biology letters
Evolutionary biology

Biol. Lett.
doi:10.1098/rsbl.2012.0774
Published online

Evidence for Permo-Triassic colonization of the deep sea by isopods

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The deep sea is one of the largest ecosystems on Earth and is home to a highly diverse fauna, with polychaetes, molluscs and peracarid crustaceans as dominant groups. A number of studies have proposed that this fauna did not survive the anoxic events that occurred during the Mesozoic Era. Accordingly, the modern fauna is thought to be relatively young, perhaps having colonized the deep sea after the Eocene/Oligocene boundary. To test this hypothesis, we performed phylogenetic analyses of nuclear ribosomal *18S* and *28S* and mitochondrial cytochrome oxidase I and *16S* sequences from isopod crustaceans. Using a molecular clock calibrated with multiple isopod fossils, we estimated the timing of deep-sea colonization events by isopods. Our results show that some groups have an ancient origin in the deep sea, with the earliest estimated dates spanning 232–314 Myr ago. Therefore, anoxic events at the Permian–Triassic boundary and during the Mesozoic did not cause the extinction of all the deep-sea fauna; some species may have gone extinct while others survived and proliferated. The monophyly of the 'munnopsid radiation' within the isopods suggests that the ancestors of this group evolved in the deep sea and did not move to shallow-water refugia during anoxic events.

Keywords: isopoda; deep sea; ancient colonization; anoxic events; molecular clock

1. INTRODUCTION

The deep sea, defined as the layer of the ocean below 200 m depth, is the largest ecosystem on Earth and contains a high species diversity [1,2]. Polychaetes, molluscs and peracarid crustaceans (amphipods, cumaceans, tanaids and isopods) are dominant groups in this environment [2]. There are divergent hypotheses concerning the timing of deep-sea colonization by these taxa. Some authors have proposed that anoxic events and increases in deep-sea floor temperatures from the end of the Palaeozoic to the early Cenozoic caused complete extinction of the deep-sea fauna (reviewed in McClain & Hardy [3]). For example, Jacobs & Lindberg [4] argued that 'all, or virtually all, of the deep marine habitat (during anoxic events in the mid-Mesozoic and Palaeocene) must have been uninhabitable for both normal marine invertebrate faunas and vent faunas' (p. 9400). Therefore, under the 'extinction and recolonization' hypothesis, the modern deep-sea fauna either arose from colonization by shallow-water fauna after the Palaeocene [4–8], or survived during anoxic events by moving to oxygenated shallow-water refuges [4].

Other authors propose that the ancestors of some deep-sea lineages colonized this environment during or prior to the Mesozoic, and survived the multiple anoxic events that occurred subsequently [3,5]. Rather than being a cause of extinction, anoxia may have contributed to speciation in these lineages via a reduction in gene flow across anoxic waters [3].

Isopod crustaceans provide an excellent opportunity for testing hypotheses concerning the evolutionary origins of deep-sea organisms. Many of the 119 families of Isopoda are found in the deep sea [9], including most of those in one of the largest suborders, Asellota. Recent studies using molecular sequence data identified at least four independent asellotan colonizations of the deep sea, with subsequent evolution and radiation of the families *in situ* [10,11]. On the basis of high endemic morphological diversity within Asellota, and an early phylogenetic origination of this group, it has been argued that members of this group may have colonized the deep sea prior to the Mesozoic [5]. On the other hand, other non-asellotan deep-sea taxa, which have relatively few representatives in this environment, may have colonized more recently [5].

Hypotheses concerning the timeframe of deep-sea colonization by isopods remain untested. A lack of fossil data for deep-sea isopods has hindered attempts to understand the time of their first appearance. To address this issue, we performed a phylogenetic analysis of isopods as a whole using molecular data. We then used fossil isopod taxa to calibrate molecular-clock estimates of the timeframe for deep-sea colonizations. This allowed us to address a fundamental question of deep-sea evolution: has the modern deep-sea fauna survived through anoxic events during the Mesozoic?

2. MATERIAL AND METHODS

We obtained nucleotide sequences of two nuclear (*18S* and *28S* rRNA) and two mitochondrial (cytochrome oxidase I, *COI* and *16S* rRNA) genes, comprising a combination of novel and published data (see the electronic supplementary material) for a list of taxa and accession numbers. We selected these genes because they were best represented among deep-sea isopods. *18S* sequences were available for all of the organisms included in our study, whereas other genes were available only for a subset of these (see the electronic supplementary material).

Nucleotide sequences were aligned by either eye or using a combination of MUSCLE v. 3.8.31 and GBLOCKS v. 0.91b (to remove ambiguously aligned regions) with default settings. Both of these alignment methods produced almost identical results; analyses based on MUSCLE/GBLOCKS are presented. Alignments and trees are available at TreeBase.org.

To estimate the phylogeny and divergence times, we analysed the concatenated sequence alignment using maximum-likelihood (ML) and Bayesian methods. In both cases, the dataset was partitioned into four subsets: *18S*, *16S*, *28S* and first + second codon sites of *COI*. We excluded the third codon sites of *COI* because of saturation. To examine the effects of composition heterogeneity, which can mislead phylogenetic inference and produce biases in estimates of branch lengths [12], we conducted a posterior predictive analysis in PHYLOBAYES [13]. On the basis of results of this test, we excluded eight ingroup taxa from subsequent analyses (see the electronic supplementary material). Using cross-validation analysis in PHYLOBAYES, we found that the CAT (so named because it classifies sites into categories)–general time-reversible (GTR) model provided a better fit

Electronic supplementary material is available at http://dx.doi.org/10.1098/rsbl.2012.0774 or via http://rsbl.royalsocietypublishing.org.

Received 20 August 2012
Accepted 12 September 2012

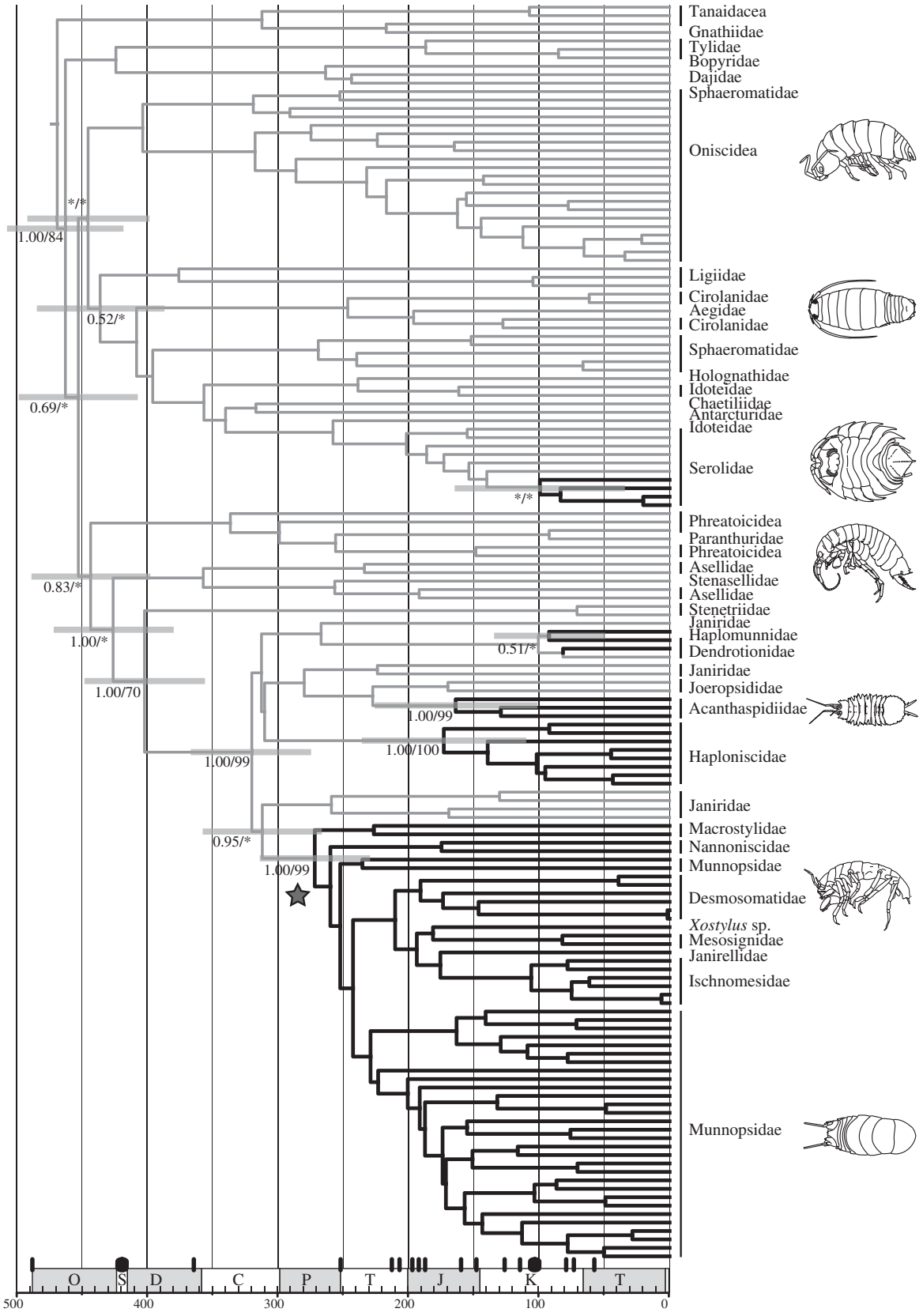

Figure 1. Bayesian phylogenetic reconstruction of isopods, showing the time of colonizations by ancestral deep-sea taxa. The tree is based on analysis of 149 species, using DNA sequences of nuclear *18S* and *28S* and mitochondrial *16S* and *COI*. Support values for deep-sea clades (thick lines) and stem nodes are given as ML bootstrap support (BS) percentages (1000 replicates) and Bayesian posterior probabilities (PP) (trees with support values for all nodes are provided in the electronic supplementary material). The tree was rooted using outgroups from the pericarid order Tanaidacea. Asterisks indicate $<50\%$ BS or <0.5 PP. Branch lengths are proportional to time and node bars denote 95% credibility intervals of the estimated node ages of interest. Black bars on the geological timescale show the major anoxic events [3]. The star indicates the clade known as the 'munnopsid radiation'. Character states on ancestral branches are based on the assumption that the common ancestor (root) inhabited shallow water. We made the conservative assumption that transitions to the deep sea occurred later rather than earlier (analogous to the DELTRAN parsimony criterion, which favours parallelisms over reversals).

to the dataset than the CAT or GTR models. The CAT–GTR model assumes a mixture of GTR matrices differing in their equilibrium base frequencies. For comparison, we also analysed the data using an ML phylogenetic approach (see the electronic supplementary material).

Estimates of divergence times were obtained using the autocorrelated lognormal-relaxed clock in PhyloBayes. For comparison, we also conducted a dating analysis using the uncorrelated lognormal-relaxed clock in the software BEAST [14] (see the electronic supplementary material). The two sets of date estimates were qualitatively similar and supported the same conclusions; the estimates from BEAST are presented only in the electronic supplementary material. The list of fossils used for calibration of the molecular clock is provided in the electronic supplementary material.

3. RESULTS AND DISCUSSION

Using a combined nuclear and mitochondrial sequence dataset, we have confirmed that isopods have colonized the deep sea on multiple occasions from shallow water [10,11,15] (figure 1). Strong support (99% likelihood bootstrap support, BS; 1.0 posterior probability, PP) was found for a large asellotan clade of deep-sea isopods known as the munnopsid radiation. Our molecular-clock analysis showed that the ancestors of this clade colonized the deep sea during the Early Permian (272 Ma, 95% credibility interval: 232–314 Myr) and diversified in the deep sea. This lineage thus survived throughout the Mesozoic and Cenozoic, when the major anoxic events are thought to have occurred and extinguished the deep-sea fauna. This result is incongruent with the 'extinction and recolonization' hypothesis, which holds that all of the deep-sea fauna became extinct during periods of anoxia and were later replaced [6–8]. Under this hypothesis, deep-sea organisms should not be older than 90 Myr, considering that subsequent anoxic events have been less severe [4]; or 57.8 Myr, the date of the Eocene/Oligocene boundary and last major anoxic event (see Wilson [5]). Another explanation for the origin of the ancient deep-sea fauna is that the lineages persisted during anoxia by taking refuge in shallow waters. This does not appear to apply to the munnopsid radiation, because its members are almost exclusively found in the deep sea [5,10].

We found strong support for parallel colonization of the deep sea by ancestors of the other asellotan families Acanthaspidiidae and Haploniscidae (99–100% BS, 1.0 PP). These colonizations were also inferred to be ancient, occurring at 164 Ma (95% CI: 101–223 Myr) and 173 Ma (95% CI: 110–234 Myr), respectively. Although these colonizations are estimated to have occurred more recently than that of the munnopsid colonization, they are nonetheless prior to key anoxia events of the late Mesozoic and early Cenozoic.

The survival of fauna through major anoxic events might have been possible if the Mesozoic deep sea contained oxygenated refuges that allowed fauna to subsist [5,6]. Such refugia, separated by anoxic areas, may have promoted speciation in the deep sea [3]. It is also possible that anoxia, in the areas where it occurred, caused the extinction of some groups and perhaps selected those able to survive at low oxygen concentration, which would then have recolonized the environment when oxygen restrictions disappeared [8,16]. Conditions of low oxygen and food supply at the end of the Permian resulted in low-diversity communities composed of small organisms such as gastropods [8,17]. Similarly, the asellotan families that evolved in the deep sea and survived the anoxic periods comprise small-bodied isopods when compared with primarily shallow-water families [5].

In contrast with the ancient groups, other lineages appear to have colonized the deep sea more recently. Members of the non-asellotan family Serolidae, the genera *Ceratoserolis* and *Cuspidocerolis* (99 Ma 95% CI: 44–172 Myr), colonized the deep sea well after the origins of the serolid shallow-water lineages 186 Ma (95% CI: 133–279 Myr), although nodal support for the grouping *Cuspidiserolis* and *Ceratoserolis* was low (<50% BS; <0.5 PP). Those genera have reduced eyes, which is a typical adaptation to deep waters. Although blind species of deep-sea serolids exist, these were not available for our study.

A molecular dating study of deep-sea echinoids found that generalist omnivore taxa have migrated to the deep sea in relatively low numbers over the last 200 Myr, with only small numbers of taxa having survived major anoxic events [18]. On the other hand, the study found several independent colonizations of the deep sea by detritivore echinoids between 75 and 55 Ma, after the last major global anoxic event at 93 Ma [18]. The authors concluded that anoxic events have played only a subsidiary role in determining diversity of deep-sea echinoids. These results are similar to ours in that deep-sea colonizations by isopods have occurred on multiple occasions over the last 210 Myr. However, the major radiation of deep-sea isopods (munnopsids) followed an ancient colonization, whereas that of echinoids (detritivores) occurred relatively recently. Our study, combined with those on echinoids and other taxa (e.g. hydrothermal vent taxa; reviewed in McClain & Hardy [3]), argues against hypotheses of a uniformly 'young' or 'old' deep-sea fauna.

Our study is, to our knowledge, the first to use a molecular-clock approach to demonstrate that the deep-sea isopod fauna has persisted since the end of the Palaeozoic Era. Our results are in conflict with the widely held notion that anoxic events and temperature fluctuations caused the extinction of most major deep-sea groups, and that recolonization from shallow water occurred after the Cretaceous Period. Multiple groups seem to show alternative patterns of deep-sea colonization, even within isopods. Date estimates for deep-sea colonizations in other groups will help us to clarify the factors that influence deep-sea biodiversity.

This research was supported by a University of Sydney International Scholarship to L.L. We thank Torben Riehl, Tim Lee and Shane Ahyong for valuable discussion, and two anonymous referees and Patrick Stewart for comments on the manuscript.

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Electronic Supplementary Materials to Lins *et al*.

Supplementary Materials and Methods

Posterior predictive analysis using PhyloBayes

To assess the extent of compositional heterogeneity in the data set, we conducted a posterior predictive analysis using PhyloBayes 3.3[1]. Initially, a phylogenetic analysis was performed with a separate CAT-GTR model for each data partition [2]. After drawing 25,000 samples from the posterior, we removed the first 2,000 samples as burn-in and drew a subsample of 20% for the posterior predictive analysis of compositional heterogeneity. Based on the results of this test, we removed eight ingroup taxa.

Phylogenetic analysis using PhyloBayes

To estimate the phylogeny, we analysed the partitioned data set using PhyloBayes. We ran separate analyses using the GTR, CAT, and CAT-GTR models of nucleotide substitution. For each model, we drew 10,000 samples from the posterior, with two replicates of each analysis. Analysis of the traces showed that the maximum discrepancy in frequencies of bipartitions was below 0.3, indicating that the samples provide a good reflection of the posterior consensus.

We used a cross-validation approach to compare the GTR, CAT, and CAT-GTR models. This analysis was performed using 10 replicates, with a learning set of 90% of the data. The CAT-GTR model was the best-fitting model for all 10 data sets, with a mean score of 11.03 (+/- 4.45) over the second-best CAT model.

Phylogenetic analysis using RAxML

Maximum-likelihood phylogenetic analysis was conducted using RAxML 7.2.8 [3]. A separate GTR+G model of nucleotide substitution was used for each data partition. Support for the estimated tree was assessed using 1000 bootstrap replicates.

Dating analysis using PhyloBayes

We estimated divergence times using the autocorrelated lognormal relaxed clock in PhyloBayes. Based on the results of the cross-validation analysis described above, we used the CAT-GTR model of nucleotide substitution. A birth-death process was used for the tree prior. We ran two replicate analyses and drew 20,000 samples from the posterior. Analysis of the traces showed that the maximum discrepancy in frequencies of bipartitions was below 0.3, indicating that the samples provide a good reflection of the posterior consensus. The first 2,000 samples were discarded as burn-in.

Dating analysis using BEAST

For comparison with the relaxed-clock estimates obtained using PhyloBayes, we conducted Bayesian phylogenetic analysis using the software BEAST 1.7.2 [4]. The best-fitting model of nucleotide substitution was chosen for each data partition using the Bayesian information criterion in Modelgenerator [5]. Rate variation among branches was modelled using an uncorrelated lognormal relaxed clock [6], with separate models for nuclear and mitochondrial markers. This clock model does not assume any correlation between rates in neighbouring branches, in contrast with the autocorrelated lognormal model in PhyloBayes. A birth-death process was used for the tree prior [7]. Fossil calibrations were implemented as uniform priors on node times, with upper limits of 499 Myr.

Posterior distributions of parameters, including the tree, were estimated via Markov chain Monte Carlo sampling. Two replicate analyses were performed, with the tree and parameter values sampled every 5×10^3 steps over a total of 10^8 steps. Acceptable sample sizes and convergence to the stationary distribution were checked using Tracer 1.5 [8].

Supplementary Table S1. Taxa and GenBank accession numbers

		Markers			
Family	**Species**	**16S**	**COI**	**28S**	**18S**
Acanthaspidiidae	*Acanthaspidia bifurcatoides*	AY691346		EU414336	AY461457
Acanthaspidiidae	*Acanthaspidia drygalskii*	AY691369		EU414338	EU414416
Acanthaspidiidae	*Ianthopsis multispinosa*	AY691342		EU414344	EU414419
Actaeciidae	*Actaecia* sp. TK 2010	GQ302701	GQ302691		GQ302703
Aegidae	*Aega antarctica*				AF255689
Alloniscidae	*Alloniscus perconvexus*				EU646199
Amphisopidae	*Paramphisopus palustris*	AF259533	EF203062		AY781425
Amphisopidae	*Eophreatoicus* sp. 04	TBA*	TBA*		TBA*
Antarcturidae	*Antarcturus spinacoronatus*	AF268206			AF279604
Anthuridae	****Cyathura carinata*	AJ388072			AF332146
Armadillidae	*Cubaris marina*				AJ287064
Armadillidiidae	*Armadillidium vulgare*	AJ388097	AF255779	AY739196	AJ267293
Armadillidiidae	*Armadillidium nasatum*	AJ388098	FN824099		AY048175
Armadillidiidae	*Armadillo officinalis*	AJ388094	FN824109		GQ302704
Asellidae	*Asellus hilgendorfii*				AB618202
Asellidae	*Lirceus fontinalis*				AF255702
Asellidae	*Proasellus coxalis*	AF532162	AY028588		AF496662
Asellidae	*Asellus aquaticus*	DQ305105	FJ749278	AY739195	AF255701
Bopyridae	*Hemiarthrus abdominalis*				AF255684
Chaetiliidae	*Glyptonotus antarcticus*	AJ269816			AF255696
Cirolanidae	****Eurydice pulcra*				AF255690
Cirolanidae	*Natatolana meridionalis*				AF255691
Cirolanidae	*Typhlocirolana moraguesi*	AF356849			AF255692
Cirolanidae	*Typhlocirolana haouzensis*	AF356848			AF453249
Cirolanidae	*Natatolana rossi*	GQ302693	GQ302696		GQ302712
Corallanidae	****Excorallana quadricornis*				AF255688
Cylisticidae	*Cylisticus convexus*	AJ388101			AJ287059
Cymothoidae	*Cymodoce tattersalli*				AF255695
Dajidae	*Zonophryxus quinquedens*				DQ008451
Dendrotionidae	*Acanthomunna spinipes*			EU414346	EU414421
Dendrotionidae	*Dendromunna* sp. 2 MR 2008			EU414348	EU414422
Dendrotionidae	*Dendrotion* sp. MR 2008			EU414349	EU414423
Desmosomatidae	*Chelator* sp.				AY461460
Desmosomatidae	*Chelator* sp. JW 2004				AY461460
Desmosomatidae	*Mirabilicoxa* sp. JW 2004				AY461461
Desmosomatidae	*Eugerdella huberti*	HQ214679	HQ214678		AY461462
Desmosomatidae	*Eugerdella natator*				AY461462
Desmosomatidae	*Eugerda* sp. JW 2004				AY461463
Gnathiidae	*Paragnathia formica*				AF255687
Haplomunnidae	*Thylakogaster* sp. 1 MR 2008				EU414424

Haploniscidae	*Antennuloniscus armatus*			EU414356	AY461468
Haploniscidae	*Mastigoniscus* spp.			EU414367	AY461469
Haploniscidae	*Haploniscus weddellensis*			EU414364	DQ435681
Haploniscidae	*Antennuloniscus* spp.	AY693397		EU414357	EU414426
Haploniscidae	*Chaulidoniscus* spp.			EU414358	EU414427
Haploniscidae	*Haploniscus rostratus*		JF283475	EU414363	EU414429
Haploniscidae	*Hydroniscus* sp. 1 MR 2008				EU414433
Haploniscidae	*Mastigoniscus polygomphios*				EU414434
Hologнathidae	*Cleantis prismatica*				AF255697
Hypsimetopidae	*Andhracoides shabbudin*				TBA*
Idoteidae	*Idotea baltica*		AF241932		AF279603
Idoteidae	*Idotea chelipes*	GQ302689	GQ302695		GQ302710
Idoteidae	*Synidotea* sp. TK 2010	GQ302692	GQ302700		GQ302715
incertae sedis	*Xostylus* sp. MR 2008			EU414413	EU414471
Ischnomesidae	*Stylomesus* spp.			EU414370	AY461471
Ischnomesidae	*Ischnomesus* sp. JW 2004				AY461472
Ischnomesidae	*Haplomesus* sp. 1			EU414368	AY461473
Ischnomesidae	*Haplomesus* sp. 2			EU414369	AY461474
Ischnomesidae	*Ischnomesus* sp. 2 MR 2008				EU414435
Ischnomesidae	*Stylomesus* sp. 2 MR 2008			EU414371	EU414436
Janirellidae	*Janirella* sp. JW 2004				AY461475
Janiridae	*Janira maculosa*				AF255700
Janiridae	*Iathrippa trilobatus*				AF279606
Janiridae	*Neojaera antarctica*			EU414374	AY461454
Janiridae	*Ianiropsis epilittoralis*		EF682303		EF682260
Janiridae	*Iais pubescens*				EU414437
Janiridae	*Iathrippa sarsi*			EU414373	EU414438
Janiridae	*Neojaera* sp. 1 MR 2008			EU414375	EU414439
Joeropsididae	*Joeropsis coralicola*				AF279608
Joeropsididae	*Joeropsis antarctica*			EU414377	EU414441
Ligiidae	*Ligidium hypnorum*	DQ182965	DQ182812		AJ287056
Ligiidae	*Ligidium germanicum*	DQ182979	DQ182798		AY048179
Ligiidae	*Ligia italica*	DQ183056	DQ182861		GQ302705
Macrostylidae	*Macrostylis* sp. 2 JW 2004				AY461477
Macrostylidae	*Macrostylis* sp. 3 MR 2008			EU414379	EU414442
Mesosignidae	*Mesosignum weddellensis*				EU414443
Mesosignidae	*Mesosignum* sp. 2 MR 2008			EU414381	EU414444
Munnopsidae	*Storthyngurella triplospinosa*				AY461482
Munnopsidae	*Acanthamunnopsis milleri*		EF682261		EF682219
Munnopsidae	*Acanthamunnopsis longicornis*		EF682265		EF682220
Munnopsidae	*Munnopsis abyssalis*		EF682273		EF682222
Munnopsidae	*Munnopsoides* sp. MB C12		EF682271		EF682224
Munnopsidae	*Munnopsis* sp. 3 C18				EF682225
Munnopsidae	*Paramunnopsis* sp. 1		EF682267		EF682227
Munnopsidae	*Paramunnopsis* sp. 2 D6		EF682270		EF682229
Munnopsidae	*Munneurycope murrayi*		EF682275		EF682232

Munnopsidae	*Acanthocope galathea*		EF682285		EF682241
Munnopsidae	*Syneurycope heezeni*		EF682295		EF682242
Munnopsidae	*Ilyarachna triangulata*				EF682244
Munnopsidae	*Ilyarachna antarctica*		EF682299	EU414400	EF682245
Munnopsidae	*Betamorpha fusiformis*	EF116541	EF682291		EF682247
Munnopsidae	*Betamorpha africana*		EF682292		EF682248
Munnopsidae	*Notopais* spp.			EU414406	EF682249
Munnopsidae	*Dubinectes acutitelson*		EF682294		EF682251
Munnopsidae	*Tytthocope* sp. 3 G8		EF682290		EF682252
Munnopsidae	*Paropsurus giganteus*		EF682287		EF682253
Munnopsidae	*Eurycope glabra*		EF682280		EF682255
Munnopsidae	*Eurycope complanata*		EF682281		EF682256
Munnopsidae	*Coperonus* sp. 1				EF682259
Munnopsidae	*Disconectes antarcticus*		EF682293		EU414449
Munnopsidae	*Dubinectes nodosus*			EU414394	EU414453
Munnopsidae	*Echinozone magnifica*			EU414393	EU414454
Munnopsidae	*Mimocopelates* sp. 1 MR 2008			EU414404	EU414460
Munnopsidae	*Storthyngurella menziesi*			EU414407	EU414464
Munnopsidae	*Storthyngura kussakini*			EU414408	EU414465
Nannoniscidae	*Austroniscus* sp. 1 MR 2008			EU414411	EU414469
Nannoniscidae	*Nannoniscus* sp. 1 MR 2008			EU414412	EU414470
Oniscidae	*Oniscus asellus*	AJ388090			AF255699
Paranthuridae	***Paranthura nigropunctata*				AF279598
Paranthuridae	*Paranthura japonica*	GQ302694			GQ302713
Philosciidae	*Philoscia muscorum*	JF309310			AJ287058
Phreatoicidae	*Colubotelson thomsoni*	AF259531	AF255775		AF255703
Phreatoicopsidae	*Phreatoicopsis raffae*	GQ302688	GQ302698		GQ302714
Platyarthridae	*Platyarthrus schoebliand*				AJ287060
Platyarthridae	*Trichorhina tomentosa*	JF309314			AY048186
Porcellionidae	*Porcellio scaber*	DQ305104	DQ305142		AJ287062
Porcellionidae	*Porcellionides pruinosus*	AJ300578	FN824139		AY048181
Porcellionidae	*Porcellionides sexfasciatus*				AY048182
Porcellionidae	*Porcellio spinicornis*				AY048183
Porcellionidae	*Trichoniscus provisorius*		DQ889123		AY048185
Serolidae	***Cristaserolis gaudichaudii*	AJ269813			AJ269828
Serolidae	*Ceratoserolis meridionalis*	AJ269800			AJ269825
Serolidae	*Ceratoserolis trilobitoides*	AJ269799	EU597422		AJ269824
Serolidae	*Serolis paradoxa*	AJ269811			AJ269827
Serolidae	*Paraserolis polita*	AJ269808			AJ269823
Serolidae	*Septemserolis* spp.	AJ269806	EU597357		AJ269821
Serolidae	*Frontoserolis waegelei*	AJ269807			AJ269822
Serolidae	*Serolella bouvieri*	AJ269804			AJ269820
Serolidae	*Acutiserolis bromleyana*	AJ269805			AJ269818
Serolidae	*Acutiserolis luethjei*	AJ269802			AJ269819
Serolidae	*Acutiserolis johnstoni*	AJ269803			AJ269817
Sphaeromatidae	*Cassidinidea* sp.				AF255693

Trachelipodidae	*Trachelipus kytherensis*	EF027528	EF027453	GQ302716
Trichoniscidae	*Hyloniscus riparius*			AJ287065
Trichoniscidae	*Haplophthalmus danicus*			AJ287066
Trichoniscidae	*Trichoniscus pusillus*	AJ388088		AJ287067
Tylidae	*Tylos europaeus*	GU097630	GU097622	EU646200
Tylidae	*Tylos ponticus*	GQ302686	GQ302699	GQ302707
Tylidae	*Helleria brevicornis*	GQ302690	GQ302702	GQ302709
Outgroup	*Parapseudes algicola*			AB618183
Outgroup	*Parapseudes arenamans*			AB618184
Outgroup	*Leptochelia itoi*			AB618197

*These sequences were oЬtained using the primers and PCR conditions described in Mattem and Schlegel (2001; Mol. Phylogenet. Evol, 18: 54-65). Sequencing was performed Ьy Macrogen (Korea). The sequences have been deposited in GenBank, with accession numbers to Ье provided upon puЬlication

** Taxa removed of the analysis after the base composition test (posterior probability on PhyloBayes).

Supplementary Table S2. Isopod fossils used to calibrate the Bayesian molecular-clock analyses.

Taxa	MY	Reference	Calibration point
Brunnaega roperi	150.8–155.7	Polz [9]	Cirolanidae
Protamphisopus baii	237-245	Fu *et al*. [10]	Amphisopidae
Hesslerella shermani	307	Schram [11]	Phreatoicidea
Joeropsididae	93.5–99.6	N.Morel, *pers.comm*	Joeropsididae
Rehbachiella	499	Walossek [12]; Harvey *et al*. [13]	Root

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Supplementary Captions

Supplementary Figure S1. PhyloBayes Bayesian phylogenetic reconstruction of isopods based on DNA sequences of nuclear *18S* and *28S* and mitochondrial *16S* and *COI*. Nodes are labelled with posterior probabilities.

Supplementary Figure S2. Maximum-likelihood phylogenetic reconstruction of isopods based on DNA sequences of nuclear *18S* and *28S* and mitochondrial *16S* and *COI*. Branch lengths are proportional to substitutions per site and nodes are labelled with bootstrap support values, estimated using 1000 bootstrap replicates.

Supplementary Figure S3. Beast Bayesian phylogenetic reconstruction of isopods based on DNA sequences of nuclear *18S* and *28S* and mitochondrial *16S* and *COI.* Nodes are labelled with posterior probabilities and branches are proportional to time. The grey bars on the nodes represent the confidence interval of the dates