Zoologica Scripta

KUNGL. VETENSKAPS-AKADEMIEN
THE ROYAL SWEDISH ACADEMY OF SCIENCES

Phylogenetic position, systematic status, and divergence time of the Procarididea (Crustacea: Decapoda)

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Submitted: 10 June 2009
Accepted: 1 September 2009
doi:10.1111/j.1463-6409.2009.00410.x

Bracken, H. D., De Grave, S., Toon, A., Felder, D. L. & Crandall, K. A. (2010). Phylogenetic position, systematic status, and divergence time of the Procarididea (Crustacea: Decapoda).—*Zoologica Scripta*, *39*, 198–212.

Ever since discovery of the anchialine shrimp, *Procaris ascensionis* Chace & Manning 1972, there has been debate as to its systematic position in relationship to other shrimp-like decapods. Several morphological characters have suggested a close affinity among Procarididae, Dendrobranchiata and Stenopodidea, whereas other physical features unite Procarididae with Caridea. Few molecular studies have examined the phylogenetic position of procaridid shrimp due to limited available material for genetic analyses. Those studies show procaridids as sister to carideans but lack sufficient taxon and locus sampling to validate the relationship. Here, we present a molecular phylogeny of selected individuals across decapod infraorders and superfamilies to clarify the phylogenetic position of procaridid shrimp. One mitochondrial (16S) and three nuclear genes (18S, 28S, H3) have been chosen to elucidate relationships. We used Bayesian molecular dating methods implemented in multidivtime to estimate and compare the divergence times among procaridids and other lineages. Findings secure the placement of the procaridids as a sister clade to carideans. Results provide evidence for the recognition of procaridids as a separate infraorder (Procarididea Felgenhauer & Abele 1983) within the Decapoda on the basis of molecular and morphological data.

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Introduction

The shrimp family Procarididae was established in 1972 to accommodate the newly discovered *Procaris ascensionis* from anchialine pools of Ascension Island (Chace & Manning 1972). Since then, four additional species of this enigmatic genus have been described, two from the Atlantic region, one from the Central Pacific, and one from the Indian Ocean (Holthuis 1973; Hart & Manning 1986; von Sternberg & Schotte 2004; Bruce & Davie 2006), all from similar anchialine environments. A conserved morphology is demonstrated among all members of *Procaris*, attributed by Hart & Manning (1986) to habitat stability and consequent lack of selective pressure. This was disputed by von Sternberg & Schotte (2004) as several other co-occurring taxa, some within the Caridea, exhibit considerable variation and conspicuous species-level synapomorphies. A related genus, *Vetericaris*, was described by Kensley & Williams (1986) from an anchialine habitat in Hawaii and included in the Procarididae, although differences between its morphology and that of *Procaris* could justify a different familial assignment.

The systematic placement of the Procarididae in relation to other decapod taxa has been debated since their discovery. Chace & Manning (1972) highlighted several features in common with Dendrobranchiata and Stenopodidea, notably the seven-articled third maxilliped, the sub-terminally attached pleurobranchs, and the large mastigobranchs extending into the branchial chamber.

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Opposing this is a suite of characters linking this group to the Caridea, such as the overlapping second pleuron, the form of the telson and uropods, and the phyllobranchiate gills (see Bauer 2004; Fransen & De Grave 2009). Notwithstanding these somewhat transitional character states, Chace & Manning (1972) placed the Procarididae in a separate superfamily (Procaridoidea) within the infraorder Caridea. An alternative viewpoint was offered by Felgenhauer & Abele (1983) who, on the basis of a comparative morphological dataset, recognized four major taxa within the shrimp-like Decapoda: Dendrobranchiata, Stenopodidea, Caridea, and Procarididea. Since then, more evidence has come to light, suggesting a close affinity between Procarididae and Caridea; including the discovery of an egg-bearing female (Felgenhauer *et al.* 1988), as well as the presence of appendix internae in *Vetericaris* (see Kensley & Williams 1986). Nevertheless, Felgenhauer & Abele (1985) did point out the close similarity in foregut morphology between *Procaris* and Dendrobranchiata, but attributed this to a retained ancestral character state rather than a close phylogenetic relationship. Felgenhauer & Abele (1985) also highlighted a unique synapomorphy of Procarididae, notably the placement of the phyllobranchiate gills on the body. These are attached very low on the body wall and extend in only one direction, as opposed to being attached much higher and extending both vertically and horizontally (along two axes), as in all Caridea so far investigated. Abele (1991) retained the name Caridea for the taxon uniting both carideans and procaridids, and coined Eucaridea for the remaining caridean taxa (excluding procaridids). This suggestion was not accepted by later authors, and the conservative viewpoint of including the Procarididae as a superfamily within the Caridea has been followed since then in all major treatments of caridean classification (Chace 1992; Holthuis 1993; Martin & Davis 2001). However, it should be noted that Schram (1986) and Christoffersen (1988) treat the procaridids as a separate infraorder within decapod crustaceans.

In recent years, there has been considerable focus on the phylogeny of Decapoda, using both morphological (Burkenroad 1963, 1981; Abele & Felgenhauer 1986; Scholtz & Richter 1995; Dixon *et al.* 2003) and molecular methods (Crandall *et al.* 2000; Ahyong & O'Meally 2004; Porter *et al.* 2005; Tsang *et al.* 2008b; Bracken *et al.* 2009a; Toon *et al.* 2009). However, the position of the Procarididae in these studies has been somewhat neglected, because of the limited availability of this taxon in museum collections and the lack of material suitable for genetic analyses. Dixon *et al.* (2003) did include *P. ascensionis* in their morphological analysis, but did not discuss its relationship to other Caridea. Kim & Abele (1990) were the first to examine the phylogenetic position of the Procarididae using genetic data (18S), suggesting a sister relationship with the Caridea; however, the 18S sequence for *Procaris* was incomplete (multiple stretches of missing characters, GenBank no. M34358) and the study lacked robust representation of caridean families (n = 2/36) and genes (n = 1). Bracken *et al.* (2009b) included *P. mexicana* in their molecular analysis of caridean families and indicated some support for infraordinal status of the taxon. However, they concluded a firmer decision must await the inclusion of more genes and a broader representation of decapod infraorders. Here, we present a molecular phylogeny of selected individuals that broadly represent decapod infraorders and superfamilies to examine the phylogenetic position of procaridid shrimp using one mitochondrial (16S) and three nuclear genes (18S, 28S, H3). Bayesian molecular dating methods were used to estimate and compare the divergence times among procaridids and other lineages. We include freshly collected material of the type species, *P. ascensionis*, and the Mexican representative, *P. mexicana*.

Materials and methods

Taxon selection

We selected 53 decapod species across the dendrobranchiate superfamilies Penaeoidea and Sergestoidea, and pleocyemate infraorders Stenopodidea, Caridea, Achelata, Astacidea, Anomura, Brachyura, Polychelida, Procarididea, Axiidea, and Gebiidea for the analysis (Table 1). Listings of these infraorders conform to conclusions of recent molecular studies that find palinurids (Scholtz & Richter 1995; Dixon *et al.* 2003; Ahyong & O' Meally 2004) and thalassinideans (Tsang *et al.* 2008a,b; Robles *et al.* 2009) to be para- or polyphyletic. The infraorders Axiidea and Gebiidea are recognized in place of Thalassinidea (Robles *et al.* 2009). We treat the procaridids as an infraordinal level taxon, as proposed by previous studies (Felgenhauer & Abele 1983; Schram 1986), and explore how this taxonomic designation reflects evolutionary relationships and divergence time, relative to other decapod infraorders.

Since we are interested in the position of procaridids among decapod infraorders, we included two species (three individuals) of the genus *Procaris*. The infraorder Caridea was also sampled more extensively than others (9 families, 18 species), since procaridids have traditionally been included within this group. One to five genera for each of the other infraorders/superfamilies (Penaeoidea, Stenopodidea, Achelata, Anomura, Brachyura, Astacidea, Polychelida, Sergestoidea, Axiidea, Gebiidea) were chosen as representatives. New sequences are indicated by accession numbers in bold, and the remaining sequences were obtained from GenBank. A majority of the GenBank sequences (EU, DQ, FJ, Table 1) in this study resulted

Table 1 . Taxonomy, voucher catalogue numbers & GenBank accession numbers for gene sequences used in study.

Taxon	Voucher	GenBank nos. 16S	GenBank nos. 18S	GenBank nos. 28S	GenBank nos. H3
Outgroup taxa					
Euphausiacea Dana, 1852					
Euphausiidae Dana, 1852					
Euphausia eximia Hansen, 1911	KCeuex	DQ079713	DQ79748	DQ079787	DQ079674
Nematoscelis sp.	KCnesp	DQ079725	DQ79760	DQ079801	DQ079690
Hoplocarida Calman, 1904					
Stomatopoda Latreille, 1817					
Squillidae Latreille, 1802					
Lysiosquillina maculata (Fabricius, 1793)	KC3832	EU920935	EU920967	EU920998	EU921076
Ingroup taxa					
Decapoda Latreille, 1802					
Dendrobranchiata Bate, 1888					
Penaeoidea Rafinesque, 1815					
Aristeidae Wood-Mason, 1891					
Aristeomorpha foliacea (Risso, 1827)	KC4280	**GQ487491**	**GQ487500**	**GQ487508**	**GQ487517**
Penaeidae Rafinesque, 1815					
Farfantepenaeus duorarum (Burkenroad, 1939)	KC4282	FJ943438	FJ943445	FJ943451	FJ943459
Penaeus semisulcatus De Hann, 1844	KC1269	DQ079731	DQ079766	DQ079809	DQ079698
Sicyoniidae Ortmann, 1898					
Sicyonia ingentis (Burkenroad, 1938)	KC4279	**GQ487492**	**GQ487501**	N/A	GQ487518
Sergestoidea Dana, 1852					
Sergestidae Dana, 1852					
Sergia sp.	ULLZ8089/KC4548	EU868710	EU868807	**GQ487509**	**GQ487519**
Pleocyemata Burkenroad, 1963					
Stenopodidea Claus, 1872					
Stenopodidae Claus, 1872					
Stenopus hispidus (Olivier, 1811)	KC4276	FJ943437	FJ943443	FJ943450	FJ943457
Spongicolidae Schram, 1986					
Microprosthema inornatum Manning & Chace, 1990	KC4278	**GQ487493**	FJ943444	FJ943452	FJ943458
Procarididea Felgenhauer & Abele, 1983					
Procarididae Chace & Manning, 1972					
Procaris ascensionis Chace & Manning 1972	KC4273	**GQ487494**	**GQ487502**	**GQ487510**	**GQ487520**
Procaris ascensionis Chace & Manning 1972	KC4274	**GQ487495**	**GQ487503**	**GQ487511**	**GQ487521**
Procaris mexicana Sternberg & Schotte, 2004	ULLZ9224	EU868715	EU868811	N/A	**GQ487522**
Caridea Dana, 1852					
Alpheidae Rafinesque, 1815					
Betaeus harrimani Rathbun, 1904	KC3103	FJ943434	FJ943440	FJ943447	FJ943454
Metabetaeus sp.	KC3109	FJ943435	FJ943441	FJ943448	FJ943455
Atyidae de Haan, 1849					
Atyoida bisulcata (Randall, 1840)	KC2138	DQ079704	DQ079738	DQ079774	DQ079661
Typhlatya pearsei Creaser, 1936	MLP85.1	DQ079735	DQ079770	DQ079813	DQ079702
Crangonidae Haworth, 1825					
Crangon crangon (Linnaeus, 1758)	KC3052	EU920915	EU920938	EU920972	EU921047
Pontophilus norvegicus (M. Sars, 1861)	KC3053	**GQ487496**	**GQ487504**	**GQ487512**	**GQ487523**
Disciadidae Rathbun, 1902					
Discias sp.	KC3108	EU920921	EU920941	EU920986	EU921054
Hippolytidae Dana, 1852					
Eualus gaimardii (H. Milne Edwards, 1837)	KC3056	EU920923	EU920940	EU920973	EU921057
Hippolyte bifidirostris Miers, 1876	KC3059	EU920927	EU920939	EU920974	EU921063
Lysmata debelius (Bruce, 1983)	MLP121	DQ079718	DQ079752	DQ079793	DQ079681
Palaemonidae Rafinesque, 1815					
Coutierella tonkinensis Sollaud, 1914	KC3068	EU920920	EU920937	EU920975	EU921053
Creaseria morleyi (Creaser, 1936)	MLP 102.1	DQ079710	DQ079746	DQ079784	DQ079671
Cryphiops caementarius (Molina, 1782)	JC1219	DQ079711	DQ079747	DQ079785	DQ079672
Macrobrachium pilimanus (De Man, 1879)	KC3110	**GQ487497**	**GQ487505**	**GQ487513**	**GQ487524**

Table 1 (Continued)

Taxon	Voucher	GenBank nos. 16S	GenBank nos. 18S	GenBank nos. 28S	GenBank nos. H3
Pandalidae Haworth, 1825					
Pandalus montagui Leach, 1814	KC3144	**GQ487498**	**GQ487506**	**GQ487514**	**GQ487525**
Processidae Ortmann, 1 890					
Nikoides danae Paulson, 1875	KC3114	FJ943436	FJ943442	FJ943449	FJ943456
Processa bermudensis (Rankin, 1900)	KC3079	**GQ487499**	**GQ487507**	**GQ487515**	**GQ487526**
Xiphocarididae Ortmann, 1895					
Xiphocaris elongata (Guérin-Méneville, 1856)	ULLZ 8882/KC3107	EU868714	EU868809	**GQ487516**	**GQ487527**
Achelata Scholtz & Richter, 1995					
Palinuridae Latreille, 1802					
Jasus edwardsii (Hutton, 1875)	KC725/KC3209	DQ079716	AF235972	DQ079791	EU921064
Palinurus elephas (Fabricius, 1787)	KC3210	EU920929	EU920959	EU920999	EU921069
Scyllaridae Latreille, 1825					
Scyllarus arctus (Linnaeus, 1758)	KC2159	DQ079732	DQ079767 EU921000	DQ079810	DQ079699
Anomura MacLeay, 1838					
Aeglidae Dana, 1852					
Aegla abtao Schmitt, 1942	KAC-Aa5/ KACaa004/ KC_Aa004	AY050067	AF439390	AY595965	DQ079658
Chirostylidae Ortmann, 1892					
Eumunida funambulus Gordon, 1930	KC3100	EU920922	EU920957	EU920984	EU921056
Galatheidae Samouelle, 1819					
Munidopsis rostrata (A. Milne-Edwards, 1880)	KC3102	EU920928	EU920961	EU920985	EU921066
Lithodidae Samouelle, 1819					
Lithodes santolla (Molina, 1792)	KClisa/KAClisa	AY595927	AF439385	AY596100	DQ079679
Pylochelidae Bate, 1888					
Pomatocheles Jeffreysii	KC3097	EU920930	EU920965	EU920983	EU921070
Astacidea Latreille, 1802					
Astacidae Latreille, 1802					
Astacus astacus (Linnaeus, 1758)	JF134	AF235983	AF235959	DQ079773	DQ079660
Cambaridae Hobbs, 1942					
Barbicambarus cornutus (Faxon, 1884)	KC1941	EU920913	EU920951	EU920993	EU921045
Nephropidae Dana, 1852					
Homarus americanus H. Milne Edwards, 1837	KChoam	HAU11238	AF235971	DQ079788	DQ079675
Parastacidae Huxley, 1879					
Astacoides betsileoensis Petit, 1923	KC1822	EU920912	EU920955	EU920992	EU921044
Euastacus robertsi Monroe, 1977	KC2781	DQ006633	EU920962	EU920988	EU921058
Brachyura Latreille, 1802					
Calappidae Milne Edwards, 1837					
Calappa gallus (Herbst, 1803)	KC3083	EU920917	EU920943	EU920976	EU921050
Cancridae Latreille, 1802					
Cancer pagurus Linnaeus, 1758	KC2158	DQ079708	DQ079743	DQ079781	DQ079668
Dorippidae MacLeay, 1838					
Ethusa sp.	KC3088	EU920925	EU920966	EU920980	EU921061
Goneplacidae MacLeay, 1838					
Carcinoplax suruguensis Rathbun, 1932	KC3087	FJ943433	FJ943439	FJ943446	FJ943453
Grapsidae MacLeay, 1838					
Cyclograpsus cinereus Dana, 1851	KC3417	EU920914	EU920945	EU920997	EU921046
Polychelida De Haan, 1841					
Polychelidae Wood-Mason, 1874					
Polycheles typhlops C. Heller, 1862	KC3101	EU920932	EU920950	EU921003-EU921004	EU921073
Axiidea Saint Laurent, 1979					
Axiidae Huxley, 1879					
Calaxius manningi Kensley *et al.*, 2000	NTOUA-0053	EF585447	EF585458	EF585469	N/A
Callianassidae Dana, 1852					
Lepidophthalmus louisianensis (Schmitt, 1935)	KC1852	DQ079717	DQ079751	DQ079792	DQ079678

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Table 1 (Continued)

Taxon	Voucher	GenBank nos. 16S	GenBank nos. 18S	GenBank nos. 28S	GenBank nos. H3
Sergio mericeae Manning & Felder, 1995	KC1865	DQ079733	DQ079768	DQ079811	DQ079700
Calocarididae Ortmann, 1891					
Calastacus crosnieri Kensley and Chan 1998	NTOUA-00212	EF585446	EF585457	EF585468	N/A
Gebiidea Saint Laurent, 1979					
Laomediidae Borradaile, 1903					
Laomedia astacina de Haan, 1841	NTOUA-00366	EF585450	EF585461	EF585472	N/A
Thalassinidae Latreille, 1831					
Thalassina anomala (Herbst, 1804)	ZRC1998-.2263	AY583896	AY583969	EF585476	N/A
Upogebiidae Borradaile, 1903					
Austinogebia narutensis (Sakai, 1896)	NTOUA-00416	EF585443	EF585454	EF585465	N/A

An 'N/A' (not available) indicates missing sequence data. New sequences are indicated in bold.

from previous work in the laboratories of one or more of the authors (Table 1).

To better resolve relationships within decapod crustaceans, we included one stomatopod (*Lysiosquillina maculata*) and two euphausiaceans (*Euphausia eximia* and *Nematoscelis* sp.) as our outgroup taxa. All outgroup sequences were obtained from GenBank (Table 1).

Gene selection

One mitochondrial (16S) and three nuclear genes (18S, 28S, H3) were selected due to their range of phylogenetic utility (Toon *et al.* 2009). The nuclear ribosomal genes 18S and 28S resolve deeper-level relationships while the nuclear protein-coding gene, H3, and mitochondrial ribosomal gene fragment, 16S, show informative resolution in family, genus, and species-level studies (Spears *et al.* 1992, 1994; Giribet *et al.* 1996; Schubart *et al.* 2000; Stillman & Reeb 2001; Tudge & Cunningham 2002; Porter *et al.* 2005; Mantelatto *et al.* 2006, 2007; Robles *et al.* 2007, 2009; Bracken *et al.* 2009a,b; Felder & Robles 2009). Since we are exploring relationships over a broad range of taxonomic levels (infraorder to species), the genes were concatenated and partitioned in the final analyses.

DNA extraction, PCR, and sequencing

Total genomic DNA was extracted from the abdomen, gills, pereopods or pleopods using the Qiagen DNeasy® Valencia, CA, USA Blood and Tissue Kit (Cat. No. 69582). Targeted gene regions were amplified by means of the polymerase chain reaction (PCR) using one or more sets of primers: 16S, large ribosomal subunit (~550 bps, Crandall & Fitzpatrick 1996); 18S, small ribosomal subunit (~1900 bps, Whiting *et al.* 1997; Apakupakul *et al.* 1999; Whiting 2002); 28S, large ribosomal subunit (~2500 bps, Whiting *et al.* 1997; Whiting 2002; Toon *et al.* 2009); H3, protein-coding gene (~330 bps, Colgan *et al.* 1998).

Reactions were performed in 25 μL volumes containing 0.5 μM forward and reverse primer for each gene, 200 μM each dNTP, PCR buffer, magnesium chloride, 1 unit HotMasterTaq polymerase (5 PRIME), and 30-100 ng extracted DNA. The thermal cycling profile conformed to the following parameters: Initial denaturation for 1 min at 94 °C followed by 30–40 cycles of 1 min at 94 °C, 1 min at 46–58 °C (depending on gene region), 1 min at 72 °C and a final extension of 10 min at 72 °C. PCR products were purified using filters (PrepEase™ PCR Purification 96-well Plate Kit, USB Corporation) and sequenced with ABI BigDye® terminator mix (Applied Biosystems, Foster City, CA, USA). An Applied Biosystems 9800 Fast Thermal Cycler (Applied Biosystems) was used in PCR and cycle sequencing reactions, and sequencing products were run (forward and reverse) on an ABI 3730xl DNA Analyser 96-capillary automated sequencer.

Phylogenetic analyses

Sequences were assembled, cleaned, and edited using the computer program Sequencher 4.7 (GeneCodes, Ann Arbor, MI, USA). Alignments were created using multiple sequence comparison by log-expectation (MUSCLE) or MAFFT, which have been found to be more accurate and faster than other alignment algorithms (Edgar 2004; Katoh *et al.* 2005). GBlocks v0.91b (Castresana 2000) was used to identify highly divergent and poorly aligned positions within 16S, 18S, and 28S datasets which were subsequently omitted from further analyses (GBlocks parameters optimized for dataset: minimum number of sequences for a conserved position (16S/18S/28S) = 27/27/27; minimum number of sequences for a flanking position (16S/18S/28S) = 44/44/41, maximum number of contiguous non-conserved positions (16S/18S/28S) = 8/8/8; minimum length of a block (16S/18S/28S) = 5/5/5; allowed gap positions = half/half/half). After highly divergent

positions were pruned, individual datasets consisted of 368 (16S), 1565 (18S), 1235 (28S), and 316 (H3) basepairs. Alignments were concatenated into a single dataset consisting of 3484 basepairs and 57 sequences.

The model of evolution that best fit the individual datasets (16S, 18S, 28S, H3) was determined by MODELTEST 3.7 (Posada & Crandall 1998). The maximum likelihood (ML) analysis was conducted using randomized axelerated maximum likelihood (RAxML) (Stamatakis *et al.* 2005, 2007, 2008) with computations performed on the computer cluster of the Cyberinfrastructure for Phylogenetic Research Project (CIPRES) at the San Diego Supercomputer Centre. Likelihood settings followed the general time reversible model (GTR) with a gamma distribution and invariable sites and RAxML estimated all free parameters following a partitioned dataset. Confidence in the resulting topology was assessed using non-parametric bootstrap estimates (Felsenstein 1985) with 1000 pseudoreplicates and values >50% are presented on the resulting phylogeny (Fig. 1).

The Bayesian (BAY) analysis was conducted in MrBayes v3.0b4 (Huelsenbeck & Ronquist 2001) on the Life Sciences Computational Cluster at Brigham Young University. Three independent BAY analyses (each consisting of four chains) were performed using parameters selected by MODELTEST. All Markov chain Monte Carlo (MCMC) algorithms ran for 20 million generations, sampling one tree every 1000 generations. To ensure that independent analyses converged on similar values, we graphically compared all likelihood parameters and scores (means and variances) using the program Tracer v1.4 (Rambaut & Drummond 2007). Observation of the likelihood (-LnL) scores in Tracer v1.4 allowed us to determine burn-ins and stationary distributions for the data. Once the values reached a plateau, a 50% majority-rule consensus tree was obtained from the remaining saved trees. Posterior probabilities (p*P*) for clades were compared for congruence and then combined between individual analyses. Values >0.5 are presented on the BAY phylogram (presented as percentages) (Fig. 1).

Fossil & time calibrations

We included 14 calibration points within our divergence time analysis across a wide variety of taxa at both shallow and deep nodes (Table 2). Thirteen of the calibration points were based on fossil data (C1–C13), while one age represented the time estimation for a vicariant event (C14). For fossil data, we chose the oldest known representative for a particular clade and then calculated the mean age for the fossil. Because we assumed the divergence time should predate the fossil occurrence, all fossil ages were set as the lower limit (minimum age) at their respective nodes. Bayesian analyses for molecular time estimation required us to set one calibration point as our upper limit (maximum age). Following previous studies (Porter *et al.* 2005; Breinholt *et al.* 2009), we set the splitting of Pangaea (185 MYA) to represent the divergence between the Northern and Southern Hemisphere crayfish superfamilies Astacoidea and Parastacoidea (Crandall & Buhay 2008).

As previously noted, the mean age was taken for most fossils and set as the lower limit, as used in previous studies (Porter *et al.* 2005; Breinholt *et al.* 2009). In some cases, we used prior knowledge to estimate dates of common ancestry and make predictions about the placement of unresolved fossils, which we discuss. The oldest penaeoid fossils were recovered from Triassic deposits in Madagascar and Europe and are known as the *Aeger* and *Antrimpos* fossils. The *Aeger* fossils, present from the Triassic to late Cretaceous, represent an extinct family Aegeridae, with three pairs of chelate pereopods, the 1st somite overlapping the second, and a petasma (Balss 1957; Burkenroad 1963). The *Antrimpos* fossils belong to a second family of penaeoid shrimp and were very similar in morphology to the present-day *Penaeus* (Burkenroad 1963). The presence of two families in the Triassic suggest the ancestral penaeoid diverged prior to the Mesozoic Era, so we chose 248 MYA to represent the most common recent ancestor of this group (taken from 1999 Geological Society of America (GSA) time scale) (Burkenroad 1963). Additional penaeoid fossils belonging to the genus *Penaeus* sensu lato (s.l.) (i.e., including *Farfantepenaeus* and *Penaeus* sensu stricto in our analysis) were first reported from the Jurassic shale and frequent throughout the Cretaceous (Glaessner 1969; Dall *et al.* 1990). We chose to place a lower limit (minimum age) of 144 MYA (taken from GSA time scale) at the end of the Jurassic for the divergence of the *Penaeus* s.l. lineage. Similar penaeoid divergence dates have been used in previous divergence time analyses (Ma *et al.* 2009).

The earliest stenopodidean fossil, *Phoenice pasinni*, was reported from the Upper Cretaceous (Cenomanian) (93.5–99 MYA). The presence of this fossil suggests the ancestor diverged prior to this period, so we assigned a lower limit of 96.3 MYA to represent the most recent common ancestor of the Stenopodidea.

The most ancient decapod fossil is *Palaeopalaemon newberryi* Whitfield 1880; recovered from the Upper Devonian approximately 360 MYA (Schram & Dixon 2004). This species has been allied with astacideans (Schram *et al.* 1978; Felgenhauer & Abele 1983; Christoffersen 1988), glypheids (Burkenroad 1983; Felgenhauer & Abele 1983), and natant groups (Felgenhauer & Abele 1983) based on morphological features it shares with these taxa. A recent

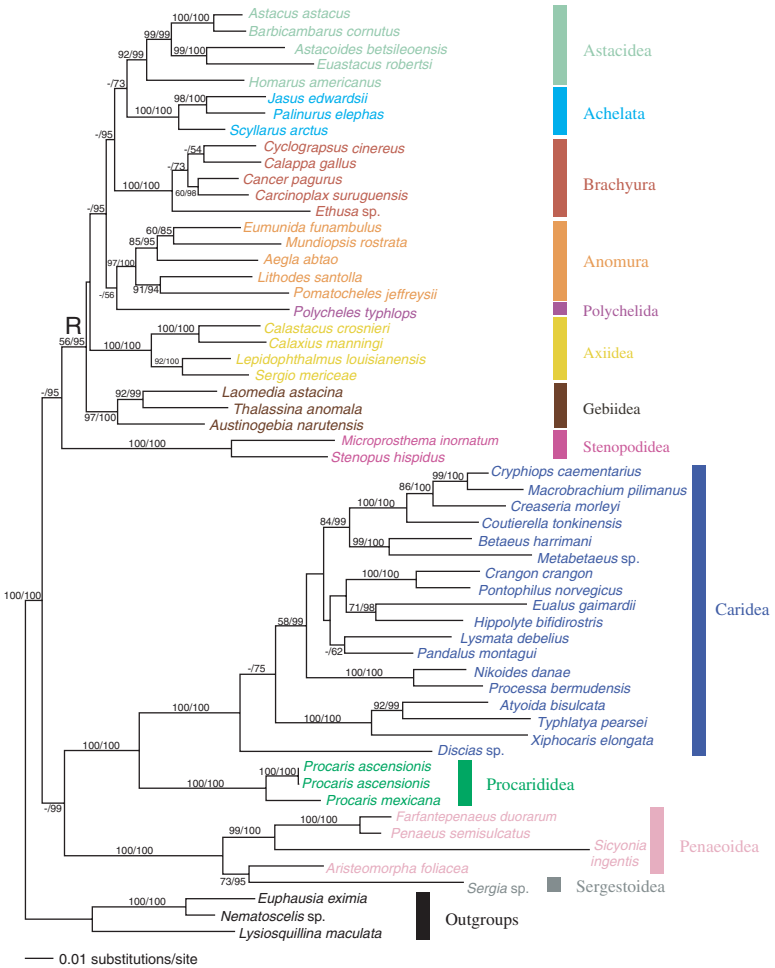

Fig. 1 Bayesian (BAY) phylogram for selected decapods (n = 54) and outgroups (n = 3) based on a 16S (mtDNA), 18S (nDNA), 28S (nDNA) and H3 (nDNA) concatenated dataset. ML bootstrap values and BAY posterior probabilities are represented as percentages and noted above or below the branches (ML/BAY). Values <50% are not shown. Vertical coloured bars indicate major infraorders/superfamilies within Decapoda. R = Reptantia.

morphological cladistic analysis placed *Palaeopalaemon* as a sister group to a clade uniting Achelata, Anomura, and Brachyura (=Eurysternalia) (Schram & Dixon 2004). Since the phylogenetic position of *P. newberryi* is uncertain, we have taken a conservative approach and used this fossil to date the Reptantia node, for primarily crawling lineages, similar to previous studies (Porter *et al.* 2005).

Divergence time estimates

We used the Bayesian molecular dating method implemented in multidivtime (Thorne *et al.* 1998; Kishino *et al.* 2001; Thorne & Kishino 2002; Thorne 2003). Multidivtime derives the posterior distribution for evolutionary rates and times by using the MCMC procedure. This Bayesian method allows us to estimate branch lengths without assuming a molecular clock and can be used on multi-locus datasets. It can accommodate missing data and multiple calibration points. Upper and/or lower limits can be assigned to nodal ages, so that the divergence estimate at a specific node is not fixed. This method has been shown to give more consistent estimates than other approaches for estimating divergence times (Perez-Losada *et al.* 2004).

Model parameters were estimated using F84 + gamma (Felsenstein 1984) in the baseml analyses (implemented in PAML (Phylogenetic Analysis by Maximum Likelihood) package). All estimations for model parameters and branch lengths were calculated separately for each gene. The prior distribution for the time separating the ingroup root from the tips (rttm) and standard deviation (rttmsd) was set to 4.37 (437 MYA), as estimated in previous studies (Porter *et al.* 2005). The prior distribution for the rate of molecular evolution at the ingroup root (rtrate) and standard deviation (rtratesd) were calculated after observing the branch lengths obtained in the estbranches program. The median of all the branch lengths was calculated (Thorne's value X) and then divided by the rttm to obtain rtrate and rtratesd of 0.04 substitutions per 100 MY. When there is little knowledge of the evolutionary rates within a group, the program authors suggest setting the rtrate equal to

Table 2 Taxonomy and calibration points used in this study.

Taxonomy	Species	Reference	Geologic age (MY A)	Node
Fossil calibrations				
Natantia				
Suborder Dendrobranchiata				
Superfamily Penaeoidea				
Family Aegeridae	*Aeger* sp.	Burkenroad (1963) Feldmann *et al.* (2007) Glaessner (1969)	Triassic – Late Cretaceous (248*) L	Cl
Family Penaeidae	*Antrimpos* sp.	Burkenroad (1936, 1963)	Early Triassic – Late Jurassic (248*) L	Cl
	Penaeus s.l.	Glaessner (1969) Dall *et al.* (1990)	Late Jurassic – Cretaceous (144*) L	C2
Suborder Pleocyemata				
Infraorder Caridea				
Family Atyidae	*Delclosia martinelli*	Rabadà (1993)	Early Cretaceous (lower Barremian) (124 – 127) L	C3
Family Palaemonidae	*Beurlenia araripensis*	Martins-Neto & Mezzalira (1991)	Early cretaceous (upper Aptian/lower Albian) (105–116) L	C4
	Palaemon antonellae	Garassino & Bravi (2003)	Early cretaceous (Albian) (99–112) L	C4
Family Crangonidae	*Morscrangon acutus*	Garassino & Jakobsen (2005)	Early Eocene (49-54.8) L	C5
Infraorder Stenopodidea				
Family Stenopodidae	*Phoenice pasinni*	Garassino (2000)	Late Cretaceous (Cenomanian) (93.5–99) L	C6
Reptantia	*Palaeopalaemon newberryi*	Whitfield (1880)	Late Devonian (Famannian) (354–364) L	C7
Infraorder Astacidea				
Family Chimaerastacidae	*Chimaerastacus pacifluvialis*	Amati *et al.* (2004)	Mid Triassic (upper Ladinian) (227–234) L	C8
Family Astacidae	*Astacus licenti*	Van Straelen (1928)	Late Jurassic (144–159) L	C9
	Astacus spinirostris	Imaizumi (1938)	Late Jurassic (144–159) L	C9
Family Parastacidae	*Palaeoechinastacus australianus*	Martin *et al.* (2008)	Early Cretaceous (106*) L	Cl0
Infraorder Anomura				
Family Aeglidae	*Protaegla miniscula*	Feldmann *et al.* (1998)	Early Cretaceous (Albian) (99–112) L	Cll
Family Chirostylidae	*Pristinaspina gelasina*	Schweitzer & Feldmann (2000a)	Cretaceous (65–144) L	C12
Infraorder Brachyura				
Family Cancridae	*Notocarcinus sulcatus*	Schweitzer & Feldmann (2000b)	Mid Eocene (41.3–49) L	C13

Time estimation: splitting of Pangaea was set at 185 MYA (U, C14) to represent the divergence of the Northern and Southern Hemisphere crayfish superfamilies Astacoidea and Parastacoidea.

The average age was taken for all fossil ages in the parenthesis and '*' represents ages used in the analysis. L, lower limit, U, upper limit.

rtratesd. Alternative values were set for rttm/rttmsd (±50–100 MY, 3.37–5.37) and rtrate/rtratesd (0.032–0.051) and the final estimations were only slightly affected (~1–3 MY). The Markov chain was sampled 1×10^4 times collected every 100th cycle with a burnin period of 10^5. When the Markov chain was sampled more extensively (5×10^4), divergence time estimates did not change much (differed by <1 MY). Default options were chosen for all other parameters. The analyses were run four times and convergence was measured by evaluating the proportion of successes (psuc) and comparing the results (nodal ages and confidence intervals) of the independent runs.

Results

Phylogenetic analyses

In total, we included 57 sequences for 16S and 18S, 56 sequences for 28S, and 52 sequences for H3 (Table 1). Missing data were designated as a '?' in the alignment. The optimal models of evolution selected in MODELTEST were the General Time Reversible (GTR) model (16S, 18S, 28S) and Transversion (TVM) model (H3) with gamma-distributed among-site rate heterogeneity and invariant sites (Table 3). Topologies derived from the ML and BAY analyses were strongly congruent, but because the BAY analysis showed better resolution at the deeper nodes (between infraorders), we present the BAY phylogram (Fig. 1).

The objective of this study was to infer the position of the procaridids in relation to other decapod crustaceans, so sampling was limited to representatives from each infraorder. Our study is not intended to test the monophyly of decapod infraorders, however, there is statistical support for nine pleocyemate infraorders (with multiple representatives): Achelata (bs = 100%, p*P* = 1.0), Astacidea (bs = 92%, p*P* = 0.99), Anomura (bs = 97%, p*P* = 1.0), Brachyura (bs = 100%, p*P* = 1.0), Stenopodidea (bs = 100%,

p*P* = 1.0), Caridea (bs = 100%, p*P* = 1.0), Procarididea (bs = 100%, p*P* = 1.0), Axiidea (bs = 100%, p*P* = 1.0), and Gebiidea (bs = 97%, p*P* = 1.0) (Fig. 1). The suborder Dendrobranchiata was supported (bs = 100%, p*P* = 1.0) but Penaeoidea was recovered as a paraphyletic clade with the inclusion of Sergestoidea. The procaridids were recovered as the sister group to the carideans with significant support (bs = 100%, p*P* = 1.0, Fig. 1). In many cases, higher-level relationships (among infraorders) were unsupported with likelihood bootstrap values, but Bayesian analyses did support Reptantia (p*P* = 0.95) among other infraordinal groupings (Fig. 1). Although BAY analyses recovered significant support for some higher-level relationships, these findings should be interpreted with caution, as many studies have shown posterior probabilities to overestimate phylogenetic support (Suzuki *et al.* 2002; Cummings *et al.* 2003; Douady *et al.* 2003), especially on short branches.

Divergence time analyses

In total, we ran 10 independent multidivtime analyses using different parameters and assumptions (see Materials and methods). For each run, the final divergence time estimates were only slightly affected (~<1–3 MY), and in our final analysis, divergence times and confidence intervals are presented for each node (Fig. 2, Table 4).

The origin of the Decapoda was placed in the Silurian (~418.56 MYA), in agreement with previous studies (Porter *et al.* 2005). This age is not unexpected since we set the prior distribution for the time separating the ingroup root from the tips at 437 MYA (rrtm). When we increased or decreased the rttm by 100 MY, a similar value was obtained (~417–418 MYA).

The Natantia, or swimming lineages (Dendrobranchiata, Procarididea, Caridea, and Stenopodidea), originated in the Devonian and represent the oldest decapod crustaceans (Fig. 2). Our results suggest the Dendrobranchiata (Penaeoidea, Sergestoidea) are an ancient group, splitting into subsequent lineages approximately 282 MYA. The procaridid and carideans represent early lineages, diverging from each other approximately 333 MYA. From the early Permian through the middle Jurassic, the carideans underwent a period of rapid radiation (~262–176 MYA), giving rise to many of the families that exist today. Our results suggest the stenopodideans split around 184 MYA into the lineages that today represent the families Spongiocolidae and Stenopodidae.

The natantian groups were followed by divergence of the crawling/walking reptant lineages, which include Axiidea, Gebiidea, Polychelida, Anomura, Brachyura, Achelata, and Astacidea. Results suggest the Reptantia originated in the Devonian (~390 MYA, Fig. 2). The radiation of the major decapod infraorders followed soon thereafter, with all groups present within the Carboniferous (290–354 MYA). Within our phylogeny, the divergence of the astacideans occurred around 280 MYA, giving rise to the crayfish (Astacoidea, Parastacoidea) and clawed lobster (Nephropoidea) clades. Following this divide, the Southern Hemisphere and Northern Hemisphere crayfish superfamilies Astacoidea and Parastacoidea radiated after the splitting of Pangaea approximately 180 MYA. Our estimates infer Anomura and Gebiidea diverged within the Carboniferous (~296, 309 MYA, respectively) while Achelata, Brachyura, and Axiidea radiated within the Jurassic (~176 MYA), Triassic (~223 MYA), and Permian (~255 MYA), respectively.

Discussion

Molecular evidence

Phylogenetic position. Few molecular studies have included procaridid shrimp and of those studies that incorporate this taxon, none have included a robust sampling of decapod infraorders and/or molecular markers (Kim & Abele 1990; Bracken *et al.* 2009b). On the basis of one mitochondrial gene, three nuclear genes, and 53 selected decapod species, our study is the first to confidently secure the placement of procaridids as the sister taxon to caridean shrimp. The infraorder Caridea was sampled exhaustively across a variety of families so that the evolutionary distance between the carideans and procaridids could be accurately portrayed in the phylogeny. The branch lengths (number of substitutions per site) uniting the carideans with the procaridids is comparable (if not longer) to the branch lengths uniting other infraorders (e.g., Astacidea

Table 3 Parameters used in BAY analysis.

Gene	Empirical base frequencies	Rate matrix	Gamma shape parameter	Proportion of invariable sites
16S	0.3906, 0.0581, 0.1519, 0.3994	1.6920, 5.4630, 1.0838, 0.5705, 11.6009,1	0.5284	0.2467
18S	0.2376, 0.2420, 0.2915, 0.2289	1.3394, 2.5424, 1.0932, 0.8185, 3.9714, 1	0.6323	0.4514
28S	0.2415, 0.2404, 0.3279, 0.1902	0.7013, 1.9876, 1.1716, 0.8877, 4.5152, 1	0.7027	0.3373
H3	0.1785, 0.2929, 0.2461, 0.2825	2.8320, 8.1229, 5.2012, 1.3928, 8.1229, 1	1.2632	0.5947

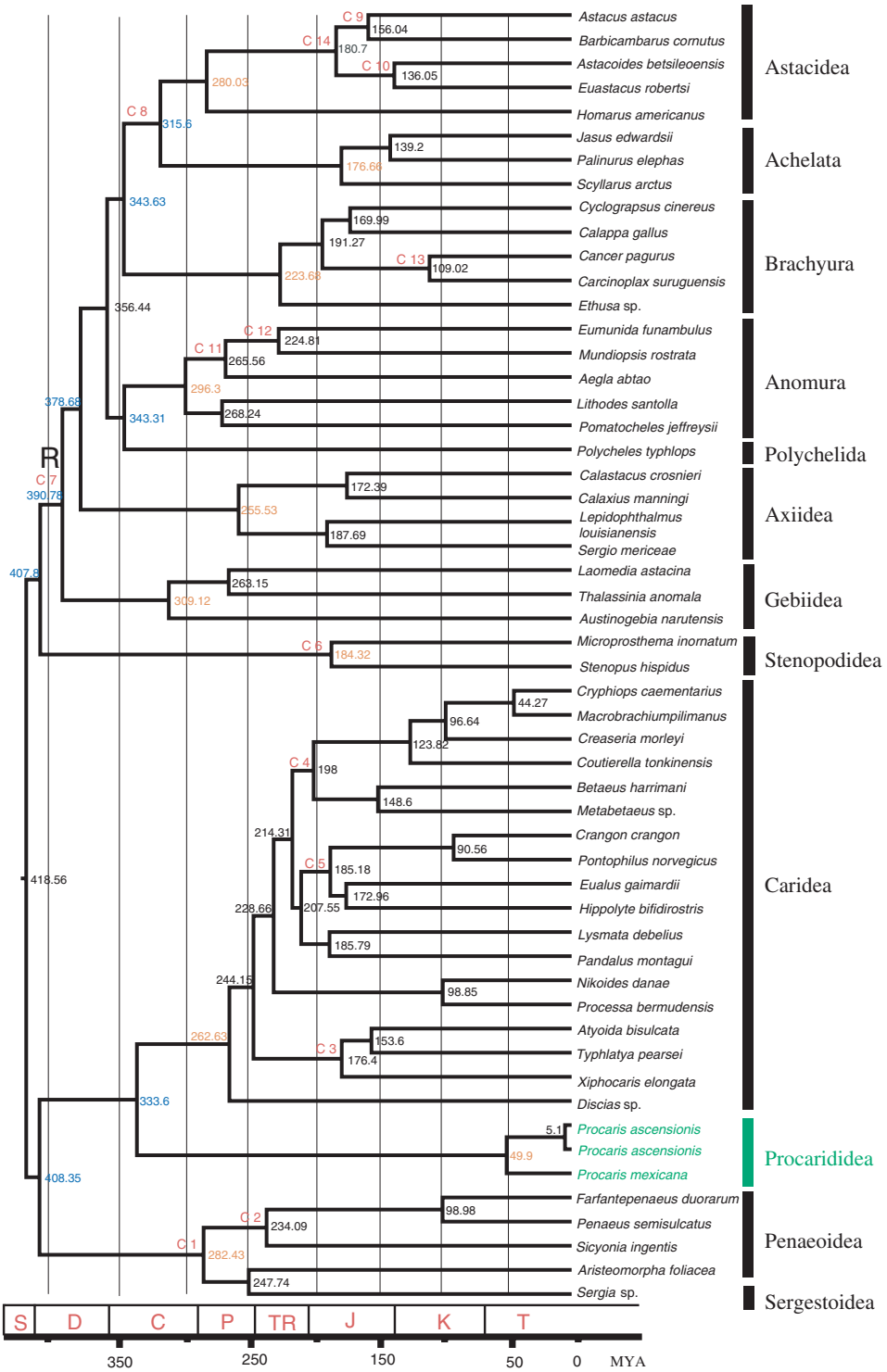

Fig. 2 Divergence time chronogram for selected decapods (n = 54) estimated using a Bayesian topology. Calibration points are indicated by C1–C14, in accordance with Table 2. Vertical bars indicate major infraorders/superfamilies within Decapoda. Divergence time estimates (MY) are noted adjacent to their respective nodes. Blues ages = divergence split times of major lineages. Orange ages = nodal ages of major clades. Geological periods are superimposed onto the phylogeny and listed as follows: S, Silurian; D, Devonian; C, Carboniferous; P, Permian; TR, Triassic; J, Jurassic; K, Cretaceous; T, Tertiary. Outgroups have been excluded from the phylogeny. R = Reptantia.

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Table 4 Divergence times and confidence intervals for all the nodes presented in the study.

Divergence times	95% Confidence intervals	Divergence times	95% Confidence intervals
5.1	0.207, 16.320	214.31	172.524, 267.814
44.27	25.020, 70.604	223.68	171.284, 286.431
49.9	15.638, 111.926	224.81	160.326, 295.191
90.56	57.624, 130.320	228.66	185.329, 284.680
96.64	62.783, 138.739	234.08	181.995, 298.066
98.85	61.757, 141.740	244.15	199.685, 302.770
98.98	51.096, 151.980	247.74	205.345, 305.711
109.02	59.942, 169.928	255.53	189.804, 330.164
123.82	87.543, 166.915	262.63	217.252, 324.222
136.05	109.687, 164.631	263.15	188.032, 344.592
139.20	85.112, 205.213	265.56	206.423, 331.898
148.60	108.703, 197.776	268.24	214.104, 332.215
153.60	112.733, 204.446	280.03	233.694, 335.111
156.04	151.148, 168.740	282.43	250.102, 341.598
169.99	119.001, 228.068	296.3	246.119, 360.728
172.39	102.500, 249.808	309.12	245.724, 383.794
172.96	130.611, 224.612	315.6	273.957, 374.262
176.40	134.332, 230.261	333.6	285.442, 403.987
176.66	120.442, 241.722	343.31	300.090, 409.701
180.7	169.708, 184.887	343.63	303.234, 406.433
184.32	125.499, 252.181	356.44	316.300, 423.106
185.18	144.188, 237.278	376.8	337.827, 445.922
185.79	144.843, 236.228	390.78	360.115, 462.470
187.69	128.767, 251.817	407.8	370.155, 484.225
191.27	140.016, 251.899	408.35	367.735, 486.030
198	155.803, 250.979	418.56	378.709, 497.065
207.55	166.091, 260.272		

vs. Achelata), consistent with treating the procaridids as an infraordinal taxon.

Divergence time analysis. The divergence time analysis suggests the caridean-procaridid lineage split approximately 333 MYA during the early Carboniferous with a confidence interval spanning between 285 MYA to 404 MYA (see Table 4). The timing of this split (333 MYA) is well within the range of divergence times for other infraordinal divergences [408 MYA (Stenopodidea-others) – 315 MYA (Astacidea-Achelata)] (Fig. 2), providing additional support for the separation of the Procarididae from the Caridea. Approximately 70 million years later, the carideans radiated into many of the present-day lineages. The divergence of the procaridid species is estimated at 50 MYA with a large confidence interval of 15–112 MYA. This is most probably due to having only two procaridid species represented in our sample and our lacking fossil calibration in this group.

Morphological evidence

Morphological support for recognizing a separate infraorder for the Procarididae is equally strong. Using an exhaustive list of morphological characters, Abele & Felgenhauer (1986) found that Procarididae share only one synapomorphy with the remaining Caridea, namely the second pleuron overlapping the first and third somite without the first one being reduced. The true phylogenetic significance of this character state remains to be investigated (and could be the result of convergence rather than ancestry) as it varies considerably within Caridea. For example, the anterior overlap is absent in *Psalidopus* and several species of *Glyphocrangon*. Although Procarididae and Caridea share phyllobranchiate gills, their differential placement casts doubt on whether this similar shape in the lamellae represents a homologous character (see Fig. 7 in Abele & Felgenhauer 1986). A similar arrangement of mastigobranchs and setobranchs for passive gill-cleaning has also been suggested as a synapomorphy to unite Caridea + Procarididae (Bauer 2004), but the present authors are not unanimous in accepting of these as equivalent arrangements. These structures do vary among carideans and are absent in several caridean families. However, it has also been argued the alteration and loss of these structures is secondary in selected caridean groups, where they have been functionally displaced by cleaning mechanisms such as cheliped brushing (Bauer 1979, 1984, 2004).

Procarididae are assumed to share a suite of characters with Dendrobranchiata (Chace & Manning 1972; Kensley & Williams 1986), including the well-developed gastric mill. The absence of a gastric mill has often been considered widespread within the Caridea, but several studies (Felgenhauer & Abele 1983, 1985, 1989) have documented the presence of a well-developed mill in several families of basally positioned carideans. Similarly, the L-shaped mastigobranch, assumed to be shared between Dendrobranchiata and Procarididae, also occurs in several basally rooted families of Caridea (De Grave, S. & Goulding, M. in preparation). We thus interpret morphological characters in Procarididae to represent a combination of ancestral states still seen in the modern Dendrobranchiata with plesiomorphic states exemplified among some modern Caridea. While an exhaustive comparison of procaridid morphology to Dendrobranchiata and Caridea is beyond the scope of the present study, a synapomorphy of the Procarididae could be the placement of the phyllobranchiate gills, as identified by Abele & Felgenhauer (1986).

Taxonomic implications and systematic status. Our results demonstrate a close phylogenetic affinity between Procarididae and the Caridea, with Procarididae being a sister group to the families of Caridea. This is effectively the same topology as inferred by Abele & Felgenhauer (1986), Christoffersen (1988), Kim & Abele (1990), Abele (1991)

and Bracken *et al.* (2009b), which were based upon a variety approaches. Abele (1991) suggested using the name Caridea for the clade uniting both of these taxa, and recognized two subclades within this: Procarididea and Eucaridea. Although not clearly stated as such, the chosen suffix (-idea) inferred that the latter two clades were to be considered infraorders, as implemented by Kensley & Williams (1986) for the classification in Abele & Felgenhauer (1986). This is at odds with the current Linnean hierarchical classification of Decapoda (Martin & Davis 2001), in which two suborders are recognized: Dendrobranchiata and Pleocyemata, the latter divided into several infraorders (see also Scholtz & Richter 1995; Ahyong & O' Meally 2004; Porter *et al.* 2005).

Our molecular results support positioning of the procaridids at the infraordinal level. The group is a strongly supported monophyletic clade separated at genetic distances comparable to those between other decapod infraorders. Divergence time estimates show chronologies also on par with the divergence times of other infraorders. Finally, a suite of morphological characters supports the infraordinal position of this taxon. Thus, we recognize infraordinal status for the family Procarididae, as Infraorder Procarididea Felgenhauer & Abele 1983; raising the number of decapod shrimp-like higher taxa to four (see Fransen & De Grave 2009): suborder Dendrobranchiata, and the infraorders Procarididea, Caridea and Stenopodidea (of the suborder Pleocyemata).

Acknowledgements

We thank the US National Science Foundation (EF-0531762 and EF-0531603) and Brigham Young University for financial support of this study. We acknowledge R. Bauer, A. Covich, S. Fredericq, E. Garcia, E. Palacios-Theil, L. M. Mejía-Ortíz, R. Robles, B. Thoma, and A. Windsor for manuscript advice and/or helping us obtain material for this analyses. SDG acknowledges the financial support of the Oxford University Fell Fund, which supported fieldwork in Ascension, under a permit granted by the Conservation Department of the Ascension Island Government. For DLF this is UL Laboratory for Crustacean Research contribution number 136.

References

Abele, L. G. (1991). Comparison of morphological and molecular phylogeny of the Decapoda. *Memoirs of the Queensland Museum*, *31*, 101–108.

Abele, L. G. & Felgenhauer, B. E. (1986). Phylogenetic and phenetic relationships among the lower Decapoda. *Journal of Crustacean Biology*, *6*, 385–400.

Ahyong, S. & O' Meally, D. (2004). Phylogeny of the Decapoda Reptantia: resolution using three molecular loci and morphology. *The Raffles Bulletin of Zoology*, *52*, 673–693.

Amati, L., Feldmann, R. M. & Zonneveld, J. P. (2004). A new family of Triassic lobsters (Decapoda: Astacidea) from British Columbia and its phylogenetic context. *Journal of Paleontology*, *78*, 150–168.

Apakupakul, K., Siddall, M. E. & Burreson, E. M. (1999). Higher level relationships of leeches (Annelida: Clitellata: Euhirudinea) based on morphology and gene sequences. *Molecular Phylogenetics and Evolution*, *12*, 350–359.

Balss, H. (1957). Decapoda. VIII. Systematik. *Klassen und Ordnungen des Tierreichs*, *5*(1), 7(12), 1505–1672, textfigs.1131–1199.

Bauer, R. (1979). Antifouling adaptations of marine shrimp (Decapoda: Caridea): gill cleaning mechanisms and grooming of brooded embryos. *Zoological Journal of the Linnean Society*, *65*(4), 281–303.

Bauer, R. (1984). Morphological trends in the genus *Heptacarpus* (Decapoda, Caridea) and their phylogenetic significance. *Journal of Crustacean Biology*, *4*(2), 201–225.

Bauer, R. (2004). *Remarkable Shrimps: Adaptations and Natural History of the Carideans*. Norman: University of Okalahoma Press.

Bracken, H., Toon, A., Felder, D. L., Martin, J. W., Finley, M., Rasmussen, J., Palero, F. & Crandall, K. A. (2009a). The decapod tree of life: compiling the data and moving toward a consensus of decapod evolution. *Arthropod Systematics and Phylogenetics*, *67*(1), 99–116.

Bracken, H. D., De Grave, S. & Felder, D. L. (2009b). Phylogeny of the infraorder Caridea based on nuclear and mitochondrial genes (Crustacea: Decapoda). In J. W. Martin, K. A. Crandall & D. L. Felder (Eds) *Decapod Crustacean Phylogenetics (Crustacean Issues 18)* (pp. 281–305). Bocan Raton: CRC Press.

Breinholt, J., Perez-Losada, M. & Crandall, K. A. (2009). The timing of the diversification of the freshwater crayfishes. In J. W. Martin, K. A. Crandall & D. L. Felder (Eds) *Decapod Crustacean Phylogenetics (Crustacean Issues)* (pp. 343–355). Bocan Raton: CRC Press.

Bruce, A. J. & Davie, P. F. J. (2006). A new anchialine shrimp of the genus Procaris from christmas island: the first occurrence of the Procarididae in the Indian Ocean (Crustacea: Decapoda: Caridea). *Zootaxa*, *1238*, 23–33.

Burkenroad, M. D. (1936). The Aristaeinae, Solenocerinae and pelagic Penaeiniae of the Bingham Oceanographic collection. *Bulletin of the Bingham Oceanographic Collection*, *5*, 1–151.

Burkenroad, M. D. (1963). The evolution of the Eucarida (Crustacea, Eumalacostraca), in relation to the fossil record. *Tulane Studies in Geology*, *2*, 1–18.

Burkenroad, M. D. (1981). The higher taxonomy and evolution of Decapoda (Crustacea). *Transactions of the San Diego Society of Natural History*, *19*, 251–268.

Burkenroad, M. D. (1983). Natural classification of the Dendrobranchiata, with a key to recent genera. In F. Schram (Ed) *Crustacean Phylogeny* (pp. 279–290). A.A. Balkema Rotterdam.

Castresana, J. (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution*, *17*, 540–552.

Chace, F. A. (1992). On the classification of the Caridea (Decapoda). *Crustaceana*, *63*, 70–80.

Chace, F. A. Jr & Manning, R. B. (1972). Two new caridean shrimps, on representing a new family, from marine pools on

Ascension Island (Crustacea: Decapoda: Natantia). *Smithsonian Contributions to Zoology*, *131*, 1–18.

Christoffersen, M. L. (1988). Phylogenetic systematics of the Eucarida (Crustacea, Malacostraca). *Revista Brasiliera de Zoologia*, *5*, 325–351.

Colgan, D. J., McLauchlan, A., Wilson, G. D. F., Livingston, S. P., Edgecombe, G. D., Macaranas, J., Cassis, G. & Gray, M.R. (1998). Histone 3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Australian Journal of Zoology*, *46*, 419–437.

Crandall, K. A. & Buhay, J. E. (2008). Global diversity of crayfish (Astacidae, Cambaridae, and Parastacidae-Decapoda) in freshwater. *Hydrobiologia*, *595*, 295–301.

Crandall, K. A. & Fitzpatrick, J. F. (1996). Crayfish molecular systematics: using a combination of procedures to estimate phylogeny. *Systematic Biology*, *45*, 1–26.

Crandall, K., A Harris, J. D. & Fetzner, J. W. (2000). The monophyletic origin of freshwater crayfish estimated from nuclear and mitochondrial DNA sequences. *Proceedings of the Royal Society of London B*, *267*, 1679–1686.

Cummings, M. P., Handley, S. A., Myers, D. S., Reed, D. L., Rokas, A. & Winka, K. (2003). Comparing bootstrap and posterior probability values in the four-taxon case. *Systematic Biology*, *52*, 477–487.

Dall, W., Hall, B. J., Rothlisberg, P. & Staples, D. J. (1990). The biology of the Penaeidae. *Advances in Marine Biology*, *27*, 1–489.

Dixon, C. J., Ahyong, S. & Schram, F. R. (2003). A new hypothesis of decapod phylogeny. *Crustaceana*, *76*, 935–975.

Douady, C. J., Delsuc, F., Boucher, Y., Doolittle, W. F. & Douzery, E. J. P. (2003). Comparison of Bayesian and maximum likelihood bootstrap measures of phylogenetic reliability. *Molecular Biology and Evolution*, *20*, 248–254.

Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, *32*, 1792–1797.

Felder, D. L. & Robles, R. (2009). Molecular phylogeny of the family Callianassidae based on preliminary analyses of two mitochondrial genes. In J. W. Martin, K. A. Crandall & D. L. Felder (Eds) *Decapod Crustacean Phylogenetics (Crustacean Issues 18)* (pp. 327–342). Bocan Raton, FL: CRC Press.

Feldmann, R. M., Vega, F. J., Applegate, S. P. & Bishop, G. A. (1998). Early cretaceous arthropods from the Tlayua Formation at Tepexi de Rodriguez, Puebla, Mexico. *Journal of Paleontology*, *72*, 79–90.

Feldmann, R. M., Vega, F. J., Martinez-Lopez, L., Gonzalez-Rodriguez, K. A., Gonzalez-Leon, O. & Fernandez-Barajas, M. D. R. (2007). Crustacea from the Muhi Quarry (Albian-Cenomanian), and a review of Aptian Mecochiridae (Astacidea) from Mexico. *Annals of Carnegie Museum*, *76*, 145–156.

Felgenhauer, B. E. & Abele, L. G. (1983). Phylogenetic relationships among shrimp-like decapods. In F. R. Schram (Ed) *Crustacean Phylogeny* (pp. 291–311). Rotterdam: A. A. Balkema.

Felgenhauer, B. E. & Abele, L. G. (1985). Feeding structures of 2 atyid shrimps, with comments on caridean phylogeny. *Journal of Crustacean Biology*, *5*, 397–419.

Felgenhauer, B. E. & Abele, L. G. (1989). Evolution of the foregut in the lower Decapoda. In B. E. Felgenhauer, L. Watling & A. B. Thistle (Eds) *Functional Morphology of Feeding and Grooming in Crustacea* (pp. 205–219). Rotterdam: A.A. Balkema.

Felgenhauer, B. E., Abele, L. G. & Kim, W. (1988). Reproductive morphology of the anchialine shrimp Procaris ascensionis (Decapoda: Procarididae). *Journal of Crustacean Biology*, *8*, 333–339.

Felsenstein, J. (1984). Distance methods for inferring phylogenies: a justification. *Evolution*, *38*, 16–24.

Felsenstein, J. (1985). Confidence-limits on phylogenies with a molecular clock. *Systematic Zoology*, *34*, 152–161.

Fransen, C. H. J. M. & De Grave, S. (2009). Evolution and radiation of shrimp-like decapods: an overview. In J. W. Martin, K. A. Crandall & D. L. Felder (Eds) *Decapod Crustacean Phylogenetics (Crustacean Issues 18)* (pp. 245–259). Bocan Raton: CRC Press.

Garassino, A. (2000). New decapod crustaceans from the Cenomanian (Upper Cretaceous) of Lebanon. *Atti della Società italiana di scienze naturali e del museo civico di storia naturale di Milano*, *141*, 237–250.

Garassino, A. & Bravi, S. (2003). *Palaemon antonellae* new species (Crustacea, Decapoda, Caridea) from the lower cretaceous 'Platydolomite' of Profeti (Caserta, Italy). *Journal of Paleontology*, *77*, 589–592.

Garassino, A. & Jakobsen, S. L. (2005). *Morscrangon acutus* n. gen. n. sp. (Crustacea: Decapoda: Caridea) from the fur formation (early eocene) of the Islands of Mors and Fur (Denmark). *Atti della Società italiana di scienze naturali e del museo civico di storia naturale di Milano*, *146*, 95–107.

Giribet, G., Carranza, S., Baguna, J., Riutort, M. & Ribera, C. (1996). First molecular evidence for the existence of a Tardigrada plus Arthropoda clade. *Molecular Biology and Evolution*, *13*, 76–84.

Glaessner, M. F. (1969). *Decapoda Part R Arthropoda 4* (pp. R400–R651). Lawrence, Kansas: The Geological Society of America, Inc., and the University of Kansas Press.

Hart, C. W. & Manning, R. B. (1986). 2 new shrimps (Procarididae and Agostocarididae, New Family) from marine caves of the Western North-Atlantic. *Journal of Crustacean Biology*, *6*, 408–416.

Holthuis, L. B. (1973). Caridean shrimps found in land-locked saltwater pools at four indo-west Pacific localities (Sinai Peninsula, Funafuti Atoll, Maui and Hawaii Islands), with the description of one new genus and four new species. *Zoologische Verhandelingen*, *Leiden*, *128*, 1–48.

Holthuis, L. B. (1993). *The Recent Genera of the Caridean and Stenopodidean Shrimps (Crustacea, Decapoda): with an Appendix on the Order Amphionidacea*. Leiden: Nationaal Natuurhistorisch Museum.

Huelsenbeck, J. P. & Ronquist, F. (2001). MRBAYES: bayesian inference of phylogeny. *Biometrics*, *17*, 754–755.

Imaizumi, R. (1938). *Fossil Crayfish from Jehol*. Japan: Sendel.

Katoh, K., Kuma, K., Toh, H. & Miyata, T. (2005). MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Research*, *33*, 511–518.

Kensley, B. & Williams, D. (1986). New shrimps (families Procarididae and Atyidae) from a submerged lava tube on Hawaii. *Journal of Crustacean Biology*, *6*, 417–437.

Kim, W. & Abele, L. G. (1990). Molecular phylogeny of selected decapod crustaceans based on 18S ribosomal-RNA nucleotide sequences. *Journal of Crustacean Biology*, *10*, 1–13.

Kishino, H., Thorne, J. L. & Bruno, W. J. (2001). Performance of a divergence time estimation method under a probabilistic model of rate evolution. *Molecular Biology and Evolution*, *18*, 352–361.

Ma, K. Y., Chan, T.-Y. & Chu, K. H. (2009). Phylogeny of penaeoid shrimps (Decapoda: Penaeoidea) inferred from nuclear protein-coding genes. *Molecular Phylogenetics and Evolution*, *53*, 45–55.

Mantelatto, F. L. M., Robles, R., Biagi, R. & Felder, D. L. (2006). Molecular analysis of the taxonomic and distributional status for the hermit crab genera *Loxopagurus* Forest, 1964 and *Isocheles* Stimpson, 1858 (Decapoda, Anomura, Diogenidae). *Zoosystema*, *28*, 495–506.

Mantelatto, F. L., Robles, R. & Felder, D. L. (2007). Molecular phylogeny of the western Atlantic species of the genus *Portunus* (Crustacea, Brachyura, Portunidae). *Zoological Journal of the Linnean Society*, *150*, 211–220.

Martin, J. W. & Davis, G. E. (2001). An updated classification of the recent Crustacea. *Natural History Museum of Los Angeles County, Science Series*, *39*, 1–124.

Martin, A. J., Rich, T. H., Poore, G. C. B., Schultz, M. B., Austin, C. M., Kool, L. *et al.* (2008). Fossil evidence in Australia for oldest known freshwater crayfish of Gondwana. *Gondwana Research*, *14*, 287–296.

Martins-Neto, R. G. & Mezzalira, S. (1991). Revisao dos Palemonideos Terciários Brasilieros (Crustacea, Caridea) com descriçao de novos taxa. *Anais da Academia Brasiliera de Ciencias*, *63*, 361–367.

Perez-Losada, M., Hoeg, J. T. & Crandall, K. (2004). Unraveling the evolutionary radiation of the thoracian barnacles using molecular and morphological evidence: a comparision of several divergence time estimation approaches. *Systematic Biology*, *53*, 244–264.

Porter, M. L., Perez-Losada, M. & Crandall, K. A. (2005). Model-based multi-locus estimation of decapod phylogeny and divergence times. *Molecular Phylogenetics and Evolution*, *37*, 355–369.

Posada, D. & Crandall, K. A. (1998). MODELTEST: testing the model of DNA substitution. *Bioinformatics*, *14*, 817–818.

Rabadà, D. (1993). Crustáceos decápodos lacustres de las calizas litográficas del Cretácico inferior de España: Las Hoyas (Cuenca) y el Montsec de Rúbies (Lleida). *Cuadernos de Geología Ibérica*, *17*, 345–370.

Rambaut, A. & Drummond, A. J. (2007). *Tracer v1.4*. Available via http://tree.bio.ed.ac.uk/software/tracer/

Robles, R., Schubart, C. D., Conde, J. E., Carmona-Suarez, C., Alvarez, F., Villalobos, J. L. *et al.* (2007). Molecular phylogeny of the American *Callinectes* Stimpson, 1860 (Brachyura: Portunidae), based on two partial mitochondrial genes. *Marine Biology*, *150*, 1265–1274.

Robles, R., Tudge, C. C., Dworschak, P. C., Poore, G. C. B. & Felder, D. L. (2009). Molecular phylogeny of the Thalassinidea based on nuclear and mitochondrial genes. In J. W. Martin, K. A. Crandall & D. L. Felder (Eds) *Decapod Crustacean Phylogenetics (Crustacean Issues 18)* (pp. 301–318). Bocan Raton, FL: CRC Press.

Scholtz, G. & Richter, S. (1995). Phylogenetic systematics of the reptantian Decapoda (Crustacea, Malacostraca). *Zoological Journal of the Linnean Society*, *113*, 289–328.

Schram, F. R. (1986). *Crustacea*. New York: Oxford University Press.

Schram, F. R. & Dixon, C. J. (2004). Decapod phylogeny: addition of fossil evidence to a robust morphological cladistic data set. *Bulletin of the Mitzunami Fossil Museum*, *31*, 1–19.

Schram, F. R., Feldmann, R. M. & Copeland, M. J. (1978). The Late Devonian Palaeopalaemonidae and the earliest decapod crustaceans. *Journal of Palaeontology*, *52*, 1375–1387.

Schubart, C. D., Neigel, J. E. & Felder, D. L. (2000). Use of the mitochondrial 16S rRNA gene for phylogenetic and population studies of Crustacea. *Crustacean Issues*, *12*, 817–830.

Schweitzer, C. E. & Feldmann, R. M. (2000a). First notice of the Chirostylidae (Decapoda) in the fossil record and new Tertiary Galatheidae (Decapoda) from the Americas. *Bulletin of the Mizunami Fossil Museum*, *27*, 147–165.

Schweitzer, C. E. & Feldmann, R. M. (2000b). Reevaluation of the Cancridea Latreille, 1803 (Decapoda: Brachyura) including three new genera and three new species. *Contributions to Zoology*, *69*, 233–250.

Spears, T., Abele, L. G. & Kim, W. (1992). The monophyly of brachyuran crabs: a phylogenetic study based on 18S rRNA. *Systematic Biology*, *41*, 446–461.

Spears, T., Abele, L. G. & Applegate, M. A. (1994). Phylogenetic study of cirripedes and selected relatives (Thecostraca) based on 18S rDNA sequence analysis. *Journal of Crustacean Biology*, *14*, 641–656.

Stamatakis, A., Ludwig, T. & Meier, H. (2005). RAxML-III: a fast program for maximum likelihood-based inference of large phylogenetic trees. *Bioinformatics*, *21*, 456–463.

Stamatakis, A., Blagojevic, F., Nikolopoulos, D. S. & Antonopoulos, C. D. (2007). Exploring new search algorithms and hardware for phylogenetics: RAxML meets the IBM cell. *Journal of Vlsi Signal Processing Systems for Signal Image and Video Technology*, *48*, 271–286.

Stamatakis, A., Hoover, P. & Rougemont, J. (2008). A rapid bootstrap algorithm for the RAxML Web Servers. *Systematic Biology*, *57*, 758–771.

von Sternberg, R. & Schotte, M. (2004). A new anchialine shrimp of the genus Procaris (Crustacea: Decapoda: Procarididae) from the Yucatan Peninsula. *Proceedings of the Biological Society of Washington*, *117*, 514–522.

Stillman, J. H. & Reeb, C. A. (2001). Molecular phylogeny of eastern Pacific porcelain crabs, genera *Petrolisthes* and *Pachycheles*, based on the mtDNA 16S rDNA sequence: phylogeographic and systematic implications. *Molecular Phylogenetics and Evolution*, *19*, 236–245.

Suzuki, Y., Glazko, G. V. & Net, M. (2002). Overcredibility of molecular phylogenies obtained by Bayesian phylogenetics. *Proceeding of the National Academy of Sciences*, *99*, 16138–16143.

Thorne, J. L. (2003). *MULTIDIVTIME v9/25/03*. http://statgen.ncsu.edu/thorne/multidivtime.html. Bioinformatics Research Center, North Carolina State University.

Thorne, J. L. & Kishino, H. (2002). Divergence time and evolutionary rate estimation with multilocus data. *Systematic Biology*, *51*, 689–702.

Thorne, J. L., Kishino, H. & Painter, I. S. (1998). Estimating the rate of evolution of the rate of molecular evolution. *Molecular Biology and Evolution*, *15*, 1647–1657.

Toon, A., Finley, M., Staples, J. & Crandall, K. A. (2009). Decapod phylogenetics and molecular evolution. In J. W. Martin, K. A. Crandall & D. L. Felder (Eds) *Decapod Crustacean Phylogenetics (Crustacean Issues 18)* (pp. 15–29). Bocan Raton, FL: CRC Press.

Tsang, L. M., Lin, F. J., Chu, K. H. & Chan, T. Y. (2008a). Phylogeny of Thalassinidea (Crustacea, Decapoda) inferred from three rDNA sequences: implications for morphological evolution and superfamily classification. *Journal of Zoological Systematics and Evolutionary Research*, *46*, 216–223.

Tsang, L. M., Ma, K. Y., Ahyong, S. T., Chan, T. Y. & Chu, K. H. (2008b). Phylogeny of Decapoda using two nuclear protein-coding genes: origin and evolution of the Reptantia. *Molecular Phylogenetics and Evolution*, *48*, 359–368.

Tudge, C. C. & Cunningham, C. W. (2002). Molecular phylogeny of the mud lobsters and mud shrimps (Crustacea: Decapoda: Thalassinidea) using nuclear 18S rDNA and mitochondrial 16S rDNA. *Invertebrate Systematics*, *16*, 839–847.

Van Straelen, V. (1928). On a fossil freshwater crayfish from eastern Mongolia. *Bulletin of the Geological Society of China*, 7, 173–178.

Whitfield, R. P. (1880). Notice of new forms of fossil crustaceans from the Upper Devonian rocks of Ohio, with descriptions of a new genera and species. *American Journal of Science*, *19*, 33–42.

Whiting, M. F. (2002). Mecoptera is paraphyletic: multiple genes and phylogeny of Mecoptera and Siphonaptera. *Zoologica Scripta*, *31*, 93–104.

Whiting, M. F., Carpenter, J. C., Wheeler, Q. D. & Wheeler, W. C. (1997). The strepsiptera problem: phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. *Systematic Biology*, *46*, 1–68.