidae (Judson, 1985) and Geogarypidae were separated from Garypidae (Harvey, 1986).

Weygoldt (1969, p. 129) postulated Feaellidae as "an old family that originated either close to Heterosphyronida or somewhere between Heterosphyronida and Diplosphyronida. They should be considered, perhaps, as a separate suborder, but further taxonomic work is necessary before this question can be settled". Nevertheless, he did not postulate any change to the classification and retained the group within Diplosphyronida.

Muchmore (1982b) rejected the subordinal system proposed by Chamberlin and Beier, and recognized six superfamilies: Chthonioidea for Chthoniidae and Tridenchthoniidae; Neobisioidea for Neobisiidae, Syarinidae, Ideoroncidae, Hyidae, Bochicidae, Vachoniidae and Gymnobisiidae; Garypoidea for Garypidae, Olpiidae, Menthidae and Pseudogarypidae; Cheiridioidea for Cheiridiidae and Sternophoridae; Feaelloidea for Feaellidae; and Cheliferoidea

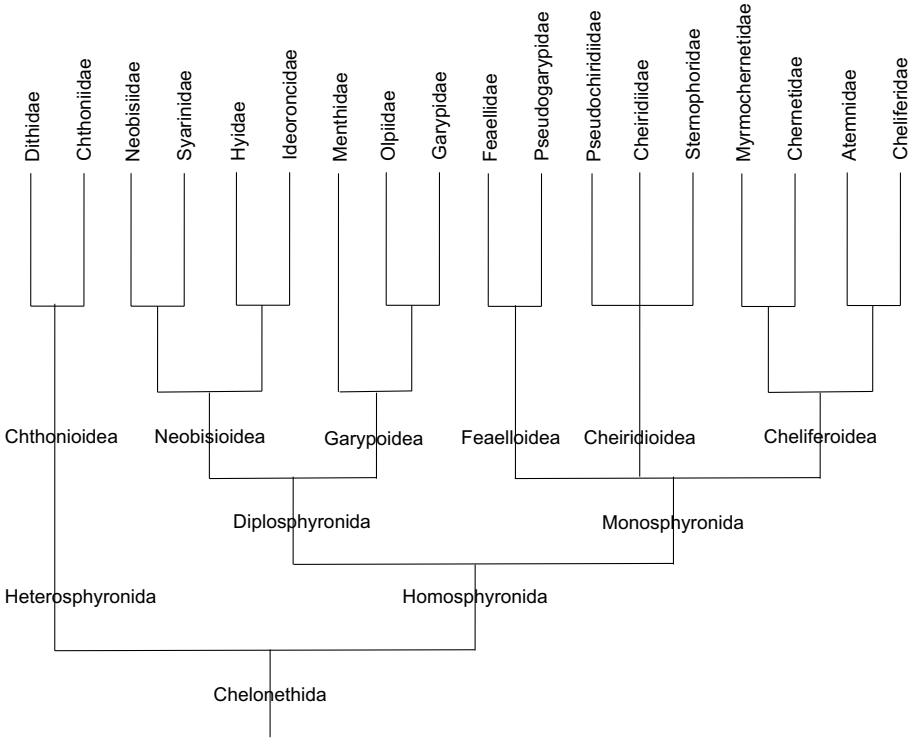

Fig. 1. The "Scheme of classification" proposed by Chamberlin (1931, Fig. 53), redrawn.

for Atemnidae, Miratemnidae, Myrmochernetidae, Chernetidae, Pseudochiridiidae, Withiidae and Cheliferidae.

Harvey (1992) presented the first cladistic analysis of the order based upon 126 characters and 24 terminal taxa (Fig. 2). The analysis, although clearly pioneering, was conducted using the standards at the time, using groundplans for supraspecific terminals, and therefore not allowing for a stricter test of monophyly of certain taxa. Substantial taxonomic changes were proposed based on the results of that analysis, including the erection of two new suborders, Epiocheirata for Chthonioidea and Feaelloidea, and Iocheirata for Neobisioidea, Garypoidea, Olpioidea, Sternophoroidea and Cheliferoidea. Several family-level changes were proposed, including the recognition of three new families, Lechytiidae (raised from the chthoniid tribe Lechytiini), Parahyidae and Larcidae. Vachoniidae was treated as a synonym of Bochicidae, and Miratemnidae was synonymized with Atemnidae.

Since then, few alterations have been made to the classification. Proctor (1993) suggested a relationship between Chernetidae and Cheliferidae based upon the morphology of the spermatophore. Judson (2005) segregated Garypinidae from Olpiidae. Judson (2000, 2007) reinstated Cheiridioidea for Cheiridiidae and Pseudochiridiidae, placing them as the sister group to Cheliferoidea. Harvey and Volschenk (2007) performed a more exhaustive morphological analysis on a variety of neobisioid species focusing on the family Hyidae. The data largely supported the results obtained by Harvey (1992), but two families, Bochicidae and Syarinidae, were never recovered as monophyletic. The bochicid subfamilies Bochicinae and Leucohyinae were always separate from each other, and the syarinid subfamily Syarininae was always located away from Chitrellinae and Ideobisiinae. These data suggest that the family-level phylogeny of Neobisioidea is still in a state of flux.

Despite the advent of DNA sequencing techniques and the eruption of molecular systematics in zoological studies in general, and arachnids in particular, molecular data for pseudoscorpions are still scarce. The first published pseudoscorpion sequence data are those of Wheeler and Hayashi (1998), who used ribosomal nuclear sequence data of a species of *Americhernes*. Since then, only a handful of papers have published sequence data on pseudoscorpions (Giribet and Ribera, 2000; Giribet et al., 2002; Mallatt and Giribet, 2006), none of which focus on pseudoscorpion systematics per se. To date, only two studies have evaluated relationships among closely related species or populations of pseudoscorpions using molecular data (Zeh et al., 2003; Moulds et al., 2007) and none have addressed deep divergences within the pseudoscorpion tree.

1.2. Aims

After several years of collecting fresh pseudoscorpion material suitable for molecular study from nearly all continental landmasses, we aimed to provide a first phylogenetic hypothesis of the arachnid order Pseudoscorpiones using DNA sequence data obtained from three markers, two nuclear ribosomal genes (18S rRNA and 28S rRNA) and the mitochondrial protein-encoding cytochrome *c* oxidase subunit I (COI).

2. Materials and methods

2.1. Taxonomic sampling

We obtained sequences from 79 species of pseudoscorpions, representing 74 genera and 22 families (Table 1). All superfamilies and the majority of currently recognized families were

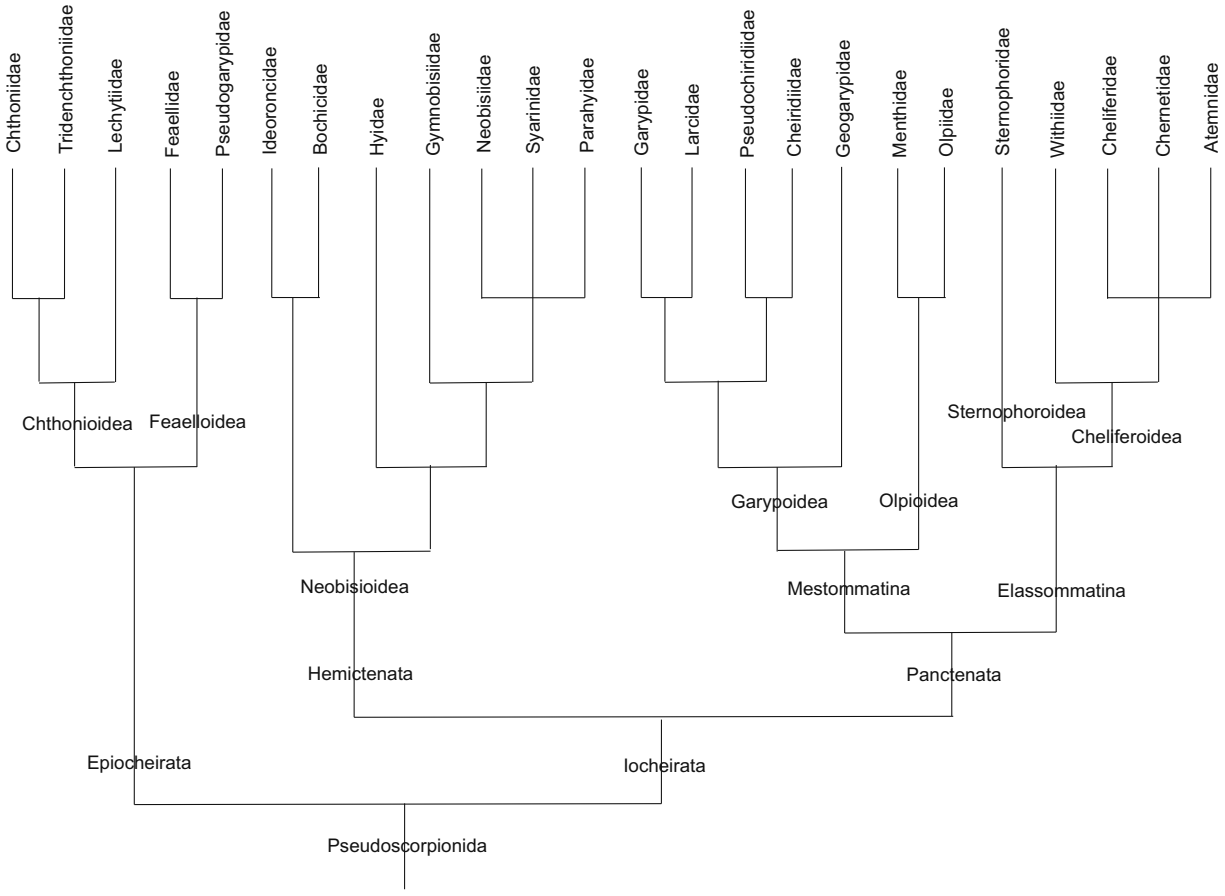

Fig. 2. Cladogram proposed by Harvey (1992) based on a parsimony analysis of 126 characters, redrawn. Consistency index = 0.60, Retention index = 0.82.

represented, but we were unable to obtain suitable specimens of the families Bochicidae, Menthidae and Pseudochiridiidae. We were able to sample multiple representatives of most families, but only single species was sampled for the families Lechytiidae (*Lechytia*), Tridenchthoniidae (*Anaulacodithella*), Feaellidae (*Feaella*), Gymnobisiidae (*Mirobisium*), Hyidae (*Indohya*), Parahyidae (*Parahya*), and Cheiridiidae (unknown genus of the subfamily Cheiridiinae).

Specimens collected by us were immediately fixed and preserved in 96% ethanol at −80 °C for long-term preservation. Most specimens collected by colleagues were preserved in a similar fashion, although some were preserved in 75% ethanol, and transferred to 96% as soon as they were received by us.

Where the entire specimen was used for the extraction of DNA, we retained one or more additional specimens as morphological vouchers, which are lodged in the Western Australian Museum, Perth. In some cases, a single appendage (such as a pedipalp or chela) was amputated and used for DNA extraction; the remainder of the body was retained as the voucher. DNA extractions and remaining vouchers are deposited at the Museum of Comparative Zoology, Department of Invertebrate Zoology DNA collection under numbers indicated as DNAXXXXXX.

For outgroups we used GenBank sequences for Xiphosura (*Limulus polyphemus*), and members of the other dromopodan orders: Opiliones (*Siro rubens*), Solifugae (*Eremobates* sp.) and Scorpiones (*Pandinus imperator*).

2.2. DNA extraction, amplification and sequencing

Molecular markers included two nuclear ribosomal genes (complete 18S rRNA [18S hereafter] and a 1 kb fragment of 28S rRNA [28S hereafter]) and one mitochondrial protein-encoding gene (cytochrome *c* oxidase subunit I [COI hereafter]). These markers have proven to be informative on many studies on arachnid systematics (e.g., Hormiga et al., 2003; Prendini et al., 2003, 2005; Boyer et al., 2005, 2007) and COI is the only marker used in previous phylogeographic studies on Pseudoscorpiones (Zeh et al., 2003; Moulds et al., 2007).

The DNEasy® tissue kit (Qiagen, Valencia, CA, USA) was used for tissue lysis and DNA purification following manufacturer's protocol. Total DNA was extracted either by crushing the whole animal or one appendage in the lysis buffer, or by incubating the entire animal or appendage in lysis buffer overnight as in Boyer et al. (2005). The intact cuticle of the animal was removed after the lysis step and kept in ethanol.

Purified genomic DNA was used as a template for PCR amplification. The complete 18S rRNA (*c.* 1.8 kb) was amplified in three overlapping fragments of *c.* 900 bp each, using primers pairs 1F/5R, 3F/18Sbi, and 18Sa2.0/9R (Giribet et al., 1996; Whiting et al., 1997). The first *c.* 1000 bp of the 28S rRNA were amplified using the primer set 28SpsF1 (5′-ATTA CCC GCC GAA TTT AAGC-3′)/28SpsR1 (5′-TCG GAG GGA ACC AGC TAC-3′) (this study) or alternatively with the more internal forward primer 28Sa (Nunn et al., 1996). COI was amplified using the primer pair LCO1490 (Folmer et al., 1994)/HCOoutout (Prendini et al., 2005; Schwendinger and Giribet, 2005) or alternatively with the more internal reverse primer HCO2198 (Folmer et al., 1994).

Polymerase chain reactions (50 μL) included 2 μL of template DNA, 1 μM of each primer, 200 μM of dinucleotide-triphosphates (Invitrogen, Carlsbad, CA, USA), 1× PCR buffer containing 1.5 mM $MgCl_2$ (Applied Biosystems, Branchburg, NJ, USA) and 1.25 U of AmpliTaq DNA polymerase (Applied Biosystems). The PCR reactions were carried out using a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems), and involved an initial denaturation step (5 min at 95 °C) followed by 35 cycles including denaturation at 95 °C for 30 s, annealing (ranging from 44 to 49 °C) for 30 s, and extension at 72 °C for 1 min, with a final extension step at 72 °C for 10 min.

The double-stranded PCR products were verified by agarose gel electrophoresis (1% agarose) and purified with a Perfectprep PCR Cleanup 96 system (Eppendorf, Westbury, NY, USA). The purified PCR products were sequenced directly with the same primer pairs used for amplification. Each sequence reaction contained a total volume of 10 μL including 2 μL of PCR product, 1 μM of one of the PCR primer pairs, 2 μL ABI BigDye 5× sequencing buffer, and 2 μL ABI Big Dye Terminator v3.0 (Applied Biosystems). The sequencing reactions involved an initial denaturation step for 3 min at 95 °C, and 25 cycles (95 °C for 10 s, 50 °C for 5 s, and 60 °C for 4 min). The BigDye-labeled PCR products were cleaned using Performa DTR Plates (Edge Biosystems, Gaithersburg, MD, USA). The sequence reaction products were then analyzed using an ABI Prism 3730xl Genetic Analyzer.

2.3. Sequence editing

Chromatograms were edited and overlapping sequence fragments for each individual were assembled using software SEQUENCHER 4.7 (Gene Codes Corporation 1991–2007, Ann Arbor, MI, USA), after BLAST searches (Altschul et al., 1997), as implemented by the NCBI website (http://ncbi.nlm.nih.gov/), were conducted to check for putative contamination. The software package MACGDE: Genetic Data Environment for MacOSX (Linton, 2005) was used to determine fragments based on internal primers and secondary structure features. As recently noted, "the use of structural information and POY are not mutually exclusive" (Kjer et al., 2007) and its use has long been suggested and applied (e.g., Giribet and Ribera, 2000; Giribet and Wheeler, 2001; Giribet, 2002). 18S rRNA and 28S rRNA were respectively divided into 29 and 15 fragments according to internal primers and secondary structure features. COI was divided in two fragments based on the position of the HCO2198 primer.

2.4. Phylogenetic analyses

Phylogenetic analyses were conducted under Direct Optimization (Wheeler, 1996; Wheeler et al., 2006) with POY version 4.0 RC builds 2398 and 2602 (Varón et al., 2007). This latest version of the software has been completely rewritten and allows conducting searches in few hours on a simple laptop while version 3.0 would have taken several days on a computer cluster. In addition, the internal parallelization library allows efficient use of multicore processors. The analyses were undertaken on a Mac Pro desktop with 2 dual-core Intel Xeon processors, thus speeding up the process by a factor of 4.

Tree searches were conducted under parsimony with different weighting schemes in a sensitivity analysis framework (Wheeler, 1995; Giribet, 2003). As all algorithms used are heuristics, numerical values for command arguments cannot be taken as optimal parameters.

Because the command structure used by POY 4.0 is different from that of previous versions, we will provide hereafter a description of the commands used to implement our search strategy: read("*.fas"); all sequence files in FASTA format are imported, namely COI.fas, 18S.fas and 28S.fas; select(terminals,files: ("taxa.txt")); only the terminals specified in the file "taxa.txt" are used for analysis. This is useful for quickly including or excluding taxa without altering the sequence files; set(root: "Limulus"); the outgroup (*Limulus*) is designated so that all the reported trees have the correct polarity; transform((all, tcm:"111")); a transformation cost matrix "111" is applied to the data to be used in the calculation of the tree cost (this matrix specifies the relative costs of indels/transversions/transitions; see examples in Giribet et al.,

Table 1
Specimens included in our analyses with reference to their current taxonomic classification

Taxon	Species	Location	MCZ voucher No.	GenBank Accession Nos.		
				18S rRNA	28S rRNA	COI
Xiphosura	*Limulus polyphemus*			U91490	AF212167	NC_003057
Xiphosura	*Limulus polyphemus*			U91490	AF212167	NC_003057
Opiliones	*Siro rubens*			U36998	AY859602	DQ513111
Solifugae	*Eremobates* sp.			AY859573	AY859572	
Scorpiones	*Pandinus imperator*			AY210831	AY210830	AY156582
Chthonioidea						
Chthoniidae, Ps	*Afrochthonius godfreyi*	South Africa	DNA103084	EU559395	EU559465	EU559507
Chthoniidae, Ps	*Pseudotyrannochthonius* sp.	Australia	DNA103085	EU559353		EU559508
Chthoniidae, Ps	*Pseudotyrannochthonius* sp.	Chile	DNA103086	EU559363	EU559466	EU559509
Chthoniidae, Ps	*Selachochthonius* sp.	South Africa	DNA103087	EU559364	EU559467	EU559510
Chthoniidae, Ch	*Apochthonius* sp.	USA	DNA103088	EU559365	EU559468	EU559517
Chthoniidae, Ch	*Austrochthonius* sp.	Australia	DNA103089	EU559354	EU559453	EU559513
Chthoniidae, Ch	*Austrochthonius* sp.	Chile	DNA103090	EU559413		EU559512
Chthoniidae, Ch	*Austrochthonius* sp.	Chile	DNA103091	EU559405		EU559514
Chthoniidae, Ch	*Chthonius* (*Chthonius*) sp.	France	DNA103092	EU559387	EU559438	EU559511
Chthoniidae, Ch	*Chthonius* (*Ephippiochthonius*) sp.	Spain	DNA103093	EU559391	EU559447	EU559504
Chthoniidae, Ch	*Drepanochthonius* sp.	Chile	DNA103094	EU559389	EU559444	EU559515
Chthoniidae, Ch	*Kleptochthonius* sp.	Chile	DNA103095	EU559366	EU559469	EU559518
Chthoniidae, Ch	*Lagynochthonius johni*	Indonesia	DNA103096	EU559415	EU559431	EU559503
Chthoniidae, Ch	*Paraliochthonius* sp.	Australia	DNA103097	EU559416	EU559433	EU559505
Chthoniidae, Ch	*Pseudochthonius* sp.	Equatorial Guinea	DNA103098	EU559367	EU559470	EU559519
Chthoniidae, Ch	*Sathrochthonius insulanus*	Lord Howe Island	DNA103099	EU559396	EU559471	EU559521
Chthoniidae, Ch	*Tyrannochthonius* sp.	Colombia	DNA103100	EU559393	EU559442	EU559506
Lechytiidae	*Lechytia hoffi*	USA	DNA103101	EU559430	EU559454	EU559516
Tridenchthoniidae	*Anaulacodithella* sp.	New Caledonia	DNA103102	EU559384	EU559434	EU559520
Feaelloidea						
Feaellidae	*Feaella anderseni*	Australia	DNA102369	EU559355		EU559500
Pseudogarypidae	*Neopseudogarypus scutellatus*	Australia	DNA103103	EU559356	EU559456	EU559502
Pseudogarypidae	*Pseudogarypus bicornis*	USA	DNA103104	EU559368	EU559472	EU559501
Neobisioidea						
Gymnobisiidae	*Mirobisium* sp.	Chile	DNA103105	EU559369	EU559473	EU559547
Ideoroncidae	*Pseudalbiorix veracruzensis*	Mexico	DNA103106	EU559427	EU559474	EU559567
Neobisiidae, Mi	*Lissocreagris* sp.	USA	DNA103107	EU559392	EU559450	EU559555
Neobisiidae, Mi	*Saetigerocreagris* sp.	USA	DNA103108	EU559412		EU559554
Neobisiidae, Mi	*Stenohya hamata*	Thailand	DNA103109	EU559370	EU559475	EU559498
Neobisiidae, Mi	*Tuberocreagris lata*	USA	DNA103110	EU559406	EU559451	EU559552
Neobisiidae, Ne	*Microbisium parvulum*	USA	DNA103111	EU559371	EU559476	EU559558
Neobisiidae, Ne	*Neobisium polonicum*	Slovakia	DNA103112	EU559357	EU559457	EU559556
Neobisiidae, Ne	*Novobisium tenue*	USA	DNA103113	EU559407	EU559452	EU559559
Neobisiidae, Ne	*Roncus transsilvanicus*	Slovakia	DNA103114	EU559372	EU559477	EU559557
Parahyidae	*Parahya submersa*	Australia	DNA103115	EU559426	EU559478	EU559548
Syarinidae, Ch	*Chitrella cala*	USA	DNA103116	EU559373	EU559479	EU559551
Syarinidae, Id	*Ideobisium* sp.	Colombia	DNA103117	EU559429	EU559458	EU559549
Syarinidae, Id	*Ideoblothrus* sp.	Colombia	DNA103118	EU559374	EU559480	EU559562
Syarinidae, Id	*Nannobisium* sp.	Equatorial Guinea	DNA103119	EU559375	EU559481	EU559561
Syarinidae, Sy	*Syarinus* sp.	Canada	DNA103120	EU559386	EU559437	EU559550
Hyidae	*Indohya* sp.	Australia	DNA103121	EU559422	EU559497	EU559564
Garypoidea						
Garypidae	*Anagarypus heatwolei*	Australia	DNA103122	EU559376	EU559482	EU559540
Garypidae	*Synsphyronus apimelus*	Australia	DNA103123	EU559358	EU559459	EU559537
Geogarypidae	*Geogarypus* sp.	South Africa	DNA103124	EU559385	EU559436	EU559560
Larcidae	*Larca lata*	Czech Republic	DNA103125	EU559425		EU559563
Cheiridiidae	Cheiridiidae sp.	Australia	DNA103126	EU559424	EU559483	EU559570
Garypinidae	*Protogarypinus giganteus*	Australia	DNA103127	EU559377	EU559484	EU559565
Garypinidae	*Pseudogarypinus cooperi*	USA	DNA103128	EU559423	EU559485	EU559566
Olpiidae, Ol	*Beierolpium bornemisszai*	Australia	DNA103129	EU559378	EU559486	EU559545
Olpiidae, Ol	*Calocheiridius termitophilus*	Equatorial Guinea	DNA103130	EU559359	EU559460	EU559544
Olpiidae, Ol	*Euryolpium* sp.	Australia	DNA103131	EU559379	EU559487	EU559546
Olpiidae, Ol	*Pachyolpium* sp.	Trinidad & Tobago	DNA103132	EU559421	EU559488	EU559542
Olpiidae, Ol	*Xenolpium* sp.	New Zealand	DNA103133	EU559403	EU559446	EU559539
Olpiidae, He	*Apolpium parvum*	Trinidad & Tobago	DNA103134	EU559380	EU559489	EU559541
Olpiidae, He	*Nanolpium* sp.	Zambia	DNA103135	EU559390	EU559445	EU559543
Olpiidae, He	*Progarypus* sp.	Chile	DNA103136	EU559420	EU559490	EU559538
Sternophoroidea						
Sternophoridae	*Afrosternophorus* sp.	Australia	DNA103137	EU559360	EU559461	EU559568
Sternophoridae	*Garyops depressus*	USA	DNA103138	EU559398		EU559569
Cheliferoidea						
Atemnidae, At	*Cyclatemnus* sp.	Zambia	DNA103139	EU559402	EU559432	EU559528
Atemnidae, At	*Oratemnus curtus*	Australia	DNA103140	EU559361	EU559462	EU559531
Atemnidae, At	*Stenatemnus* sp.	Indonesia	DNA103141	EU559394	EU559448	EU559529
Atemnidae, At	*Titanatemnus* sp.	South Africa	DNA103142	EU559401	EU559441	EU559530

Table 1 (*continued*)

Taxon	Species	Location	MCZ voucher No.	GenBank Accession Nos.		
				18S rRNA	28S rRNA	COI
Atemnidae, Mi	*Miratemnus* sp.	Botswana	DNA103143	EU559388	EU559440	EU559535
Xiphosura	*Limulus polyphemus*			U91490	AF212167	NC_003057
Atemnidae, Mi	*Caecatemnus* sp.	Colombia	DNA103144	EU559404	EU559449	EU559534
Cheliferidae, Ch	*Nannochelifer* sp.	Australia	DNA103145	EU559400	EU559439	EU559532
Cheliferidae, Ch	*Protochelifer victorianus*	Australia	DNA103146	EU559428	EU559463	EU559533
Cheliferidae, Ph	*Philomaoria* sp.	New Zealand	DNA103147	EU559419	EU559491	EU559536
Chernetidae, Cr	*Apatochernes* sp.	New Zealand	DNA103148			EU559527
Chernetidae, Cr	*Haplochernes* sp.	Australia	DNA103149	EU559381	EU559492	EU559524
Chernetidae, Cr	*Maracherneslbellus*	Australia	DNA103150	EU559382	EU559493	EU559522
Chernetidae, Cr	*Nesidiochernes* sp.	Australia	DNA103151	EU559397		EU559499
Chernetidae, Cr	*Neochelanops* sp.	Chile	DNA103152	EU559408		
Chernetidae, Cr	*Nesochernes* sp.	New Caledonia	DNA103153	EU559409	EU559494	EU559573
Chernetidae, Cr	*Parazaona?* sp.	Colombia	DNA103154	EU559399	EU559495	EU559574
Chernetidae, Cr	*Pseudopilanus kuscheli*	Chile	DNA103155	EU559418	EU559455	EU559553
Chernetidae, Cr	*Lustrochernes* sp.	Colombia	DNA103156	EU559411		EU559523
Chernetidae, Cr	*Incachernes* sp.	Colombia	DNA103157	EU559410	EU559496	EU559525
Chernetidae, Go	*Calymmachernes angulatus*	Australia	DNA103158	EU559383	EU559464	EU559526
Chernetidae, Go	*Conicochernes crassus*	Australia	DNA103159	EU559362	EU559435	EU559571
Withiidae	*Withius* sp. 1	Zambia	DNA103160	EU559417	EU559443	EU559572

Vouchers numbers from the Museum of Comparative Zoology and GenBank accession numbers are indicated. Subfamilies are indicated by a two letter code, from top to bottom: Ps, Pseudotyrannochthoniinae; Ch, Chthoniinae; Mi, Microcreagrinae; Ne, Neobisiinae; Ch, Chitrellinae; Id, Ideobisiinae; Sy, Syarininae; Ol, Olpiinae; He, Hesperolpiinae; At, Atemninae; Mi, Miratemninae; Ch, Cheliferinae; Ph, Philomaoriinae; Cr, Chernetinae; Go, Goniochernetinae.

2002: Appendix 4); build(1); a first tree-building step is performed by random addition sequence; transform((all, auto_sequence_partition:true)); once a first tree is obtained, each fragment is evaluated and subdivided into smaller fragments for subsequent analysis whenever an unambiguous fragment homology can be established. This strategy should not affect the result but only the computation time by greatly accelerating the search; build(-trees:20, randomized); 20 random addition sequences are performed; swap(); each of the 20 trees is subjected to alternating SPR and TBR; select(unique, optimal); after completion of branch swapping, only optimal and topologically unique trees are retained; perturb(iterations:1, ratchet:(0.18,3), swap(tbr, trees:1)); in order to check if trees were not trapped in a local optimum, resulting trees are subject to one round of ratchet (Nixon, 1999), re-weighting 18% of the fragments by a factor of three. Note that in the context of Dynamic Homology, fragments are re-weighted and not individual nucleotides; select(unique, optimal); finally, only optimal and topologically unique trees are retained.

A total of twelve parameter sets were used for a range of indel-to-tranversion and transversion-to-transition ratios. Congruence was used as a meta-optimality criterion to choose a parameter set that minimizes incongruence among the partitions. Congruence was measured by a modification of the ILD metric (Mickevich and Farris, 1981; Farris et al., 1994, 1995). The value is calculated by subtracting the sum of the scores of all partitions from the score of the combined analysis of all partitions, and normalizing it for the score of the combined length. When the ILD is employed as a simple heuristic measure of congruence, as in this study, objections (e.g., Cunningham, 1997a,b; Dolphin et al., 2000; Yoder et al., 2001; Barker and Lutzoni, 2002; Darlu and Lecointre, 2002; Dowton and Austin, 2002) for its use for determining incongruence between partitions do not apply (Prendini et al., 2005). The ILD has also received criticism as a measure of congruence (Aagesen et al., 2005; Wheeler et al., 2006), but results of the alternative measures are very similar. Since we explore also "suboptimal" parameter sets, our major conclusions should not be affected by the type of congruence measure employed. The parameter set that minimizes character conflict is considered the optimal parameter set and the resulting tree is taken as the best estimate of the phylogeny. Results from the other parameter sets were also considered for assessing nodal stability (sensu Giribet, 2003; contra Kluge, 1997a,b; Frost et al., 2001; Grant and Kluge, 2003).

After completing the sensitivity analysis, we used all resulting trees of each parameter set as input for 50 replicates of tree fusing (Goloboff, 1999, 2002) with the commands: read("parenthetical*"); fuse(iterations:50, swap()). This technique, informally called SATF (Sensitivity Analysis Tree Fusing), allows quick estimation if the first strategy was sufficient by using a genetic algorithm that performs inter-tree branch swapping (Giribet, 2007).

Nodal support was estimated by jackknifing (Farris et al., 1996; Farris, 1997) with 100 replicates. In the context of dynamic homology (Wheeler et al., 2006), the characters being sampled during pseudoreplicates are entire sequence fragments, not individual nucleotides. Therefore we used the command transform(auto_sequence_partition:true), which increases the number of fragments—and thus of characters—used for jackknifing. This command breaks up the fragments, possibly into a large number of pieces, improving the number of characters for resampling while maintaining certain amount of freedom to adjust the tree cost between different topologies. As opposed to the search strategy where this command is not mandatory, we recommend to use this command for jackknife because too few fragments could lead to exaggerate jackknife values (either too high or too low).

The data for all genes were analyzed simultaneously (Nixon and Carpenter, 1996). In addition, we performed separate analyses for COI alone and for the combined nuclear genes (as both ribosomal genes are found in the same gene array).

3. Results

Of the 12 parameter sets under which the data were analyzed, the one that minimized overall incongruence is indel:transversion ratio 1:1 and a transversion:transition ratio 2:1 (parameter set 121, ILD = 0.1219). This will hereafter be referred to as the optimal parameter set. Under the first search strategy (RAS + SPR + TBR + ratchet), one tree of length 35,952 was found. After the Tree Fusing step, one tree of length 35,940 was obtained. The two trees are topologically identical and we attribute the differences in tree length to the improvement of optimization procedure between POY 4.0 RC build 2398 (used for sensitivity analysis) and POY 4.0 RC build 2602 (used for Tree Fusing). We re-diagnosed the 35,952 steps tree with POY 4.0 RC build 2602 and found a length of 35,940. This result suggests that our first search strategy was sufficient for obtaining the optimal tree.

The combined analysis of all three genes under the optimal parameter set (121; Fig. 3) shows monophyly of Feaelloidea (95% jackknife frequency; JF hereafter)—which is the sister group to the remaining Pseudoscorpiones. The monophyly of the group and its position both appear highly supported by resampling techniques and stable under all parameter sets examined. The monophyly of Chthonioidea is retrieved under 83% of the parameter sets. All Cheliferoidea except for *Neochelanops* sp. form a clade retrieved under 75% of the parameter sets. In addition, Sternophoridae is sister to Cheiridiidae (66% JF, 100% of parameter sets), this group being sister group to the main clade of Cheliferoidea under 83% of the parameter sets. Neobisiodea and Garypoidea are represented by a paraphyletic assemblage at the base of the Cheiridiinae + Sternophoriidae + Cheliferoidea group, which also includes *Neochelanops*. The spurious placement of *Neochelanops* sp. could be attributed to missing data. This species has only been sequenced for the a2.0/9R fragment of the 18S rRNA gene and thus the taxon is represented with the most missing data in our dataset. A more "classical" position (i.e., grouping with the remaining Cheliferoidea) is however retrieved under alternative parameter sets (111).

Internal relationships within the main clades also show some clear patterns. If we exclude *Parazaona* sp. and *Neochelanops* sp. the monophyly of Chernetidae appears to be fairly stable, under 75% of the parameter sets. The subfamily Goniochernetinae is monophyletic but is included inside Chernetinae rendering the latter paraphyletic. Cheliferidae is not monophyletic because Philomaoriinae (1 sp.) does not group with Cheliferinae (monophyletic, with 93% JF). Atemnidae is not monophyletic because Miratemninae (2 spp.) does not group with Atemninae (monophyletic, with 77% JF). Indeed the two Miratemninae (*Miratemnus* and *Caecatemnus*) do not group together, with *Miratemnus* being sister to Cheliferinae (60% JF) and *Caecatemnus* sister to Philomaoriinae (83% JF).

Sternophoridae are found monophyletic under the optimal parameter set (54% JF, and in 92% parameter sets) and always group with Cheiridiidae (66% JF; 100% parameter sets). This group is found to be the sister group to Cheliferoidea and this result is fairly stable (83% of parameter sets).

Garypoidea are almost never found monophyletic (except under parameter set 281). *Geogarypus* sp. groups within Neobisioidea but this position cannot be attributed to missing data. Garypinidae and Larcidae always form a paraphyletic (monophyletic with the inclusion of *Syarinus* sp. and *Neochelanops* sp. as in Fig. 3) or a monophyletic group. In addition they almost always (83% of the parameter sets) form a distinct lineage from the remaining Garypoidea. Garypidae (100% JF) form a monophyletic group within Olpiidae, rendering the latter paraphyletic in most of the analyses (83% of parameter sets). In order to check whether the dubious position of *Geogarypus* sp. and *Syarinus* sp. was due to a problem of mixlabeling, new tissue samples from the same *Geogarypus* specimen (with identification re-checked by MSH) were sent to the molecular laboratory. We sequenced the 18S rRNA gene and confirmed that the original sequence was indeed the correct one and concluded that there was no mislabeling or vial mixing errors.

Neobisioidea never form a monophyletic group but are most of the time paraphyletic because of the position of Ideoroncidae, Hyidae and Syarinidae, and for the inclusion of *Geogarypus* mentioned earlier. Neobisiidae are monophyletic (83% of parameter sets) with Neobisiinae monophyletic (65% JF) and Microcreagrinae paraphyletic.

The same general topology is retrieved when the genes are analyzed separately (Figs. 4 and 5). In particular, the monophyly of Cheliferoidea is always retrieved as well as the position of Cheiridiidae forming a clade with Sternophoridae. However, some discrepancies exist between markers. The 18S and 28S rRNA genes both separately (not shown) and in combination (Fig. 4) agree on the "basal" position of Feaelloidea where COI indicates a basal position of Chthonioidea.

4. Discussion

The first molecular analysis of deep pseudoscorpion relationships shows good resolution and high support and stability in many deep nodes of the tree (see Fig. 3), indicating that our data are able to resolve many key aspects of the phylogeny of this arachnid order. Major traditional groupings appear in our phylogeny, although paraphyly is suggested for an important number of taxa, especially those towards the base of the tree. A formal revision of pseudoscorpion systematics must await future combined analyses of the morphological evidence in conjunction with the current molecular data (author's work in progress).

4.1. Higher classification hypotheses

The present analyses only partially support Chamberlin's (1929, 1930, 1931) hypothesis of three suborders. Heterosphyronida (=Chthonioidea) are recovered, but Homosphyronida are not, due to the position of Feaelloidea which is always found to be sister to the remaining pseudoscorpions. These data are congruent with Harvey (1992) who found a monophyletic Chthonioidea, and a paraphyletic Homosphyronida. Feaelloidea do not group with the remaining Monosphyronida (c.f. Chamberlin, 1931), and Diplosphyronida form a paraphyletic assemblage at the base of Monosphyronida, therefore not corroborating either hypothesis. Whilst Iocheirata is supported in all analyses with high jackknife support (Figs. 3 and 6), Epiocheirata are not supported, with Chthonioidea grouping with Iocheirata in all analyses (with moderate jackknife support), and Feaelloidea always being sister to the remaining Pseudoscorpiones. Our results favor Cheiridiidae + Sternophoridae [i.e., Chamberlin's, 1931 Cheiridioidea] being almost always sister group to Cheliferoidea. However, Mestommatina form a paraphyletic assemblage at the base of Elassommatina.

4.1.1. Feaelloidea

Our molecular data support a monophyletic Feaelloidea with high support, upholding the hypotheses of Chamberlin (1931) and Harvey (1992), but refuting the suggestion by Muchmore (1982b) that Pseudogarypidae belong to Garypoidea. Furthermore, the inclusion of *Synsphyronus* within Feaelloidea (Beier, 1932a) is likewise not substantiated, as this genus always groups with *Anagarypus* within Garypidae, as maintained by all subsequent studies (e.g., Chamberlin, 1943; Harvey, 1987). Harvey (1992) identified 20 morphological features supporting monophyly of Feaelloidea of which eight were unique to the group.

The systematic relationships of Feaelloidea have varied the most of all pseudoscorpion taxa over the past century, having been included in Monosphyronida (Chamberlin, 1931), Neobisiinea (Beier, 1932a), "close to Heterosphyronida or somewhere between Heterosphyronida and Diplosphyronida" (Weygoldt, 1969), or within Epiocheirata (Harvey, 1992). The molecular data do not support a relationship with Monosphyronida or Diplosphyronida, but suggest they represent the most basal pseudoscorpion clade.

4.1.2. Chthonioidea

Chthonioidea is consistently recovered as a monophyletic group in the majority of our analyses, supporting the hypotheses of all classifications and analyses since Chamberlin (1929, 1931). Harvey (1992) delineated 10 apomorphies for the superfamily, of which nine were autapomorphic. As presently constituted, Chthoniidae lacks any support for monophyly based upon morphological

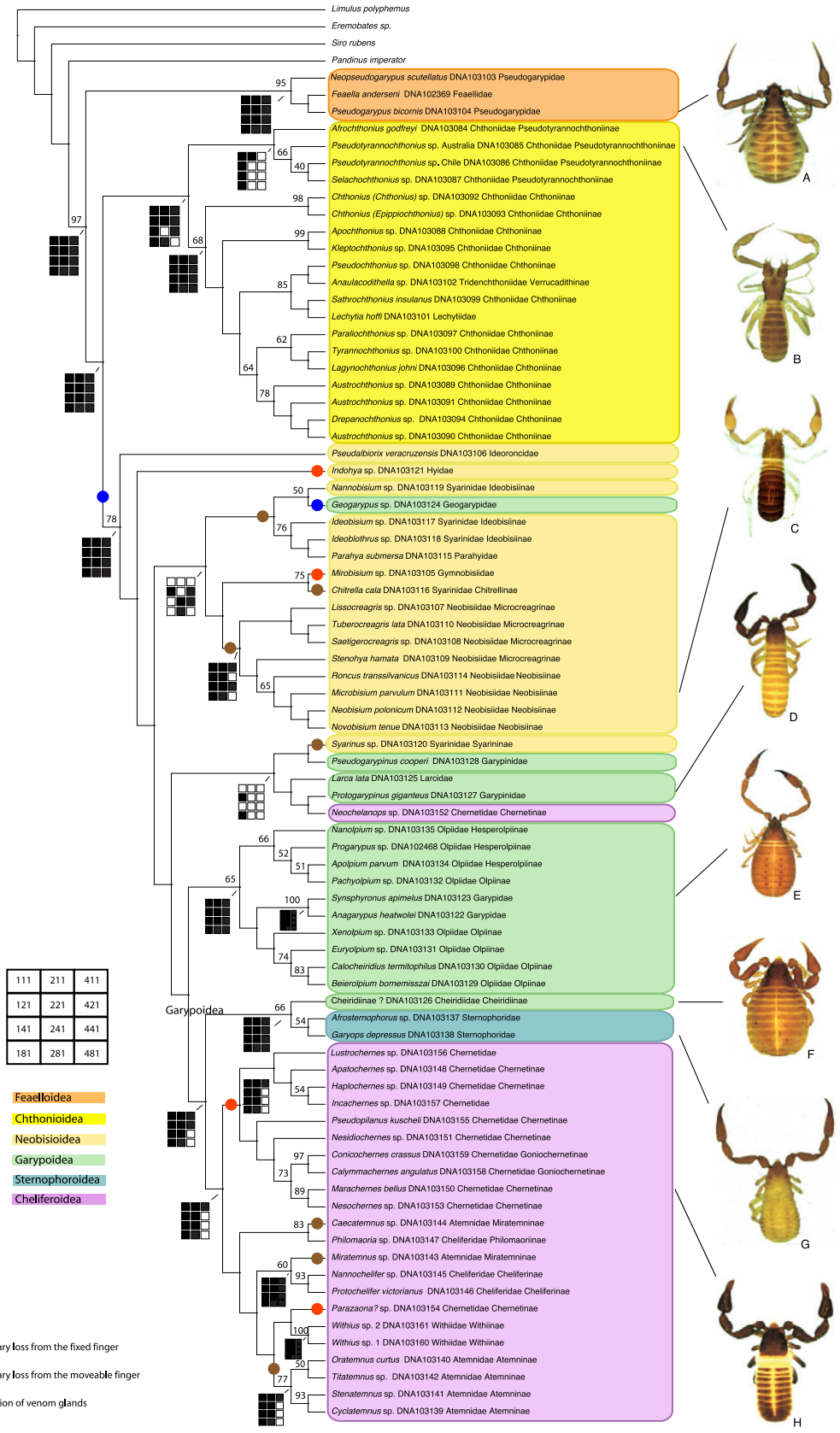

Fig. 3. Phylogeny of Pseudoscorpiones based on the direct optimization combined analysis of 18S rRNA, 28S rRNA and COI data under the parameter set that minimizes incongruence. Support values on branches indicate jackknife frequencies. Each weighting scheme is named by a three-digit code corresponding to the ratio of indel/transversion, transversion/transition and transition values. Black indicates that the monophyly of the group is retrieved under the particular parameter set. Representative pseudoscorpions of all recognized superfamilies are illustrated: (A) *Pseudogarypus bicornis* (Banks) (Pseudogarypidae) from CA, USA; (B) *Pseudotyrannochthonius* sp. (Chthoniidae), from Western Australia; (C) *Neobisium carcinoides* (Leach) (Neobisiidae), from Scotland; (D) *Protogarypinus giganteus* Beier (Garypinidae), from Western Australia; (E) *Synsphyronus* sp. (Garypidae), from Western Australia; (F) unidentified Cheiridiinae (Cheiridiidae), from Western Australia; (G) *Garyops depressus* Banks (Sternophoridae), from FL, USA; (H) *Marachernes bellus* Harvey (Chernetidae), from Victoria. All images by M.S. Harvey.

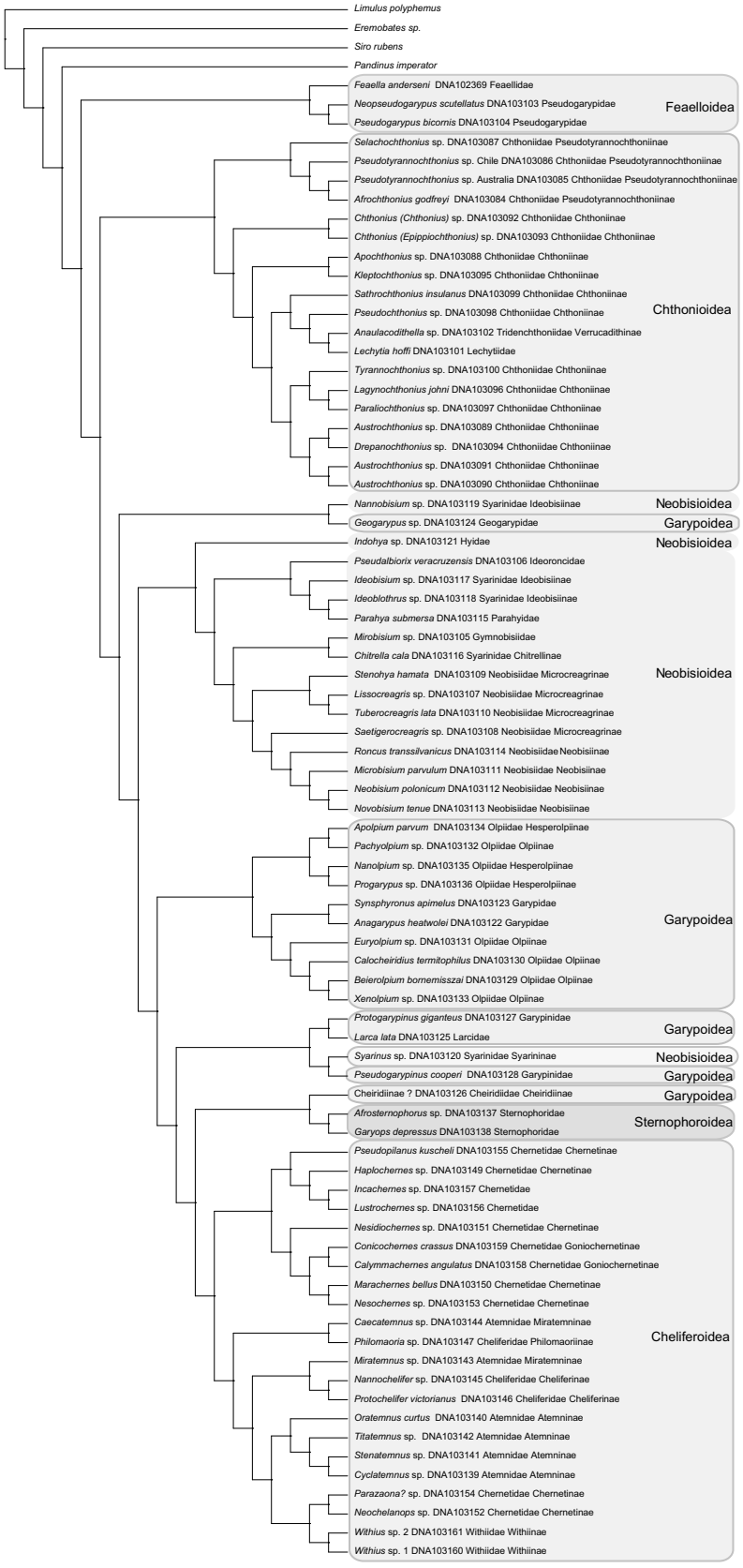

Fig. 4. Phylogeny of Pseudoscorpiones based on the direct optimization analysis of the nuclear genes 18S rRNA and 28S rRNA under the parameter set that minimizes incongruence.

characters (Harvey, 1992), or in the current molecular analysis where it is rendered paraphyletic with the inclusion of *Lechytia* (Lechytiidae) and *Anaulacodithella* (Tridenchthoniidae). The inclusion of these two families within Chthoniidae is retrieved under

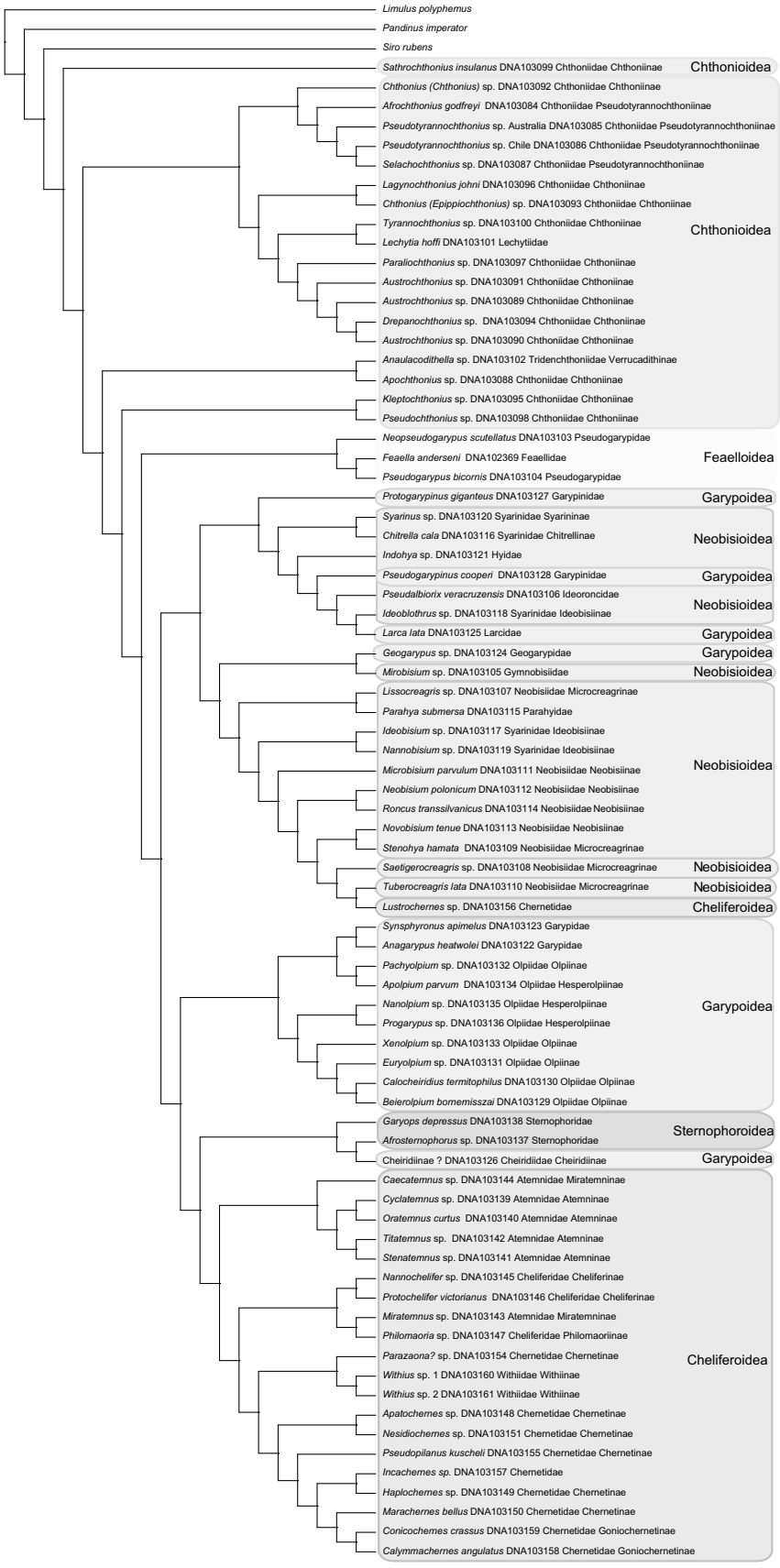

Fig. 5. Phylogeny of Pseudoscorpiones based on the direct optimization analysis of the mitochondrial COI gene under the parameter set that minimizes incongruence.

all parameter sets examined. To maintain a monophyletic Chthoniidae, it will be necessary to either synonymize Lechytiidae and Tridenchthoniidae with Chthoniidae, or to divide Chthoniidae into a number of smaller families. The former option seems to be the

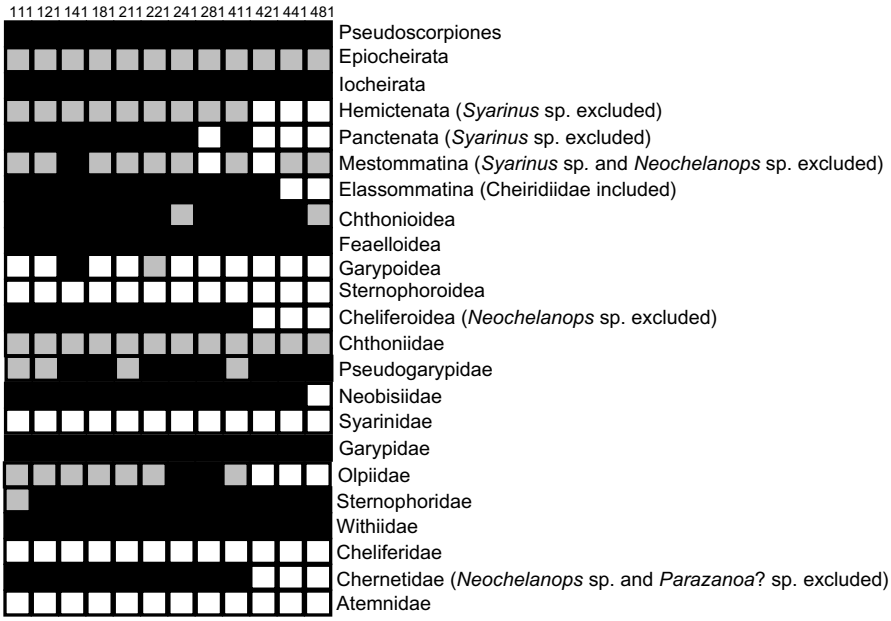

Fig. 6. Sensitivity analysis plot representing the monophyly (black), paraphyly (grey) or polyphyly (white) of the groups recognized in Harvey (1992), to date, the only Pseudoscorpiones classification based on a cladistic analysis.

most practical and will depend upon a larger study using multiple exemplars and morphological data. The pseudotyrannochthoniine chthoniids (*Pseudotyrannochthonius*, *Afrochthonius* and *Selachochthonius*) nearly always group as the sister to the remaining chthonioids, suggesting that the group is monophyletic. In some analyses, monophyly of the group is not retrieved because *Afrochthonius godfreyi* groups with the remaining chthoniids. Schawaller (1980) presented a diagram illustrating the potential relationships within Chthonioidea based upon Hennigian logic, in which Dithidae (=Tridenchthoniidae) and Chthoniinae could be construed as monophyletic entities, but the pseudotyrannochthoniines were a paraphyletic assemblage without any synapomorphies.

4.1.3. Neobisioidea

A monophyletic Neobisioidea was not recovered in any analysis we performed, as it was always paraphyletic at the base of Iocheirata. *Pseudalbiorix* (Ideoroncidae) and then *Indohya* (Hyidae) were situated as sister taxa to Iocheirata. Both of these genera were found to be relatively basal within Neobisioidea by Harvey (1992) and Harvey and Volschenk (2007). Our analysis could not test the other basal neobisioid family, Bochicidae, due to the lack of specimens. Harvey (1992) found only two characters supporting Neobisioidea.

The syarinid *Syarinus* was placed within Garypoidea, contrary to all previous morphological hypotheses where it has been considered a member of Neobisioidea (e.g., Chamberlin, 1930, 1931; Beier, 1932a; Muchmore, 1982b; Harvey, 1992; Harvey and Volschenk, 2007). This placement seems to be essentially due to the nuclear ribosomal genes signal since *Syarinus* appears sister group to Syarinidae Chitrellinae when the COI data are analyzed alone (Fig. 5). In addition, this position was unstable to variation in parameter sets and appears poorly supported. The molecular data conflict with the morphological data, and such conflict requires further scrutiny.

Syarinidae contain 16 genera placed in three subfamilies (Harvey, 2008), but a recent analysis of neobisioid relationships based upon morphological features (Harvey and Volschenk, 2007) suggested that the family may be paraphyletic. The molecular data also suggest that Syarinidae is polyphyletic, with *Ideobisium* and *Ideoblothrus* grouping with *Parahya* (Parahyidae), *Chitrella* with *Mirobisium* (Gymnobisiidae) and *Syarinus* with the garypoids, but the latter result, appears flawed based on morphology (e.g., Chamberlin, 1930; Muchmore, 1982a; Harvey and Volschenk, 2007).

Neobisiidae is well supported in this analysis, and is almost consistently monophyletic, supporting the morphological data (e.g., Chamberlin, 1930; Muchmore, 1982b; Harvey, 1992). Whilst Neobisiinae are monophyletic, with limited support, Microcreagrinae are not. This correlates with the sole morphological feature used to separate these subfamilies, the plesiomorphic presence (Microcreagrinae) or apomorphic absence (Neobisiinae) of a galea on the chelicera.

4.1.4. Garypoidea and Olpioidea

Chamberlin (1930, 1931) included Garypidae, Olpiidae and Menthidae within Garypoidea, which was accepted in most later classificiations (e.g., Beier, 1932a; Muchmore, 1982b), although Harvey (1986) segregated Geogarypidae from Garypidae. Harvey (1992) restricted Garypoidea to Garypidae, Geogarypidae and Larcidae, and proposed Olpioidea for Olpiidae and Menthidae, and Judson (2005) separated Garypinidae from Olpiidae. The molecular data suggest that neither Olpioidea nor Garypoidea can be corroborated since Olpiidae + Garypidae form a distinct lineage from Garypinidae + Larcidae. The picture is further confused by *Geogarypus* situated within Neobisioidea, and *Syarinus* and *Neochelanops* within Garypoidea. Futhermore, Garypoidea sensu lato, is not supported as a monophyletic group.

Only one garypoid family—Garypidae with *Anagarypus* and *Synsphyronus*—was found to be monophyletic in our analyses. Garypinidae (*Pseudogarypinus* and *Protogarypinus*) were never recovered, and was rendered paraphyletic by the inclusion of Larcidae and, bizarrely, *Syarinus* and *Neochelanops*. Olpiidae was made paraphyletic by the inclusion of Garypidae.

4.1.5. Cheiridioidea

The superfamily Cheiridioidea was first proposed by Chamberlin (1931) for Cheiridiidae, Pseudochiridiidae and Sternophoridae, and substantiated by the morphology of the legs in which the fem-

oral and patellar joints of each leg are similar to each other. Harvey (1992) disbanded Cheiridioidea, removing Cheiridiidae and Pseudochiridiidae to Garypoidea, and placing Sternophoridae in its own superfamily which was sister to Cheliferoidea. Whilst we only had a single cheiridiid (unknown genus of Cheiridiinae) and two sternophorids (*Afrosternophorus* and *Garyops*) within our analysis, the results indicate that Chamberlin's superfamily Cheiridioidea is corroborated. This result is retrieved under all parameter sets and even when 28S rRNA and COI are analyzed separately. The molecular results are in conflict with the solution proposed by Judson (2000) who suggested that Cheiridioidea (Cheiridiidae + Pseudochiridiidae) was sister to Cheliferoidea, supported by several morphological features; this hypothesis has yet to be tested empirically.

4.1.6. Cheliferoidea

Cheliferoidea are retrieved as monophyletic in most analyses, confirming the traditional composition of the superfamily (e.g., Chamberlin, 1931; Beier, 1932b; Harvey, 1992). We cannot test the hypothesis of Pseudochiridiidae within Cheliferoidea proposed by Muchmore (1982b) due to lack of sequence data for this family.

The analysis presented by Harvey (1992) placed Withiidae as sister to the remaining families (Atemnidae, Cheliferidae and Chernetidae), and Proctor (1993) suggested a relationship between Chernetidae and Cheliferidae based upon the morphology of the spermatophore. Our molecular data suggest Chernetidae is sister to the other three families, followed by a mixture of atemnids, cheliferids and withiids.

Most Chernetidae sampled in this study formed a monophyletic group, but *Neochelanops* grouped with Garypoidea in some analyses, and *Parazaona* sister to Withiidae. Neither of these results are credible, and the monophyly of the family seems well supported based on morphological grounds (e.g., Chamberlin, 1931; Beier, 1932b; Muchmore, 1982b; Harvey 1992). The subfamily Goniochernetinae (*Conicochernes* and *Calymmachernes*) is monophyletic but is included inside Chernetinae rendering the latter paraphyletic.

A monophyletic Atemnidae was never recovered, with a monophyletic Atemninae sister to Withiidae + *Parazaona* (Chernetidae), and a non-monophyletic Miratemninae grouping elsewhere, *Caecatemnus* with the cheliferid *Philomaoria*, and *Miratemnus* with the cheliferids *Nannochelifer* and *Protochelifer*. These results are not in agreement with traditional circumscriptions of the family which found the loss of a venom apparatus in the moveable chelal finger as diagnostic (e.g., Beier, 1932b; Chamberlin, 1931; Muchmore, 1982b) and synapomorphic (Harvey, 1992). More recently, Klausen (2005) suggested that Atemnidae is monophyletic based upon the morphology of the male genitalia.

Similarly, Cheliferidae was not found to be monophyletic, with the two cheliferines (*Nannochelifer* and *Protochelifer*) situated away from the philomaoriine (*Philomaoria*).

4.2. Evolution of the venom apparatus

The acquisition of venom by arachnids has occurred within three different lineages. Spiders (Araneae) possess venom glands within the prosoma that discharge via the chelicerae. Scorpions (Scorpiones) possess a pair of venom glands after the terminal metasomal segment, discharging via the sting. Pseudoscorpions have long been recognized as possessing venomous capabilities, with the presence of venom apparatus in one or both of the chelal fingers discharging via the tips of the fingers which bear an enlarged distal tooth. It was first described by Croneberg (1888), these observations augmented and strengthened by Chamberlin (1924a, 1931) and many other authors. Chamberlin (e.g., 1930, 1931) noted the taxonomic usefulness of the presence or absence of venom glands, and used the lack of venom glands in either chelal finger to diagnose individual families. Chamberlin (1931, p. 132) suggested that the venom glands are homologous with the pedipalpal sperm receptacle of male spiders, and that the lack of venom glands in Chthonioidea was secondary. Chamberlin's (1924a,b, 1938) observation that venom glands were totally absent in Cheiridioidea seems to be in error (e.g., Mahnert, 1982; Muchmore, 1982b; Harvey, 1992). Although Chamberlin (1931) also noted the complete absence of venom apparatus in Feaelloidea, he makes no specific mention of secondary loss in this superfamily, concentrating his discussion on the loss of venom glands in individual fingers of some families.

Harvey (1992) used the presence of venom glands in all pseudoscorpions except Chthonioidea and Feaelloidea to propose Iocheirata—"poison hand"—for this large clade. Iocheirata was also supported by three other apomorphies: Character 5, presence of posterior maxillary lyrifissure; Character 24, trichobothrium *isb* on external face of fixed chelal finger (reversed in some taxa); and Character 69, subdistally placed slit sensillum of legs I and II. The latter character state is not universal within Iocheirata, as some taxa have subbasal sensillae.

Our molecular data corroborate the monophyly of Iocheirata in all parameter sets tested, with high jackknife support (Fig. 3). Chamberlin's (1931) hypothesis that venom glands were secondarily lost in Chthonioidea is not supported by molecular or morphological analyses, and we conclude that venom glands have evolved only once within pseudoscorpions, with subsequent losses in either chelal fingers in some clades. It is absent or strongly reduced in the fixed chelal finger of Gymnobisiidae, some Bochicidae (*Vachonium*, and *Paravachonium*), some Hyidae (*Indohya*) and Chernetidae, and in the moveable finger in Neobisiidae, Syarinidae, Parahyidae, Menthidae and Atemnidae. The importance of these antagonistic and/or defensive structures to their lifestyle is exhibited by the absence of total loss of venom glands in any iocheiratan.

Although the earliest pseudoscorpion—*Dracochela deprehendor*—is Devonian, it is unlikely to have venom glands (Schawaller et al., 1991; Harvey, 1992), thus excluding it from Iocheirata. The next earliest pseudoscorpions are from Cretaceous ambers, and have been reported from Myanmar (Cockerell, 1917; Judson, 2000; Grimaldi et al., 2002), Lebanon (Whalley, 1980), USA (Grimaldi et al., 2002), Canada (Schawaller, 1991) and France (Perrichot, 2004). Of those that have been identified or described with any accuracy, all are iocheiratans belonging to extant families, suggesting that venom glands had evolved in pseudoscorpions prior to the Lower Cretaceous, even though venom glands cannot be discerned in the actual fossils. The lack of pseudoscorpion fossils between the middle Devonian and Lower Cretaceous hampers analysis of the early diversification of iocheiratans.

5. Conclusions

Comparing our results with the classifications of Chamberlin (1931) and Harvey (1992), it is apparent that the groups that are congruent between those classifications are also retrieved in our analyses. For example the position of Chthonioidea and Monosphyronida/Elassommatina is retrieved even if our results favor Chamberlin's scheme for the position of Cheiridiidae. One point of disagreement between the two previous classifications was the position of Feaelloidea. Our results reject any of the previous hypotheses and place it as sister to the remaining Pseudoscorpiones. Another point of disagreement was the position of Garypoidea (Chamberlin)/Mestommatina (Harvey) group. In the same way, our results do not support any of these hypotheses since Hemictenata and Mestommatina are paraphyletic. The nestedness of the groups is however closer to Harvey's classification.

Although this study represents an important step forward and introduces molecular data for most pseudoscorpion lineages, further study of combined morphological (Harvey, 1992; Judson, 2000) and molecular data are required to more fully understand pseudoscorpion phylogeny. Nevertheless high support was detected for the monophyly of the following clades: unnamed clade comprising all pseudoscorpions except Feaelloidea; Iocheirata sensu Harvey (1992); Feaelloidea; Chthonioidea (=Heterosphyronida) sensu Chamberlin (1929, 1931); Cheiridioidea sensu Chamberlin (1931); and Sternophoroidea sensu Harvey (1992). These can be considered for now well supported clades based on the data in hand. Cheliferoidea sensu Chamberlin (1931) was retrieved with the exception of *Neochelanops* which grouped with some garypoids. Neobisioidea and Garypoidea, both sensu Chamberlin (1930, 1931), were not retrieved under any parameter set.

Although we have sequenced 74 genera and 79 species for this study, this represents 16.8% of the 439 currently recognize and named pseudoscorpion genera and 2.3% of the 3385 species (Harvey, 2008). Additional gene regions for species already sequenced for this study, as well as additional taxa and morphology, would allow refining the evolutionary patterns within this ancient and diverse group of arachnids.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2008.06.002.

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