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The first phylogenetic analysis of Palpigradi—the most enigmatic arthropod order

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Abstract. Palpigrades are a poorly understood group of tiny soil arthropods, often found in caves. Concomitantly, they have been neglected from a phylogenetic point of view. Here we present the first molecular phylogeny of palpigrades based on specimens collected in different environments (caves and soil) from Australia, Africa, Europe, South America and North America. Analyses of two nuclear ribosomal genes and COI under an array of methods and homology schemes found monophyly of Palpigradi, Eukoeneniidae, and a division of Eukoeneniidae into three main clades, each including samples from multiple continents. This supports either ancient vicariance or long-range dispersal, two alternatives we cannot distinguish with the data at hand. In addition, we show that our results are robust to homology scheme and analytical method, encouraging further use of the markers employed in this study to continue drawing a broader picture of palpigrade relationships.

Additional keywords: Arachnida, micro whip scorpions, speleology, biogeography.

Introduction

The arachnid order Palpigradi (micro whip scorpions) is one of the smallest, rarest and most neglected groups of terrestrial arthropods, and the last order of arachnids to be discovered—first reported in 1885 (Grassi and Calandruccio 1885). To provide an example of such neglect, the first photographs of living palpigrades did not appear published until 2002 (**Errore. Riferimento a collegamento ipertestuale non valido.**; Beccaloni 2009). Another example is illustrated by the small amount of DNA sequence data available in GenBank, with only 64 sequences, 56 of which are for *Prokoenenia wheeleri*, a species that was part of a multi-gene phylogeny of arthropods (Regier *et al.* 2010); The remaining eight sequences are unidentified specimens from three studies on chelicerate phylogenetics (Giribet *et al.* 2002; Pepato *et al.* 2010; Arabi *et al.* 2012); No data appear in the Barcode of Life website (http://www.barcodinglife.org). (In comparison, GenBank has 100 DNA sequences for Uropygi, 187 for Schizomida, 200 for Ricinulei, 251 for Amblypygi, and 493 for Pseudoscorpiones, to mention some of the other smaller orders [checked on June 5th, 2013]).

Palpigrades walk sensing the floor with what seems a nervous behavior of the first pair of walking legs, and use their highly unmodified palps for walking, unlike all other arachnids. While moving, most palpigrades keep the flagellum upward, moving it laterally. Accordingly, it is possible that the uplifted flagellum is associated with perception of the environment (Ferreira and Souza 2012). This small, depigmented and highly translucent arachnids, range in size from 0.65 mm in *Eukoenenia grassi* to 2.8 mm in the "giant" *E. draco* from Spain (Beccaloni 2009).

The living members of the order are currently divided in two families, Eukoeneniidae Petrunkevitch, 1955, with 4 genera and 75 species, and Prokoeneniidae Condé, 1996, with 2 genera and 7 species (Harvey 2002a; Prendini 2011). Eukoeneniidae includes the genera *Allokoenenia* (*A. afra* from West Africa and 3 undescribed species from Brazil), *Eukoenenia* (the largest genus, Pantropical distribution, in temperate regions restricted to caves), *Koenenioides* (8 Palaeotropical species) and *Leptokoenenia* (*L. gerlachi* from Saudi Arabia, *L. scurra* from Congo and 2 undescribed species from Brazil). Prokoeneniidae includes the genera *Prokoenenia* (6 spp.) and *Triadokoenenia* Condé 1991, the latter a monotypic genus from Madagascar (Condé 1991).

The position of Palpigradi among the arachnid orders remains highly debated. The largest set of data analysed to date for palpigrades places them as the sister group to Acariformes mites in a basal position within arachnids, although without support (Regier *et al.* 2010), while the most recent morphological cladistic analysis of arachnid relationships leaves it mostly unresolved among the clades Stomothecata, Haplocnemata, Pantetrapulmonata, and Acaromorpha (Shultz 2007). Earlier studies combining morphology and a small set of molecular data placed them as the sister group of Ricinulei + Tetrapulmonata or as sister to pycnogonids when fossils were considered, although again, without significant clade support (Giribet *et al.* 2002); or as sister to a clade including two clades of Acari and Solifugae, based on the same two markers used in earlier studies (Pepato *et al.* 2010); or in an unresolved position within arachnids (Arabi *et al.* 2012). Even less is known about the internal relationships of the group, since no published study—molecular or morphological—has yet incorporated information for more than one palpigrade species, and only one unpublished undergraduate thesis has explored palpigrade relationships cladistically using morphology (Montaño Moreno 2008).

To ameliorate this gap in knowledge, although acknowledging the difficulties in sampling and identification of these elusive animals, we obtained samples for as many species of palpigrades as possible with the aim to obtain molecular DNA sequence data to generate a preliminary hypothesis of palpigrade relationships.

Materials and Methods

Taxon sampling

Palpigrades are difficult to obtain and identify, and many samples obtained for this study were collected indirectly in caves and bore holes [Western Australia]. In other places they can be abundant and fresh specimens have recently become available for molecular study in different cave systems and soil samples. We have collaborated to obtain specimens for this study, although in some cases older specimens were used, especially from the prolific cave systems in Brazil, where several new species have been collected and described (Souza and Ferreira 2010; Ferreira *et al.* 2011; Souza and Ferreira 2011a; Souza and Ferreira 2011b; Souza and Ferreira 2012a; Souza and Ferreira 2012b). While a recently collected specimen of *Eukoenenia ferratilis* amplified well for some of the studied markers, none of the six specimens of *Allokoenenia* spp.

and the two specimens of *Leptokoenenia* sp. collected from the caves yielded workable DNA. We also obtained a relatively large collection of specimens from the Western Australian bore holes from Barrow Island and from near Homestead or Pannawonica, but these were collected from pitfall traps collected sporadically and many specimens did not amplify or only yielded some amplicons. Some of these specimens are probably related to the Western Australian endemic *E. guzikae* Barranco & Harvey, 2008, but unrelated to the more widespread species *E. mirabilis*, also found in Western Australia (Harvey *et al.* 2006). A single specimen of *Prokoenenia wheeleri* was obtained from the Austin area (Texas, USA), but worked well and amplified for all fragments attempted. In addition, we obtained samples of *Eukoenenia mirabilis* from Italy (Christian *et al.* 2010), specimens from multiple localities from the hanseni-chilanga group of *Eukoenenia* from Mexico and the USA (Montaño-Moreno 2012), *Eukoenenia spelaea* from Slovakia (**Errore. Riferimento a collegamento ipertestuale non valido.**; Král *et al.* 2008). Additional specimens come from Mexican caves and South Africa. Details on collecting localities are available in Table 1. Vouchers or additional specimens are deposited in the Museum of Comparative Zoology, Harvard University (MCZ), and in the Western Australian Museum (WAM).

We included three species available in GenBank, one from South Africa sequenced by Giribet et al. (2002), one from Brazil from Pepato et al. (2010), and one of unknown origin published by Arabi et al. (2012). Here we added sequences from an additional South African specimen from the same collection of Giribet et al. (2002), and a specimen of *E. ferratilis* from Brazil, which was identical to the specimen reported by Pepato et al. (2010) as *Eukoenenia* sp., and to which we refer to as *E.* cf. *ferratilis* in the present study. Outgroup taxa were selected from GenBank (Table 2), mostly from previous studies on arthropod or arachnid phylogeny using nuclear ribosomal genes (Giribet *et al.* 2002; Mallatt and Giribet 2006).

Molecular methods

Although we attempted to amplify and sequence five molecular markers typically used in other analyses of arachnid systematics, the mitochondrial 16S rRNA gene only amplified for one sample and the nuclear protein-encoding gene histone H3 amplified for several samples, but did not produce clean reads. We thus restricted our study to the two broadly available nuclear ribosomal genes, the complete 18S rRNA and ca. 2.2 Kb of 28S rRNA, and the mitochondrial

protein-encoding cytochrome *c* oxidase subunit I (COI hereafter), although the latter gene only amplified for about half of the specimens. For two of the bore-hole Western Australian specimens, poorly preserved, only the middle amplicon of 28S rRNA worked.

Total DNA was extracted from whole specimens or from the opisthosomal region using Qiagen's DNEasy® tissue kit (Valencia, CA, USA). Although we were aiming to preserve the digested carcass as a morphological voucher, it was completely digested and not recoverable. Purified genomic DNA was used as a template for Polymerase chain reactions (PCR) amplification. PCR, visualization by agarose gel electrophoresis, and direct sequencing were conducted for most specimens as described in earlier work, e.g., Edgecombe and Giribet (2009). Chromatograms obtained from the automatic sequencer were read and sequences assembled using the sequence editing software Sequencher™ (Gene Codes Corporation, Ann Arbor, MI, USA). Sequence data were edited in MacGDE (Linton 2005). The three genes were analysed as follows:

18S rRNA: This marker was amplified in three amplicons (*a, b, c*), as in previous studies (Edgecombe and Giribet 2009; Giribet *et al.* 2010; Giribet *et al.* 2012). In the present study we include 15 palpigrade specimens plus 2 outgroups, for a total of 1760-1771 bp per complete sequence (up to 1805 bp for one of the outgroups). From the 15 palpigrade sequences all but two were complete; *E. spelaea* is missing fragment *a* and the sample of *Eukoenenia* from South Africa (DNA100456.2) is missing fragment *b*. For the direct optimization analyses the three amplicons were treated as a single input file, containing 23 sequences, and divided into six fragments. The three amplicons were concatenated for the static alignment analyses.

28S rRNA: This nuclear gene was amplified in three amplicons (*a, b, c*), as described in Giribet and Shear (2010). The data set includes 17 palpigrade specimens plus 2 outgroups, for a total of 2,080 to 2,114 bp, with some length variation among species (2,199 bp for *Anoplodactylus*). These three fragments correspond to primer pairs 28S rd1a—28D rd4b, 28Sa—28S rd5b, and 28S rd4.8a—28S rd7b1. Some of the published sequences were amplified with a shorter fragment *b*, generated with primers 28Sa—28Sb (Whiting *et al.* 1997), and therefore fragment *b* was divided into fragments *b1* and *b2* to accommodate these two amplicons. Fragment *a* was available for 18 taxa (10 palpigrades) and divided into three fragments, fragment *b* for 25 taxa (17 palpigrades) and three fragments, and fragment *c* for 20 taxa (12

palpigrades) and analysed as a single fragment. These were treated as three different amplicons for the dynamic homology analyses, and aligned independently for the static homology approaches.

COI: This widely used mitochondrial marker amplified for seven palpigrade terminals in a single amplicon using primers LCO—HCO, showing no length variation (654 bp analysed), plus one was available in GenBank. Five outgroup sequences were obtained from GenBank, but these were 3 bp longer in all cases except for the pseudoscorpion. It was analysed as a single fragment; not pre-aligned due to the length difference with some outgroups.

Phylogenetic analyses

Parsimony analyses were based on a direct optimization (DO) approach (Wheeler 1996) using POY v. 5 (Varón *et al.* 2012). Tree searches were performed using the timed search function in POY, i.e., multiple cycles of (a) building Wagner trees, (b) subtree pruning and regrafting (SPR), and (c) tree bisection and reconnection (TBR), (d) ratcheting (Nixon 1999), and (e) tree-fusing (Goloboff 1999, 2002) [command: `search (max_time:00:01:00, min_time:00:00:10, hits:20, memory:gb:2)`]. For the individual partitions, timed searches of 1 hour were run on 4 processors under six parameter sets, as in Giribet et al. (2012) (see Table 3). For the combined analysis of the three markers we started with the same search strategy, and the resulting trees were given as input for a second round of analyses (sensitivity analysis tree fusing; SATF), as described by Giribet (2007), and continued until the tree lengths stabilised (Giribet *et al.* 2012). The optimal parameter set was estimated using the modified the $_{W}$ILD metrics (Wheeler 1995; Sharma *et al.* 2011), as a proxy for the parameter set that minimizes overall incongruence among data partitions (Table 4). Nodal support for the optimal parameter set was estimated via jackknifing (250 replicates) with a probability of deletion of e^{-1} (Farris *et al.* 1996) using `auto_sequence_partition`, as discussed in earlier work (Giribet *et al.* 2012).

Maximum likelihood (ML) analyses were conducted on static multiple sequence alignments (MSA) inferred in MUSCLE v. 3.6 (Edgar 2004). We also used an implied alignment (IA) generated in POY (Wheeler 2003; Giribet 2005) for subsequent analyses based on static alignments, as recently explored by Giribet and Edgecombe (in press) for a centipede data set. The MUSCLE alignments were conducted for each gene independently, as well as for each of the

three amplicons of 28S rRNA. The IA and MSA therefore were based on the same data (see length for each gene in Table 5). In order to evaluate the impact of the hypervariable regions in the data set, MSAs and IAs were subsequently trimmed with Gblocks v. 0.91b (Castresana 2000; Talavera and Castresana 2007) to cull positions of ambiguous homology (see length for each trimmed gene in Table 5). These data sets are thus based on different data from their original sources and from each other, but the remaining data use the same homology scheme as the source. Data sets were concatenated with SequenceMatrix (Vaidya *et al.* 2011).

In total we analysed five data sets accounting for different optimality criteria, homology schemes, and/or amount of data, as follows:

- Analysis 1. Direct optimization/dynamic homology under parsimony (full sensitivity analysis of 6 parameter sets) analysed in POY
- Analysis 2. Static homology from the implied alignment for the optimal parameter set under ML (analysed in RaxML)
- Analysis 3. Static homology from the implied alignment for the optimal parameter set trimmed with Gblocks under ML (analysed in RaxML)
- Analysis 4. Static homology based on MUSCLE multiple sequence alignment (analysed in RaxML)
- Analysis 5. Static homology based on MUSCLE/Gblocks (analysed in RaxML)

ML analyses were conducted using RAxML ver. 7.2.7 (Stamatakis *et al.* 2008b) in CIPRES (Miller *et al.* 2010). For the maximum likelihood searches, a unique General Time Reversible (GTR) model of sequence evolution with corrections for a discrete gamma distribution (GTR + Γ) was specified for each data partition (the three 28S rRNA amplicons were treated as a single partition), and 100 independent searches were conducted. Nodal support was estimated via the rapid bootstrap algorithm (1000 replicates) using the GTR-CAT model (Stamatakis *et al.* 2008a). Bootstrap resampling frequencies were thereafter mapped onto the optimal tree from the independent searches.

Results and Discussion

All phylogenetic analyses yielded very similar results with respect to the ingroup relationships—although the outgroups relationships were incongruent from analysis to analysis and unsupported for the most part, something expected given the small amount of data and outgroup taxa and the poor resolution in deep arachnid relationships in other studies (e.g., Wheeler and Hayashi 1998; Giribet *et al.* 2002; Pepato *et al.* 2010; Regier *et al.* 2010). The optimal parameter set under parsimony direct optimization was *121* (where indels cost 2, transversions cost 2 and transitions cost 1; $_{W}$ILD = 0.00703), with a cost of 8683 weighted steps (Fig. 2). Nearly all examined parameter sets concurred on the topology of the optimal parameter set. Likewise, the analyses of the four data sets analysed under maximum likelihood were identical, with the exception of some of the shallowest relationships. One of these trees, the one for the multiple sequence alignment trimmed with Gblocks—the one that could be potentially the most different from the POY analysis—is presented in Fig. 3, and it is virtually identical to the direct optimization trees. From the 12 nodes depicted in Figure 2 summarizing the six direct optimization and the four maximum likelihood analyses, 10 were recovered in all analyses. Support values for all these nodes is near optimal for most analyses (jackknife values are lower by definition). Basically, all analyses concur on the overall topology of the palpigrade tree, with a few minor incongruences in the position of GenBank sample "Palpigradi sp. JA2011" and on the internal resolution of the clade including *Eukoenenia ferratilis* and the Western Australian *Eukoenenia.*

Our analyses show another case of high congruence between alternative methods (parsimony and maximum likelihood) based on identical raw data with different homology schemes (implied alignments versus multiple sequence alignments), or different data sets (trimmed implied alignments and trimmed multiple sequence alignments). There are very few cases with such consistency across weighting schemes, homology schemes, and methodologies, but a recent case was documented for scutigeromorph centipedes (Giribet and Edgecombe in press). In that case, the fossil record and denser sampling allowed for accurate molecular dating and analyses of diversification of lineages through time, and it was suggested that the congruence across analyses was due to constant rates of diversification through the more than 400 million years evolution of the group. We can only guess this for our palpigrades, as the fossil record is rare, and only a Pliocene specimen is known (Rowland and Sissom 1980), although the group must be much older in origin (Giribet and Edgecombe 2013).

Phylogenetic analysis of the three molecular markers combined and for all analyses performed resolves into Prokoeneniidae (although represented by a single species) and Eukoeneniidae, supporting the monophyly of Eukoeneniidae—palpigrades without sternal opisthosomal vesicles (Condé 1996). We were, however, unable to obtain samples of *Triadokoenenia* or of additional *Prokoenenia* species, thus not being able to test the taxon Prokoeneniidae. Within Eukoeneniidae, three main clades are supported in all analyses, one including *Eukoenenia spelaea* and four specimens of *Eukoenenia* from Texas (USA) and the Mexican states of Guerrero and Yucatán. The Yucatán and Texan specimens show nearly identical COI sequences and identical nuclear ribosomal RNA sequences, suggesting that they may be conspecific (see Edgecombe and Giribet 2008; Vélez *et al.* 2012). Another clade includes the Western Australian samples and *Eukoenenia ferratilis* from the Iron caves of Minas Gerais (Brazil). Difficulties in amplifying the Australian samples and the lack of COI information for any of the members of the clade precludes us from understanding genetic variability within this clade of geographically distant species (both between the continents, but also among the Western Australian localities), and the more conservative nuclear genes fail to consistently resolve this clade of six individuals; in some cases there is reciprocal monophyly of the two geographic regions, but in general the Brazilian samples appear nested within the Australian samples, although without significant support. Fresh samples are difficult to obtain in Western Australia using current sampling methods, but the importance of this subterranean fauna (Guzik *et al.* 2010) and the high incidence of short-range endemism in this region of the world (Harvey 2002b; Harvey *et al.* 2011) stresses the importance of additional faunistic and taxonomic work in the region. Further Australian samples are needed that yield good COI sequences before reaching any further conclusions. The third eukoeneniid clade, although with less support than the other two, includes the sample sequenced by Arabi et al. (2012) and the cosmopolitan *E. mirabilis*, including two specimens from Italy and two putative members of this species from South Africa. Two of the parameter sets examined under direct optimization, however place the GenBank sequence one node more basally, supporting the remainder of the clade as the sister group to the Australian/Brazilian clade.

Given the sampling of this study it is still too early to make any firm conclusions about palpigrade relationships. We were not able to test for the monophyly of Prokoeneniidae, and monophyly of *Eukoenenia* is not thoroughly tested either. Attempts to sequence *Allokoenenia* and *Leptokoenenia* were unsuccessful, and we were unable to obtain specimens of the

Palaeotropical *Koenenioides* and *Triadokoenenia*. Few studies have looked at variation among palpigrade species, but Král *et al.* (2008) investigated the karyotypes of *E. spelaea* and *E. mirabilis*, which appear in different clades in our study. However, the karyotypes of both species showed no variation, both consisting of a low number of tiny chromosomes that decrease gradually in size and a lack of morphologically differentiated sex chromosomes.

Morphologically, the characters used to differentiate *Eukoenenia* species are restricted to the number of lobules in the lateral organs, the number of setae in different body regions, among others, but the significance of these characters has not been tested phylogenetically—for example, *E. mirabilis* and *E. ferratilis* are very similar morphologically (Souza and Ferreira 2011a). with many somatic traits, considered important for taxonomy, virtually identical. However, these two species belong to different clades, reflecting that their differences in genital morphology and chaetotaxy may be better systematic characters than the ones outlined above. Our study thus provides a new framework for adding new sequences and testing the significance of these characters. However, additional samples and genera must be added before we can attempt a taxonomic revision of the higher taxa in Palpigradi.

Conclusions

Palpigrades are a poorly understood group of tiny soil arthropods, often found in caves, and have received little attention from a phylogenetic point of view. Here we were able to amass specimens from different environments (caves and soil) from Australia, Africa, Europe, South America and North America with the aim of generating a molecular phylogenetic hypothesis for the group. The difficulty in obtaining well-preserved material for molecular work is reflected in the large number of specimens that did not yield DNA of enough quality for sequencing, but we were able to propose the first phylogenetic hypothesis of the group based on molecular data to find monophyly of Eukoeneniidae and a division into three main clades, each including samples from multiple continents. Given the absence of denser sampling and proper clock calibrations, our data cannot discern whether palpigrades are a very old group that diversified prior to the breakup of Pangea, or a group of animals that disperses across large geographic distances, as suggested by some widespread species, although probably due to human introduction (Harvey *et al.* 2006). Long-range dispersal is however difficult to reconcile with the narrow ecological

conditions and the facility with which these animals desiccate once removed from their environments.

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Fig. 1. Photos.

Eukoenenia spelaea – general habitus, Ardovská Cave (Slovak Karst, Slovakia). Photo: Ľ. Kováč & V. Kóňa

Eukoenenia spelaea – detail of cheliceral dentes. Photo: Ľ. Kováč & J. Mourek

Fig. 2. Left: Optimal tree at 8683 weighted steps obtained from the direct optimization analysis under parameter set 121 of the combined analysis of the three genes. Numbers on branches indicate jackknife support values. Right: Parsimony jackknifing tree with nodes below 50% support value collapsed and Navajo rugs for the resolved nodes; Black square indicates monophyly, white square non-monophyly. Specific parameter sets or analyses indicated in the figure. Numerals indicate parameter set under parsimony direct optimization; IA (ML analysis using implied alignment under parameter set 121); IAg (Idem, Gblocked); MSA (ML analysis of the Muscle multiple sequence alignment); MSAg (Idem, Gblocked).

Fig. 3. Optimal maximum likelihood tree (-Ln*L* = -22968.240827) of the combined data set using the Muscle multiple sequence alignment trimmed with Gblocks. Numbers on nodes are the bootstrap support values for the four ML analyses; from top to bottom: Implied alignment; implied alignment trimmed with Gblocks; multiple sequence alignment; multiple sequence alignment trimmed with Gblocks.

Table 1. Palpigrade specimens, accession numbers, collecting information and amplified loci with GenBank accession numbers

IZ: Department of Invertebrate Zoology, Museum of Comparative Zoology, Cambridge; DNA: MCZ DNA collection; WAM: Western Australian Museum, Perth; MNHN: Muséum national d'histoire naturelle, Paris. A dash (-) indicates a missing amplicon

					18S rRNA			*28S rRNA*			*COI*
	MCZ No.			***a***	***b***	***c***	***a***	***b***	***c***	
Prokoenenia wheeleri	IZ-134477	DNA107078	Texas, USA	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Eukoenenia ferratilis	IZ-127609		Brazil	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	-
Eukoenenia cf. *ferratilis*	-	GenBank	Brazil	-	HM070336	HM070336	-	HM070299	-	-
Eukoenenia mirabilis	IZ-127901		Italy	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Eukoenenia mirabilis	IZ-127902		Italy	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Eukoenenia spelaea	IZ-135126	DNA106786	Slovakia	-	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	-
Eukoenenia sp.	IZ-134549	DNA107079	Texas, USA	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Eukoenenia sp.	-	DNA100456.1	S. Africa	AF207648	AF207648	AF207648	-	AF207653	-	-
Eukoenenia sp.	-	DNA100456.2	S. Africa	XXXXXX	-	XXXXXX	-	XXXXXX	-	-
Eukoenenia sp.	IZ-127598.1		Mexico	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Eukoenenia sp.	IZ-127598.2		Mexico	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Eukoenenia sp.	IZ-128499		Mexico	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX
Eukoenenia sp.	IZ-127636	WAM T81111	Australia	-	-	-	-	XXXXXX	-	-
Eukoenenia sp.	IZ-127639	WAM T116012	Australia	XXXXXX	XXXXXX	XXXXXX	-	XXXXXX	XXXXXX	-
Eukoenenia sp.	IZ-127640	WAM T111422	Australia	-	-	-	-	XXXXXX	-	-
Eukoenenia sp.	IZ-127643	??	Australia	XXXXXX	XXXXXX	XXXXXX	-	XXXXXX	XXXXXX	-
Palpigradi sp.	-	MNHN-JAA76	???	JN018286.1	JN018286.1	JN018286.1	JN018383.1	JN018383.1	JN018383.1	JN018169.1

Table 2. Outgroup sampling with GenBank accession numbers

		18S rRNA	28S rRNA	COI
Anoplodactylus portus	Pycnogonida	AY859551	AY859550	GQ912859
Limulus polyphemus	Xiphosura	U91490	AF212167	AF216203
Pandinus imperator	Scorpiones	AY210831	AY210830	AY156582
Metasiro americanus	Opiliones	DQ825542	DQ825595	DQ825645
Calocheiridius termitophilus	Pseudoscorpiones	AY859559	AY859558	EU559544
Dermacentor sp.	Acari	Z74480	AY859582	-
Eremobates sp.	Solifugae	AY859573	AY859572	-
Mastigoproctus giganteus	Uropygi	AF005446	AY859587	JN018215

Table 3. Result of the POY timed searches (search) and improvement after each round of SATF for the six explored parameter sets

	search	SATF2	SATF3	SATF4
111	5608	5599	5599	5599
121	8683	8683	8683	8683
211	6505	6504	6504	6504
221	10260	10260	10259	10259
3211	9005	9004	9001	9000
3221	11637	11634	11634	11634

Table 4. Number of weighted steps for each data partition, the combination of them (MOL) and $_{w}$ILD value

The optimal parameter set is indicated in italics

	18S	28S	COI	MOL	$_{w}$ILD
111	1016	3463	1075	5599	0.00804
121	*1493*	*5502*	*1627*	*8683*	*0.00703*
211	1125	4223	1102	6504	0.00830
221	1685	6814	1652	10259	0.01053
3211	1530	5758	1643	9000	0.00767
3221	2089	7240	2203	11634	0.00877

Table 5. Length of each data partition (28S rRNA is divided into three amplicons) and total length of alignment

IA (121) is for implied alignment under parameter set 121; IA+Gb is for implied alignment trimmed with Gblocks; Muscle is for Muscle multiple sequence alignment; Muscle+Gb is for multiple sequence alignment trimmed with Gblocks

	18S	28Sa	28Sb	28Sc	COI	TOTAL
Unaligned	1760-1805	773-852	503-543	762-819	654-657	
IA (121)	1850	1149	615	891	684	5189
IA+Gb	1698	449	471	716	606	3940
Muscle	1817	998	566	858	657	4896
Muscle+Gb	1740	641	490	751	624	4246