Drawn to the dark side: A molecular phylogeny of freshwater shrimps (Crustacea: Decapoda: Caridea: Atyidae) reveals frequent cave invasions and challenges current taxonomic hypotheses

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ABSTRACT

Atyid freshwater shrimps are globally distributed and form an important part of freshwater ecosystems, particularly in the tropics and subtropics. Despite their widespread distribution and ecological importance, their phylogenetic relationships are largely unresolved. Here we present the first comprehensive molecular phylogeny of the Atyidae investigating the evolutionary relationships among 32 of the 42 genera using mitochondrial and nuclear markers. Our data indicate that the established classification of the Atyidae is in need of substantial taxonomic revision at all taxonomic levels. We suggest a new suprageneric systematization of atyids and discuss problematic issues at the generic level, particularly in the most speciose genus, *Caridina*. Molecular clock based divergence time estimates for atyids vary widely, but invariably support the assumption that atyids are an ancient freshwater lineage with an origin in the mid-Cretaceous at the very latest. Atyid distribution patterns are the result of instances of both long-distance dispersal and vicariance, depending largely on the reproductive mode of taxa. From an evolutionary perspective, the high frequency of independent origin of both a complete (landlocked) freshwater life cycle and a cave-dwelling mode of life is remarkable and unparalleled among crustaceans.

1. Introduction

"The family Atyidae, although a small one, comprises a considerable number of ill-defined species and genera, since most authors in creating such did not investigate their relations to those already known."

Ortmann (1894, p. 397)

Atyid freshwater shrimps are the most species-rich group of shrimps and are found in freshwater habitats worldwide bar Antarctica. The family currently contains 42 extant genera (Table 1) with at least 469 species (De Grave and Fransen, 2011) and

the exclusively fossil genus *Delclosia* from the Lower Cretaceous in Spain (Rabadá, 1993; De Grave et al., 2009). At least 16 genera are monotypic (they have only one species) (Table 1). Morphologically, the Atyidae are characterized by chelipeds (claw bearing legs) that are tipped with setae and are used for filtering or detritus feeding (Fryer, 1977; De Grave et al., 2008). Atyid ecology is rather diverse and it has been recognized that atyids play an important role as components of stream food webs in tropical freshwater habitats (see overview in Yam and Dudgeon, 2006). In addition, some species of atyids provide examples of ecological specialization that are more typically observed in marine habitats, i.e., they form commensal associations with a freshwater sponge in an ancient Indonesian lake (von Rintelen et al., 2007) and with a freshwater clam in Lake Tanganyika (Roth-Woltereck, 1958).

Ortmann's critical remark in the introduction to the first revision of the family Atyidae (Crustacea, Decapoda, Caridea) from 1894 is still relevant today. Taxonomic subdivisions at every level within the Atyidae are used inconsistently and a comprehensive phylogeny of the family has been lacking. Building on earlier

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Table 1
List of extant genera of atyid freshwater shrimps (Crustacea, Decapoda, Caridea).

Genus	Distribution	Subterranean representatives	Genus present in this study
Antecaridina Edmondson, 1954 (monotypic genus)	Wide but disjunct distribution on islands of the Indo-West Pacific region: Fiji, Madagascar, Sinai Peninsula (Red Sea), Hawaii, Solomon Islands, Ryukyus, Philippines	Can enter anchialine caves	+
Archaeatya Vilalobos, 1959 (monotypic genus)	Central America and islands in the Pacific (Cocos Islands, Galapagos, Perlas Archipelago)	None	−
Atya Leach 1816	Americas & West Africa	Possibly one species[a]	+
Atyaephyra de Brito Capello, 1867	Europe, North Africa, Middle East	None	+
Atydina Cai, 2010 (monotypic genus)	Indonesia	None	−
Atyella Calman, 1906	Lake Tanganyika, East Africa	None	+
Atyoida Randall, 1840	Indo-Pacific region	None	+
Atyopsis Chace, 1983	Widely distributed in Southeast Asia and Indo-Pacific		+
Australatya Chace, 1983 (monotypic genus)	Eastern Australia	None	+
Caridella Calman, 1906	Lake Tanganyika, East Africa	None	+
Caridina H. Milne Edwards, 1837	Indo-Pacific region, Africa	Some species	+
Caridinides Calman, 1926 (monotypic genus)	Northern Australia	None	+
Caridinopsis Bouvier, 1912 (monotypic genus[b])	West Africa	None[b]	+
Dugastella Bouvier, 1912	Morocco & Spain	None	+
Edoneus Holthuis, 1978	Luzon, Philippines	All	+
Gallocaris Sket and Zakšek, 2009 (monotypic genus)	Southern France	One species	+
Halocaridina Holthuis, 1963	Hawaii	All (anchialine)	+
Halocaridinides Fujino and Shokita, 1975	Ryukyus, Palau, Guam, Zanzibar	All (anchialine)	+
Jolivetya Cals, 1986 (monotypic genus)	New Britain, Papua New Guinea	One species	−
Jonga Hart, 1961 (monotypic genus)	West Indies and Central America	None	+
Lancaris Cai and Bahir, 2005	Sri Lanka	None	+
Limnocaridella Bouvier, 1913 (monotypic genus)	Lake Albert, East Africa	None	−
Limnocaridina Calman, 1899	Lake Tanganyika, East Africa	None	+
Mancicaris Liang, Guo and Tang, 1999 (monotypic genus)	Hunan, China	One species	−
Marosina Cai and Ng, 2005	Sulawesi, Indonesia	All	+
Micratya Bouvier, 1913 (monotypic genus)	Caribbean, Costa Rica, Panama	None	+
Neocaridina Kubo, 1938	China, Taiwan, Japan, Korea, Hawaii (introduced)	None	+
Palaemonias Hay, 1902	Alabama & Kentucky, USA	All	+
Paracaridina Liang and Guo, in Liang et al., 1999	Hunan, China	None	+
Paratya Miers, 1882	Wide but disjunct distribution in the Pacific region: Japan, East Siberia, Korea, India, southern Vietnam, Indonesia, Australia, New Zealand, New Caledonia, Lord Howe, Norfolk Island	None	+
Parisia Holthuis, 1956	Madagascar, Australia (Northern Territory), Philippines (Luzon), Indonesia (Sulawesi)	All	+
Potimirim Holthuis, 1954	Central and South America, Caribbean, Florida (introduced)	None	+
Puteonator Gurney, 1987 (monotypic genus)	Iraq	One species	−
Pycneus Holthuis, 1986 (monotypic genus)	Western Australia	One species	+
Pycnisia Bruce, 1992 (monotypic genus)	Northern Territory, Australia	One species	+
Sinodina Liang and Cai, 1999	Southwest China	None	+
Stygiocaris Holthuis, 1960	Western Australia	All	+
Syncaris Holmes, 1900	California, USA	None	+
Troglocaris Dormitzer, 1853	Southern Europe	All	+
Typhlatya Creaser, 1936	Caribbean, oceanic islands in the Atlantic and eastern Pacific oceans, Europe	All	+
Typhlocaridina Liang and Yan (1981)	Guangxi, southern China	All	−
Typhlopatsa Holthuis, 1956 (monotypic genus)	Madagascar	One species	−

[a] Two specimens of *A. brachyrhinus* Hobbs and Hart, 1982 were found in a single cave on Barbados, but nothing is known about the life history of this species (Hobbs and Hart, 1982).
[b] A second species, *C. brevinaris*, was described by Holthuis, 1956 from subterranean waters in Guinea, West Africa and subsequently synonymized with *C. chevalieri* by Rutherford (1975), though this has been questioned (Tiefenbacher 1993). However, Hobbs et al. (1977) mentioned the lack of troglobitic morphological traits in *C. brevinaris*.

informal classifications (Bouvier, 1925; Holthuis, 1965), Holthuis (1986) recognized four subfamilies (Atyinae, Caridellinae, Paratyi-nae, and Typhlatyinae) to which new genera (e.g., *Lancaris* Cai and Bahir, 2005) were later added in some cases. The validity of these

subfamilies is disputed (Page et al., 2008b; Sket and Zakšek, 2009) or they are simply not recognized (De Grave et al., 2009). One reason for the unsatisfying state of atyid systematics is the challenge posed by attempts to reconstruct atyid phylogeny based on morphological characters. Many of the taxonomic characters utilized can be quite variable, especially when larger populations are studied (e.g., in *Paratya*: Smith and Williams, 1980; in *Caridina*: von Rintelen and Cai, 2009; in *Troglocaris*: Jugovic et al., 2010). Previous studies using molecular (and morphological; YC, unpublished data) phylogenies questioned the monophyly of several genera within the Atyidae (Bracken et al., 2009), especially within the most speciose genus *Caridina* (Page et al., 2007a,b; von Rintelen et al., 2008), but also in smaller groups such as *Atya* (Page et al., 2008a) and *Parisia* (Page et al., 2008b). These results led to novel taxonomic divisions at the generic level, for example the split of the newly erected *Gallocaris* from *Troglocaris* (Sket and Zakšek, 2009) and several other species from the genus *Caridina* (e.g., *Lancaris*; Cai and Bahir, 2005).

The Atyidae comprise both epigean and subterranean taxa with widely different geographic distributional ranges (Table 1), the latter resulting from respective reproductive strategies. Larval metamorphosis can be divided roughly into two types (based on Lai and Shy, 2009): species with a small number of relatively large eggs (around 1 mm) that lack planktonic larval stages in their life history and generally live in landlocked freshwater habitats for their entire life; species with a distinctly larger number of smaller eggs that produce planktonic larvae and need brackish water to complete development (amphidromy; McDowall, 2007). These larvae can potentially be dispersed via the sea and long-distance dispersal has been suggested for some atyid taxa (Page et al., 2005, 2008a).

In the context of geographical distribution, the term freshwater shrimp is usually used in a broader sense including species in anchialine caves, or species in which only the adults are obligatory freshwater dwellers, but where the juveniles occur under brackish or fully marine conditions (Hobbs and Hart, 1982; De Grave et al., 2008).

Some of the subterranean species appear to be more stygophilic than stygobitic (obligate cave-dwellers); for example several species of *Caridina* from Sulawesi, Indonesia (Cai and Ng, 2009; KvR, personal observation), or of *Edoneus* from the Philippines (Cai and Husana, 2009) can have either reduced or fully developed eyes and enter subterranean waters, but otherwise do not necessarily seem to be obligate cave-dwellers. In contrast, there are several stygobitic shrimps that live exclusively in caves and are adapted to life underground (Table 1), such as the Indonesian genus *Marosina* (Cai and Ng, 2005), the European genera *Troglocaris* and *Gallocaris* (Sket and Zakšek, 2009), and the Australian genera *Stygiocaris* (Page et al., 2008b) and *Pycnisia* (Bruce, 1992; Page et al., 2007b). In Madagascar, for example, there are at least seven species of *Caridina* that have been found in caves, but only three of them show morphological adaptations (Cai, 2005). Molecular phylogenies including subterranean species or genera were so far biogeographically focused on Australia (Page et al., 2007b, 2008b), southern Europe (Zakšek et al., 2007, 2009), the Caribbean (Hunter et al., 2008; Page et al., 2008a) and Sulawesi, Indonesia (KVR et al., unpubl. data).

Our aim here is (1) to test previous hypotheses on atyid systematics by using the largest sampling of epigean and subterranean atyid genera so far from localities worldwide, in particular the monophyly of the subfamilies and genera as currently recognized. In addition the patterns revealed by the data will be used (2) to discuss biogeographic patterns and the frequency and distribution of landlocked taxa and (3) the evolution of troglophily and troglomorphy within the group.

2. Material and methods

We used representatives from 34 atyid genera worldwide (Fig. 1, Table 1). Thirty specimens were collected by the authors and two obtained from pet shops in the USA and Germany (with a known origin). Sixty-four specimens were kindly provided by other collectors (Supplementary Table S1). We tried to include as many genera as possible and limited the number of specimens in genera of which we had sufficient material, e.g., *Caridina*, to representatives with a broad geographic coverage (Fig. 1). For some taxa, e.g., the European cave shrimp *Troglocaris*, we had to rely on 11 additional sequences from GenBank (Supplementary Table S1). This study is mainly lacking species from monotypic genera with a limited distribution (Table 1) that proved too difficult to obtain, for example the genus *Puteonator* from Iraq.

As in previous studies using atyid phylogenies (Page et al., 2008a; Bracken et al., 2009), we also included the amphidromous Caribbean freshwater shrimp *Xiphocaris elongata* (Xiphocarididae), which was previously considered a basal atyid (Fryer, 1977; Christoffersen, 1986). We used *Macrobrachium australe* from Papua New Guinea and *M. tolmerum* from Australia (Palaemonidae) as outgroups.

DNA was extracted exclusively from abdominal tissue or pleopods using a CTAB extraction protocol (Winnepenninckx et al., 1993) for most samples. Fragments of the mitochondrial 16S rRNA (~540 bp), nuclear 28S rRNA (~1090 bp) and nuclear Histone 3 (328 bp) genes were amplified by polymerase chain reaction (PCR) and sequenced using the primers listed in Supplementary Table S2. Amplifications were conducted in 25 µL volumes containing 50–100 ng DNA, 1× PCR buffer, 200 mM of each dNTP, 0.5 mM of each primer, 2 mM $MgCl_2$ and 1 U of Taq polymerase (and 5% DMSO for 28S to avoid the formation of secondary structures during amplification). After an initial denaturation step of 3 min at 94 °C, 35 cycles of 30 s at 94 °C, 60 s at 50–62 °C (see Supplementary Table S2) and 60–120 s (see Supplementary Table S2) at 72 °C were performed, followed by a final extension step of 5 min at 72 °C. PCR products were purified using NucleoSpin Extract II Kits (Macherey–Nagel) or ExoSap (US Biochemicals). Both strands of the amplified gene fragments were cycle-sequenced using the primers employed in PCR with Big Dye Terminator chemistry version 1.1 (Applied Biosystems Inc.). Sequences were visualized on an Applied Biosystems 3130xl or 3730xl Genetic Analyzer.

Alignments of forward and reverse strands were conducted using CodonCode Aligner v. 3.0.3 (CodonCode Corporation, Dedham, MA, USA) and corrected by eye. 16S rRNA and 28S rRNA alignments were computed using MAFFT (Katoh and Toh, 2008). ALISCORE (Misof and Misof, 2009) was used to identify and delete ambiguous sites using a Monte Carlo approach. After the ALISCORE run 76.5% for 16S (571 bp vs. 437 bp) and 67.6% for 28S (1507 bp vs. 1019 bp) remained of the original MAFFT alignment. For both MAFFT-ALISCORE alignments ML trees (see below) were computed to test if the ALISCORE algorithm results in different topologies in comparison to the original MAFFT alignment, and if the deletion of the ambiguous sites results in a collapse of clades. No obvious topology discrepancies among the major clades were found in both genes.

Substitution models for single gene datasets (16S, 28S, and H3) and the concatenated sequences datasets (16S + 28S, 16S + 28S + H3; i.e., for the individual single gene datasets contained in these) were obtained from MrModeltest v. 2.3 (Nylander, 2004; Supplementary Table S3).

In total, 117 specimens were included in the analyses, of which 111 were used for the analysis of 16S, 96 for 28S, and 97 for H3. In the concatenated datasets, the total number of included sequences was reduced: 16S + 28S (90), 16S + 28S + H3 (83).
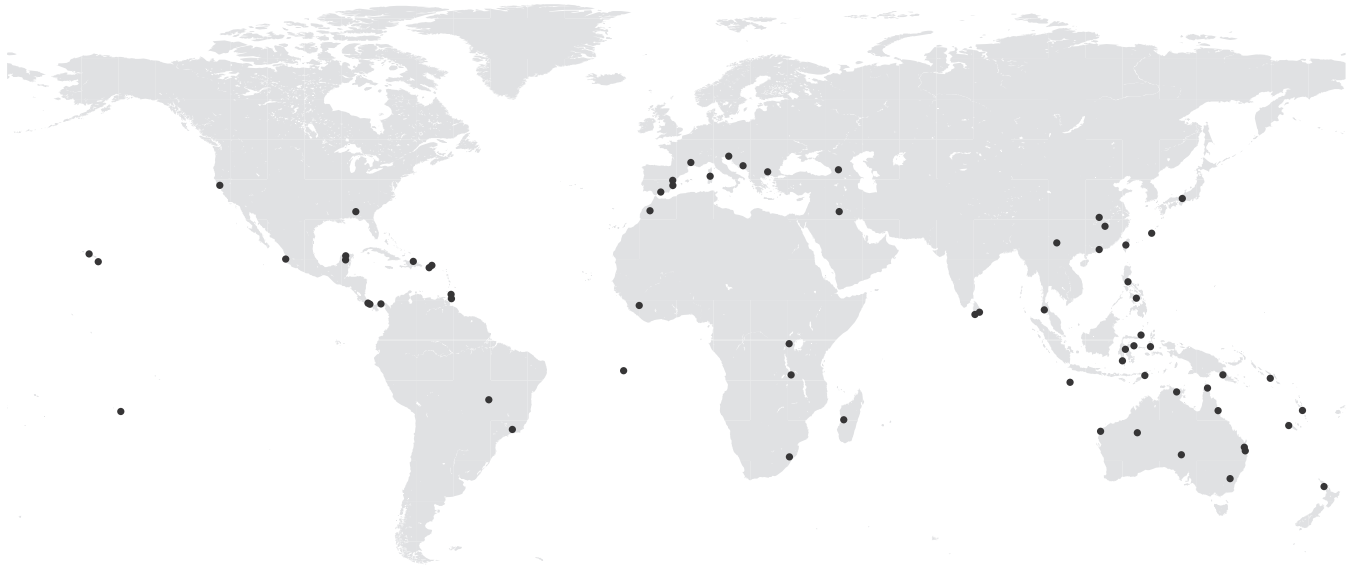
Fig. 1. Sample origin of sequenced specimens from worldwide localities (for details see Supplementary Table S1).

Phylogenetic analyses were performed using maximum parsimony (MP) as implemented in PAUP* v. 4.0b010 for Microsoft Windows (Swofford, 2002), maximum likelihood (ML) using TREE-FINDER v. June, 2008 (Jobb et al., 2004) and Bayesian inference using MrBayes v. 3.1.2 (Ronquist and Huelsenbeck, 2003. The substitution model obtained from MrModeltest was used for the ML and BI analyses, and the concatenated datasets were partitioned by genes. Other parameters (among-site rate variation and base frequencies) were allowed to vary freely.

MP parameters: heuristic search with 10 random additions (maximum number of saved trees), tree bisection and reconstruction (TBR) branch swapping, No. of bootstrap replicates = 10,000 (multrees = no). In H3, heuristic search was repeated with number of saved trees = 500,000, because the first analysis (maximum number of saved trees) ran out of memory. ML parameters: search depth = 2, No. of bootstrap replicates = 1000; BI parameters: 5000,000 generations (H3 ngen = 15,000,000), samplefreq = 100 (H3 = 300), No. of chains = 4, burnin value = 35,001 to keep 15,000 trees.

All sequences have been deposited at GenBank and EMBL (for accession numbers see Supplementary Table S1).

Distribution data and a stygobitic mode of living were mapped onto the molecular BI consensus topology of the combined dataset comprising all three genes using Mesquite, version 2.5 (Maddison and Maddison, 2007), which was also employed to reconstruct ancestral states using MP with the unordered states assumption and ML. For the ML reconstruction of character states, a Markov k-state 1-parameter model (Pagel, 1994) was employed for the multistate distribution data and an Asymmetrical Markov k-state 2-parameter model. Table S1 lists the character states for each taxon employed in the ancestral state reconstructions.

For the molecular clock analyses, the concatenated dataset was reduced to unique haplotypes and single gene datasets were tested for nucleotide substitution saturation using the test by Xia and Xie implemented in DAMBE v. 5.1.1 (Xia and Xie, 2001). Tests revealed no significant saturation ($p > 0.05$) for symmetrical trees. Molecular analyses revealed divergent branch lengths among the ingroup taxa. Thus, a relaxed uncorrelated log-normal clock was performed using BEAST v. 1.5.3 (Drummond and Rambaut, 2007; parameters used: Speciation: Yule process, ngen = 20–50,000,000 (depending on ESS values), log = 400/1000, burnin value = 35,001, calibration point settings: normal distribution, see Table 2 showing node ages

and 95% HPD). Four different calibration points (Table 2) based on two fossils – with two alternative taxonomic placements for the only atyid fossil included (Table 2; 1a/1b) – and one secondary calibration point were used in six different calibration schemes (calibration points: 1a, 2, 3, 1a & 2, 1b & 2, 2 & 3). Since a run using all three calibration points yielded major topological conflicts of ingroup relationships indicating inherent problems with the dataset, the resulting tree was discarded.

3. Results

3.1. Resolution and support for different datasets

A combined analysis of all three genes (Fig. 2) and a combination of 16S and 28S (Fig. 4) yielded the topologies with the best resolution and support irrespective of method (BI, ML, MP). In addition, the 16S topology (Fig. 5) is shown, as several taxa could only be sequenced for 16S (compare Supplementary Table S1). Its topology differs in some aspects significantly from the other analyses, but the deviant nodes are rather poorly supported (differences are discussed in detail below). Single gene analyses for H3 and 28S showed similar results as well (Supplementary Figs. 1 and 2), but include a higher number of unresolved nodes. The lowest resolution was obtained in the separate analysis of the H3 dataset (Supplementary Fig. 2), although the number of available sequences was similar to those for 28S (97 vs. 96; compare Supplementary Table S1).

3.2. Atyid systematics

The position of *Xiphocaris* (family Xiphocarididae) as sister group to the remaining Atyidae is highly supported in four of seven analyses, (Figs. 2–4, Supplementary Figs. 1 and 3). In three analyses, this genus is nested within the Atyidae, but without good support or resolution (Fig. 5, Supplementary Figs. 2 and 4). The Atyidae fall into three major clades, but none of these corresponds fully to the four subfamilies *sensu* Holthuis (1986), i.e., none of these subfamilies is monophyletic. The genus *Syncaris* (Paratyinae) is basal to all other atyid genera with high support values (Figs. 2–4; Supplementary Figs. 1 and 3) and thus distinctly split from the majority of Paratyinae (including *Atyaephyra*, *Dugastella*, *Palaemonias*,

Table 2
Molecular clock calibration points.

Calibration point	Taxon	Age		Comments
		Stage	My	
1a	*Atyoida roxoi* Beurlen, 1950	Early Cretaceous (Aptian)	118.5 ± 6.5 (112–125)	Assigned to stem of Atya group based on original taxonomic assignment
1b				Assigned to stem of Atyidae without *Syncaris*
2	*Palaemon antonellae* Garassino & Bravi, 2003 *Alburnia petinensis* Bravi & Garassino, 1998	Early Cretaceous (Albian)	105.5 ± 6.5 (99–112)	Assigned to stem of Palaemonidae
3	Secondary calibration from Porter et al., 2005		269.5 ± 52.5 (217–322)	Node 54 in Fig. 2 from Porter et al., 2005

Paratya and *Troglocaris*; Figs. 2 and 3). In other analyses, *Syncaris* is nested within the remaining Atyidae but either unresolved (Supplementary Fig. 4) or poorly supported (e.g. Fig. 5). The remaining Paratyinae form a monophyletic group without strong support (Figs. 2–4), although they appear in a strongly supported clade of anchialine cave dwellers also comprising members of the subfamilies Typhlatyinae (e.g., *Stygiocaris, Typhlatya*) and Caridellinae (*Halocaridina, Halocaridinides*). Sister group to this larger clade is another well-supported group including the Atyinae and the remaining Caridellinae that appear in two subclades (Figs. 2–4), one mainly consisting of *Caridina* and *Caridina*-like shrimps, the other one mainly *Atya* and *Atya*-like shrimps. Again, the assignment to one of these subfamilies is not possible as subfamily members are mixed within both subclades: The *Caridina*-like clade includes *Caridina, Neocaridina* (Atyinae) and *Limnocaridina, Edoneus, Parisia, Pycneus* (Caridellinae); the *Atya*-like clade e.g., *Atya, Atyoida, Atyopsis, Jonga* (Atyinae) and *Micratya* (Caridellinae). While the implications of these results for the validity of the currently recognized subfamilies are being discussed below, these are still used for reference in the remaining parts of the 'results' section.

The monophyly of 10 genera – considering the incomplete dataset for some genera – is confirmed. These are *Atyoida* (all analyses), *Atyopsis* (Fig. 5; only one sequence available for other analyses), *Dugastella* (all analyses), *Limnocaridina* (all analyses), *Neocaridina* (all analyses, H3 unresolved), *Palaemonias* (all analyses), *Paratya* (all analyses, except for 16S in Fig. 5), *Potimirim* (all analyses), *Stygiocaris* (all analyses), and *Troglocaris* (all analyses; no sequence available for H3). The monophyletic status of eight genera (*Atyella, Caridella, Halocaridina, Halocaridinides, Lancaris, Marosina, Paracaridina, Sinodina*) could not be tested, because sequences were only available from a single species (Supplementary Table S1). The nine monotypic genera included in the analysis were not considered (see Table 1).

Four genera could not be recovered as monophyletic. The genus *Atya* is represented in all analyses in one well-supported clade but always excluding the species *A. ortmannioides* (e.g., Fig. 2). Instead, this species is nested within a strongly supported clade comprising *Micratya, Potimirim* and *Jonga* (Figs. 2–4). In all analyses (e.g., Figs. 2–4), the genus *Caridina* is not monophyletic, with individual species groups being sister group to other atyid genera (often cavernicolous, such as *Marosina* or *Pycnisia*). The genus *Parisia* with sequenced specimens from Australia is split as well into two separate clades (Fig. 5, Supplementary Figs. 2 and 4). One species (*P. gracilis*) clusters with *Pycneus*, the other species (*P. unguis*) with *Pycnisia*. In most analyses, the genus *Typhlatya* does not appear monophyletic as well (except for Fig. 2 and 3 but unsupported): either *Typhlatya miravetensis* from Spain (Figs. 4 and 5; Supplementary Fig. 3) or *T. rogersi* from Ascension Island is excluded when sequences of *T. miravetensis* are absent (Supplementary Figs. 1, 2 and 4). However, a clade including *T. rogersi* but excluding *T. miravetensis* is well supported in the 16S/28S analysis (Fig. 3). Instead, *T. miravetensis* clusters with the genus *Stygiocaris* with a good sup-

port in the combined 16S and 28S dataset (Fig. 4), and with lower or no support in the single 16S (Fig. 5) and 28S datasets (Supplementary Fig. 3).

3.3. Divergence time estimates

Divergence time estimates for the origin of atyids, i.e., the split from their sister group Xiphocarididae, range from the early Carboniferous to the early Cretaceous, depending on the calibration scheme (Fig. 6 and Table 3). Considering confidence intervals, the range is even larger (Table 3). In two analyses (Fig. 6D and E), *Xiphocaris* is shown as sister to *Syncaris* instead of all atyids, and under two other calibration schemes (Fig. 6B and F) Typhlatyinae are paraphyletic with respect to Paratyinae.

3.4. Distribution of atyid taxa

The distribution of all atyid genera is confined by ocean boundaries, and so no genus has a worldwide distribution (Fig. 2) with many genera or clades endemic to a much more restricted area (Fig. 3–5 – land-locked groups). Ancestral state reconstructions have revealed that the basal diversification of atyids occurred in the Indo-Pacific (Fig. 2), where 75% of atyid genera are found. The Atlantic region was colonized on three independent occasions by different lineages in two of the three major atyid clades (Fig. 2). Two of the Atlantic clades (in the Paratyinae and Atyinae/Caridellinae) have representatives on both sides of the Atlantic (Europe – North America in the Paratyinae and West Africa – Central/South America in the Atyinae/Caridellinae). In the former clade, taxa from both sides of the Atlantic are separated by deep splits, but in the latter some species are found both in West Africa and the Caribbean.

Landlocked genera or, in *Caridina*, species groups have evolved repeatedly in all three major clades. The epigean *Syncaris* and the subterranean *Palaemonias* are the only genera native to continental North America (Martin and Wicksten, 2004), whereas the majority of the Atydiae occur in the tropics (Table 1). Species of the genus *Dugastella* are either restricted to Spain or Morocco, the subterranean genera *Troglocaris* and *Gallocaris* (Figs. 4 and 5) to Southern Europe (for details see Sket and Zakšek, 2009), whereas *Atyaephyra* (Europe/N Africa/Middle East) and *Paratya* (wide but disjunct distribution in the Pacific region) are not landlocked (compare Table 1).

The two subclades of the largest major clade of atyids comprising the Atyinae and most Caridellinae show a very different pattern. Within the *Atya*-like subclade, only *Australatya* is landlocked in Australia, species within other genera are widely distributed (Fig. 4; Table 1). In contrast, within the *Caridina*-like subclade, several landlocked groups can be found: two Australian groups including members the genera *Caridina, Parisia, Caridinides, Pycneus* in one clade, and *Pycnisia* and *Parisia* in the other (Figs. 3–5); a Lake Tanganyika group from Africa with the typical endemics *Limnoca-*
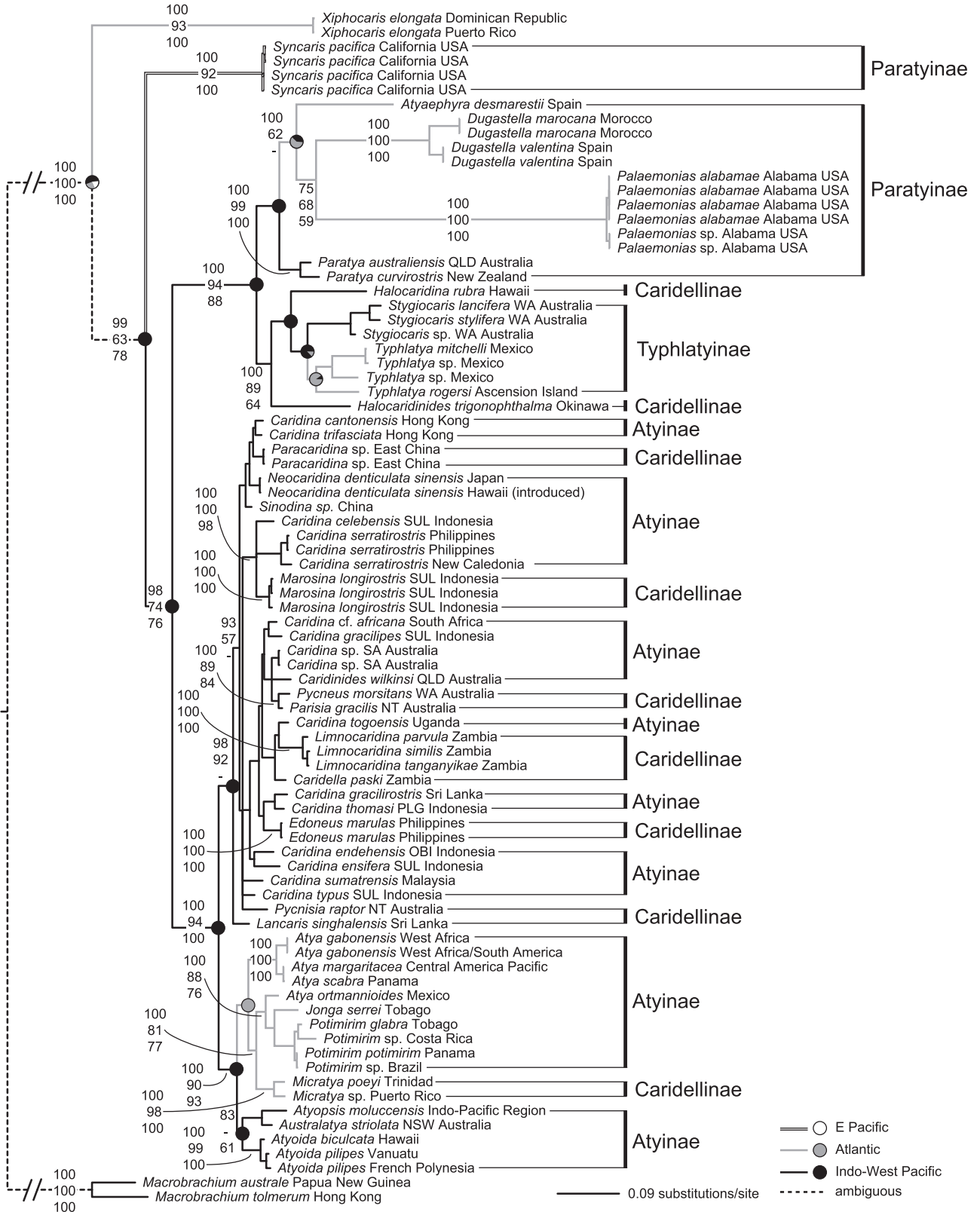
16S+28S+H3

Fig. 2. Bayesian inference phylogram of atyids based on a combined mitochondrial and nuclear 16S/28S/H3 dataset with ancestral state reconstruction of distributions. Numbers on branches are, from top, Bayesian posterior probabilities, ML and MP bootstrap values. Branch and nodal pie diagram color correspond to geographic regions (see legend), branches show the result of the MP ancestral state reconstruction, pie diagrams at nodes show ancestor probabilities of the ML reconstruction.

ridina and *Caridella* (Fryer, 2006) clustering with the wider distributed African species *Caridina togoensis*; a second clade with

the endemic subterranean species *Caridina steineri* from Madagascar clustering with an epigean and widespread African species *Car-*
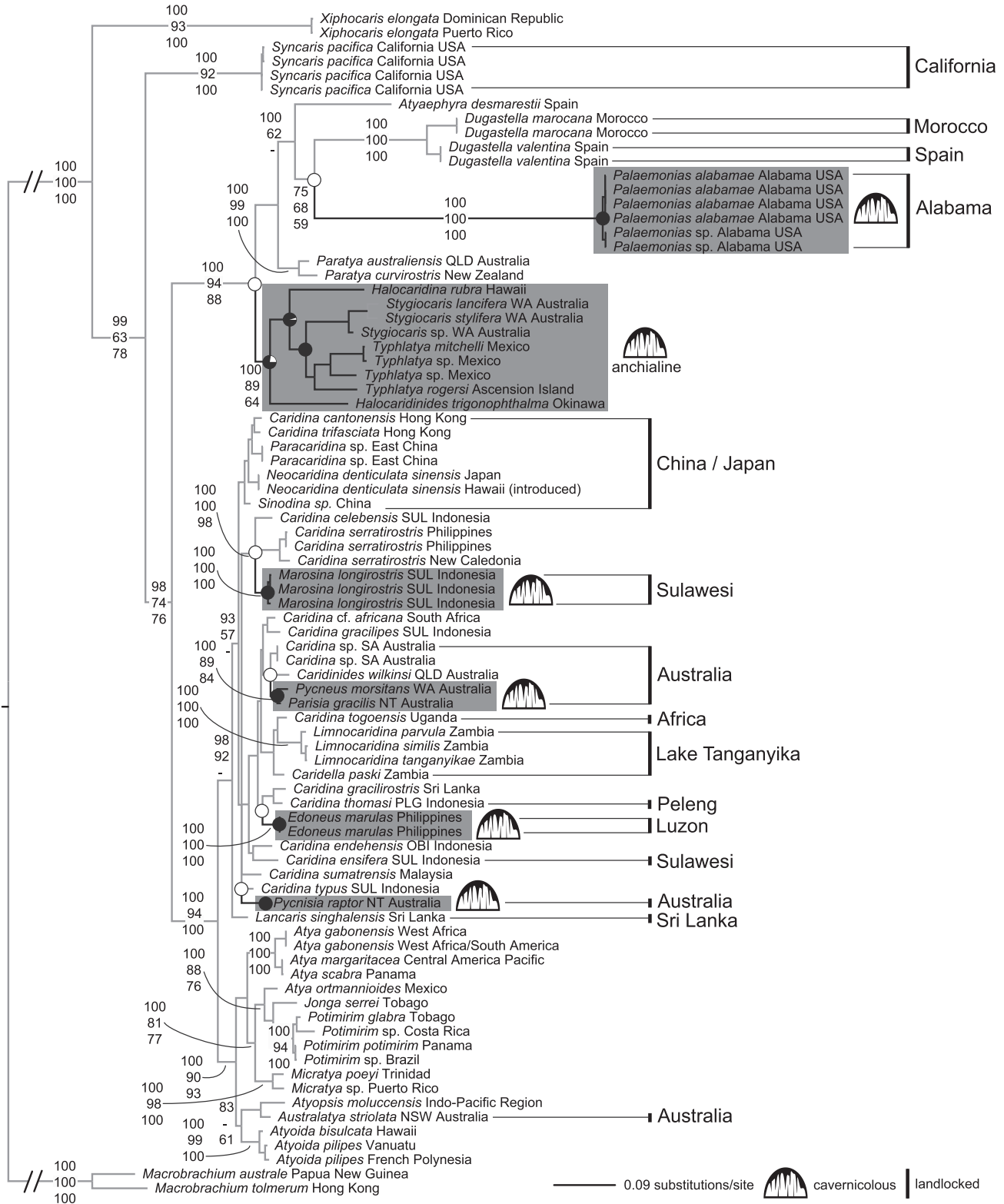
16S+28S+H3

Fig. 3. Bayesian inference phylogram of atyids based on a combined mitochondrial and nuclear 16S/28S/H3 dataset with ancestral state reconstruction of troglobity. Numbers on branches are, from top, Bayesian posterior probabilities, ML and MP bootstrap values. Landlocked clades and cavernicolous taxa are indicated by right-hand brackets and cave symbols, respectively. Branch and nodal pie diagram color correspond to an epigean or subterranean mode of living (see legend), branches show the result of the MP ancestral state reconstruction, pie diagrams at nodes show ancestor probabilities of the ML reconstruction (white – epigean, black – subterranean).

idina cf. *africana* from South Africa (Fig. 5 only); several Asian clades with landlocked species (e.g., *Caridina thomasi*, *Caridina*

ensifera) and genera (*Marosina, Edoneus, Lancaris*; Table 1) from Indonesia, the Philippines, Sri Lanka, China, Japan and Taiwan,
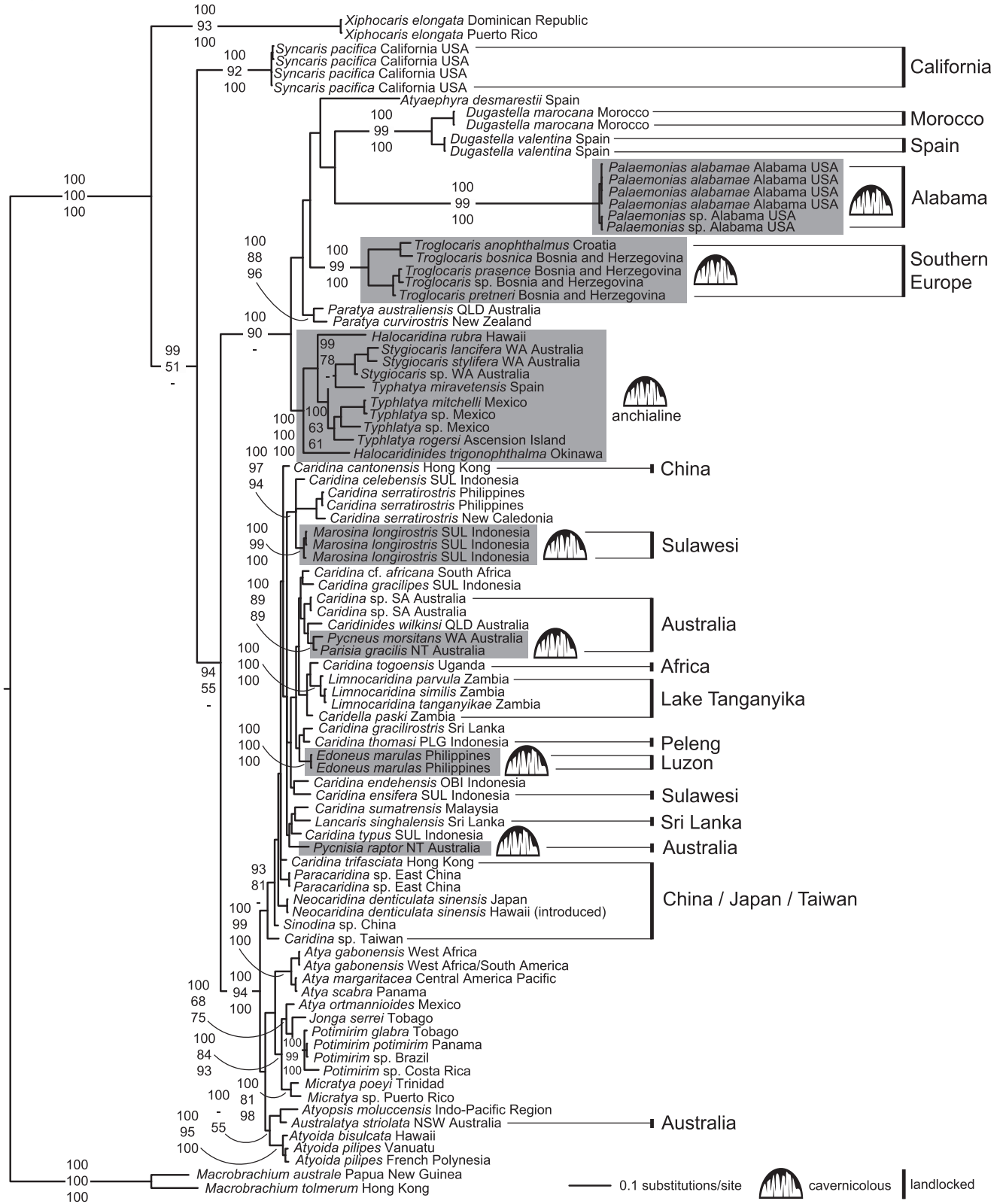
16S+28S

Fig. 4. Bayesian inference phylogram of atyids based on a combined mitochondrial and nuclear 16S/28S dataset. Numbers on branches are, from top, Bayesian posterior probabilities, ML and MP bootstrap values. Landlocked clades and cavernicolous taxa are indicated by right-hand brackets and cave symbols, respectively.

which often cluster with more widespread species (e.g., *Caridina typus*, *Caridina sumatrensis*, *Caridina endehensis*) from the Indo-Pacific region (Figs. 3–5). Several East-Asian genera (*Neocaridina, Sin-* *odina, Paracaridina*) cluster with the *Caridina cantonensis* species group and all species of this cluster are landlocked and endemic to Eastern Asia.
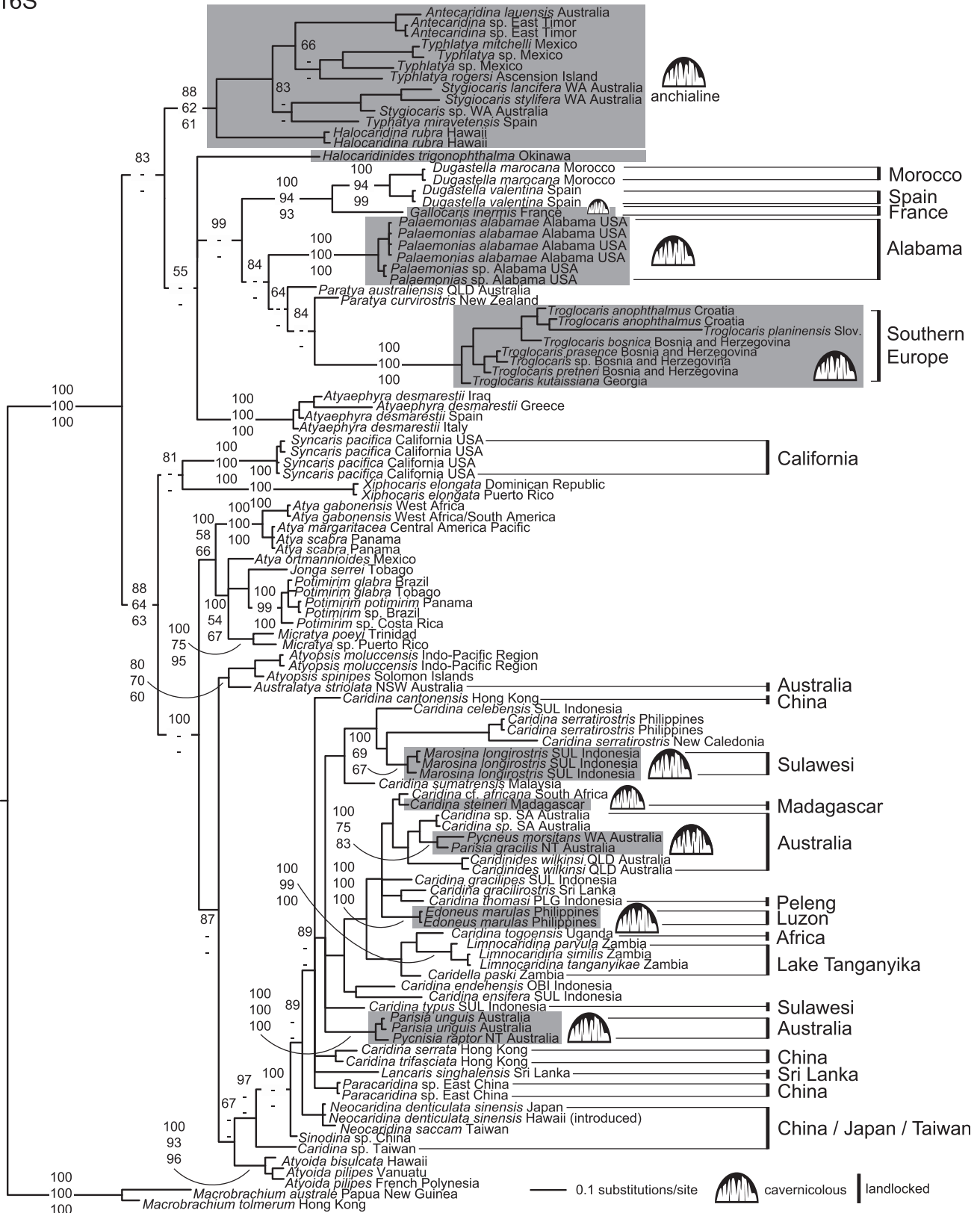
16S

Fig. 5. Bayesian inference phylogram of atyids based on the mitochondrial 16S dataset. Numbers on branches are, from top, Bayesian posterior probabilities, ML and MP bootstrap values. Landlocked clades are indicated by right-hand brackets. Landlocked clades and cavernicolous taxa are indicated by right-hand brackets and cave symbols, respectively.

3.5. Subterranean taxa

Subterranean genera or species are found in two of the three major atyid clades, only the basal *Syncaris* clade is lacking stygobitic species (Fig. 3). Among the remaining atyids, the molecular phylogeny revealed nine clades of exclusively cavernicolous (subterranean) species: (1) *Palaemonias* with landlocked species from Alabama (Figs. 3–5), (2) *Troglocaris* with landlocked species from southern Europe (Figs. 4 and 5), (3) the landlocked genus *Gallocaris* from France (with only one 16S sequence, Fig. 5), (4) *Marosina* with landlocked species from Sulawesi (Figs. 3–5), (5) Australian landlocked species of the genera *Parisia* and *Pycneus* (Figs. 3–5), (6) *Edoneus* with landlocked species from Luzon (Figs. 3–5), (7) Australian landlocked species of the genera *Parisia* and *Pycnisia* (Figs. 3–5), (8) the landlocked species *C. steineri* from Madagascar (with only one 16S sequence, Fig. 5), and (9) a clade including all non-landlocked anchialine cave-dwellers of the genera *Antecaridina*, *Halocaridina*, *Halocaridinides*, *Stygiocaris*, and *Typhlatya* from various localities (Figs. 3–5). All of these clades are well supported in the combined analyses of two and three genes (Figs. 3,4), only in the single 16S analysis, *Halocaridinides* from Okinawa does not cluster with the other anchialine cave-dwellers (Fig. 5). However, this is not strongly supported.

All cave clades are found in terminal positions and have epigean sister groups (Figs. 3–5), except for the well-supported basal position of the clade of anchialine cave-dwellers to three groups of cave dwellers (*Palaemonias*, *Troglocaris*) and otherwise epigean genera (Figs. 3 and 4). The relationship between *Troglocaris*, *Palaemonias* and some epigean genera is poorly supported (Fig. 4). The majority of landlocked cave dwellers can be found within the *Caridina*-like subclade of the largest major atyid clade, which contrasts with the almost complete lack of troglobitic taxa in its sister-group, the *Atya*-like subclade. The only stygobitic species of that clade, *Atya brachyrhinus* Hobbs and Hart, 1982 from karst caves on Barbados is not included in our sampling as it is very rare and may be extinct (A. Karge, pers. comm.).

4. Discussion

4.1. Systematics at the family and subfamily level

The present study, which is the most comprehensive molecular phylogeny of atyid freshwater shrimps to date (32 of the 42 extant genera are included), suggests that previous concepts of atyid systematics and taxonomy need to be revised considerably.

The close relationship between the genus *Xiphocaris* (Xiphocarididae) and the Atyidae as a well-supported monophyletic group was already shown by Bracken et al. (2009), who used a combination of mitochondrial and nuclear gene sequences (16S/18S) for a higher-level phylogeny of the infraorder Caridea. These authors found neither clear support for a basal position of *Xiphocaris* to the Atyidae nor its position within the family, which left them with the expression "the enigmatic position of *Xiphocaris*". Our study now clearly supports *Xiphocaris* as being the basal lineage sister to the Atyidae. In consequence, the Atyidae turn out as a well-supported monophyletic group.

In our analyses none of the established four atyid subfamilies Atyinae, Caridellinae, Paratyinae, and Typhlatyinae, updated by Holthuis (1993), was recovered as monophyletic. The validity of the atyid subfamilies, which are all based on morphological characters, has recently been questioned. De Grave et al. (2008) chose not to recognize these subfamilies at all and Page et al. (2007b, 2008b) suggested an informal systematization using "Atyinae" (as "*Caridina*-like" and "*Atya*-like" shrimps including members of the Atyinae and Caridellinae) and "Non-Atyinae" (including members of the Paratyinae and Typhlatyinae) as higher-level clades. With regard to our data, the latter approach is not necessarily more consistent than the prior division into subfamilies, as two independent groups of "Non-Atyinae" would result. In our opinion, the major clades and subclades recovered in our analyses should be named in order to ease communication, which is a major objective of taxonomy. However, for four of the five major clades recovered in our analysis, morphological apomorphies or at least diagnostic characters are not known presently. For *Syncaris* the fusion of the dactylus and propodus of the second maxilliped has been regarded as an apomorphy (Martin and Wicksten, 2004). This character state is apparently found in some other atyids as well, though (compare, e.g., Smith and Williams (1981) on *Antecaridina* or Cai and Ng (2005) on *Marosina*). This would just leave a behavioral character, i.e., a unique (for atyids) winter breeding pattern, when generally all adult females become ovigerous around the same time between September and October (Hedgpeth, 1968; Born, 1968), as a potential apomorphy of *Syncaris*. For the time being, we therefore suggest using informal names for all five subclades (Table 4): (1) The *Atya* group should be restricted to the *Atya*-like genera only, which Chace (1983) defined as the group of genera with the "carpus of second pereopod deeply excavate and little longer than broad". (2) The *Caridella* group should be redefined to comprise the cluster of *Caridina*-like genera, i.e., *Caridina* together with all genera previously assigned to Caridellinae except for *Halocaridina* and *Halocaridinides*. Bouvier's (1925) definition of the "série Caridinienne" for genera with a complete branchial formula and "Caridellienne" for genera with an incomplete branchial formula were fully adopted by Holthuis (1986; 1993) for the subfamilies Atyinae and Caridellinae. This definition, however, is misleading phylogenetically. The number of gills and the size of a particular gill are probably habitat-related, in some cases even at the species level, and hence are not suitable characters for defining phylogenetic relationships. (3) The *Typhlatya* group should also comprise *Halocaridina* and *Halocaridinides*. (4) The *Paratya* group should not include (5) *Syncaris*, which should be recognized as a separate group. Our informal names are largely based on the old subfamily names and further advances in the understanding of atyid morphology might allow a formalization of our informal groups by redefining Holthuis' subfamilies. We employ our new informal systematization also below in the discussion.

4.2. Systematics at the genus level and below

Our analyses show that the genus *Caridina* is polyphyletic, which is not a new result (compare introduction). Based on morphology, molecular data and distribution, several *Caridina*-subclades still await their assignment to new genera (YC, unpublished data) such as the newly erected and now monotypic genus *Atydina* (Cai, 2010a) for the former *Caridina atyoides* from Indonesia, which is morphologically more closely related to *Atyopsis* than to other species of *Caridina*. Given the position of the type species, *C. typus*, in the trees, it seems likely that most species presently assigned to *Caridina* will end up in a different and possibly new genus. This is evident in all cases where species of *Caridina* are sister group to a different genus, like *Caridina serratirostris* and *Caridina celebensis*, which always cluster with the cavernicolous genus *Marosina* in our analyses and which have been split from other *Caridina* species in previous molecular phylogenies (Page et al., 2007a,b; von Rintelen et al., 2008). Other examples include the sister group to the cavernicolous genus *Edoneus*, i.e., *Caridina. thomasi* and *C. gracilirostris*. Based on morphological novelties (reduced gills), a possible new generic status at least for *C. thomasi* has been discussed previously (von Rintelen et al., 2008). *Caridina cantonensis* and *C. trifasciata*, both of which have been assigned to the *Caridina serrata* group (Cai and Ng, 1999; Yam and Cai, 2003), cluster with
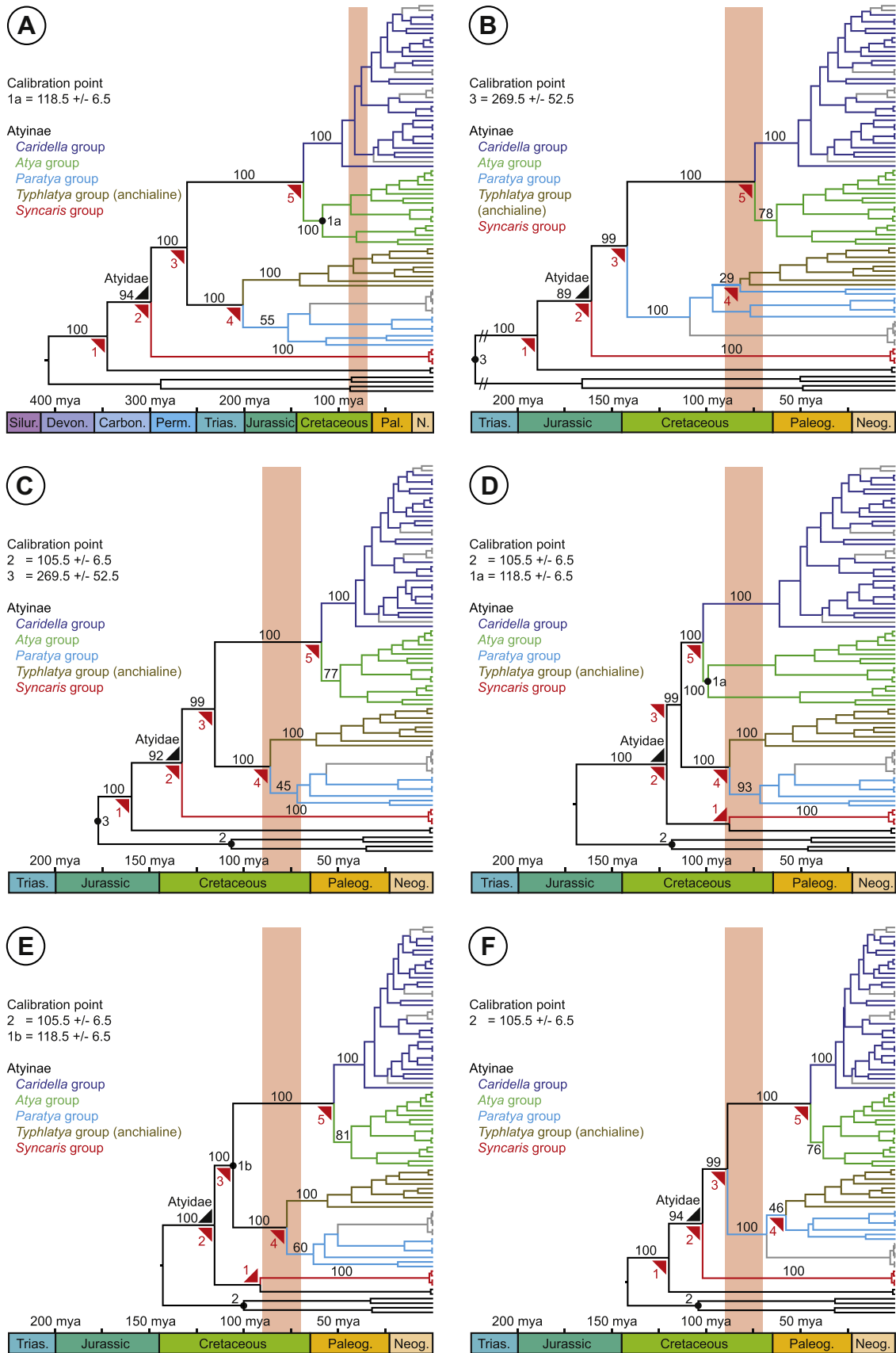
Fig. 6. Bayesian inference chronogram of atyids under different calibration schemes based on a combined mitochondrial and nuclear 16S/28S/H3 dataset. Black dots are calibration points as listed in Table 2. Red triangles indicate the nodes listed in Table 3, the basal split within atyids is marked by a black triangle. Cave lineages are indicated by gray shading except for the Typhlatya group, which are all anchialine cave dwellers. The pink vertical bar indicates the geological timeframe for the opening of the northern Atlantic (Funnell and Smith, 1968).

Table 3

Estimated mean node ages from relaxed clock analyses for the concatenated dataset (16S, 28S, H3). Node numbers correspond to those in Fig. 6. ESS – effective sample size.

Panel	Mean node age [95% highest posterior density (HPD)] in my					ESS Node 1 (tmrca *Syncaris* + all other Atyidae)
	Node 1	Node 2	Node 3	Node 4	Node 5	
A	348.33 [205.65, 508.23]	297.60 [177.88, 426.59]	258.35 [159.83, 366.06]	196,63 [123.18, 284.83]	138.52 [106.77, 176.11]	557
B	189.63 [84.97, 297.81]	160.96 [68.72, 256.64]	142.07 [61.48, 230.72]	82.22 [N.A.][b]	74.47 [27.02, 121.88]	1594
C	159.55 [95.08, 226.20]	133.05 [74.62, 191.27]	116.00 [63.48, 166.55]	87.05 [49.31, 127.74]	59.04 [27.80, 91.64]	561
D	87.42 [39.97, 121.31][a]	120.45 [109.81, 131.01]	112.81 [101.53, 124.05]	87.31 [68.22, 105.16]	61.06 [38.39, 83.85]	2605
E	91.49 [64.31, 116.33][a]	115.72 [105.66, 125.95]	105.92 [95.67, 115.87]	77.43 [59.95, 94.06]	52.52 [32.26, 73.35]	3352
F	116.46 [64,38, 169.44]	99.22 [54.60, 144.12]	86.66 [48.88, 128.31]	58.26 [N.A.][b]	44.22 [23.28, 70.24]	327

[a] Node 1 differs in Panel D and E, Xiphocarididae are sister to *Syncaris*.
[b] Node support below 50%, 95% HPD not available.

Paracaridina sp., while *C. togoensis* clusters with three species of *Limnocaridina*. It remains to be seen whether morphological synapomorphies can be found for all emerging clades in the subclade of *Caridella* group which comprises *Caridina*. For the present, as the threshold for generic subdivisions is somewhat arbitrary, it might be more prudent and heuristic to keep the number of names down to a minimum.

Caridina is not the only atyid genus in need of revision. A molecular phylogeny based on mitochondrial gene sequences (16S/COI) has already shown a strongly supported clade of *Atya* species that excluded *A. ortmannioides* from western Mexico (Page et al., 2008a). In addition to the same 16S sequences, we also used nuclear markers and found the same result. As Page et al. (2008a) already pointed out, this rather large and robust species has not been very well studied (Hobbs and Hart, 1982). Our analyses suggest that it is more closely related to the physically smaller genera *Jonga*, *Potimirim* and *Micratya* than to other similarly large *Atya*. However, this hypothesis is lacking morphological support so far.

A further case of non-monophyly can be found in the genus *Parisia* (Fig. 5). As already shown by Page et al. (2008b), two species of that genus from the same cave in northern Australia (Cutta Cutta Caves; Supplementary Table S1) are not closely related despite their morphological similarity. The authors interpret this result as independent cave colonization in Australia (which we can confirm here) along with morphological convergence. Unfortunately, we could not sequence species of *Parisia* from Madagascar, Sulawesi, Papua New Guinea (Cai and Ng, 2009; Cai, 2010b) and the Philippines (compare Table 1). Nevertheless, the genus *Parisia* seems to be in an urgent need of taxonomic revision.

Molecular data show that the genus *Typhlatya* is not monophyletic. Several species of *Typhlatya* have been sequenced with mitochondrial (16S, COI) and nuclear (28S, H3) sequences in other studies, although either the genus *Stygiocaris* from Australia (Zakšek et al., 2007) or the species *T. miravetensis* (Page et al., 2008b) were not included. A study by Jaume and Bréhier (2005) already hinted at some morphological characteristics in *T. miravetensis* (see detailed discussion in that paper) and the general need of thorough revision of the genus. A genetic split between this and other *Typhlatya* species is already visible in Zakšek et al. (2007). In our study, *T. miravetensis* was generally excluded from the otherwise monophyletic clade of members of the genus *Typhlatya*. According to Jaume and Bréhier (2005), it is morphologically closely related to *Typhlatya arfeae* from southern France. They assumed a sister-group relationship of both species. A closer look at those two species (morphology and a more detailed molecular phylogeny) in comparison with other species of *Typhlatya* and the Australian *Stygiocaris* might be helpful in the future.

4.3. The timing of atyid origin and diversification

The large differences between the six divergence estimates for the origin of atyids ranging from the early Carboniferous to the early Cretaceous (Fig. 6 and Table 3) suggests that the three calibration dates used (see Table 2) are not compatible with each other, which is also suggested by deviations from the unconstrained topology (Fig. 2) revealed in most of the clock analyses (Fig. 6B and D–F), and the spurious results of an analysis using all three calibrations points (compare Section 2). The only atyid fossil used in our clock analyses illustrates the difficulties stemming from the use of fossils of uncertain taxonomic assignment. Described as an *Atyoida* (Beuren, 1950), it is very likely an atyid but cannot be assigned to any recent lineage. Here, it has been used to constrain the age of the *Atya* group (Fig. 6A and D; Table 2, calibration point 1a) and alternatively the entire Atyinae (Fig. 6E; Table 2, calibration point 1b), with vastly different results (Table 3). Leaving out this calibration point does not lead to more consistency, though, as the secondary calibration point derived from Porter et al. (2005) (see Fig. 6B) in combination with a date derived from two palaemonid fossils (see Table 2) yields widely diverging estimates as well. Porter et al. (2005) used eight fossil calibration points for an analysis of the entire Decapoda, and found that divergence time estimates derived from various combinations of these fossils were significantly incongruent as well. Clearly, the taxonomic assignment of decapod fossils, and atyid fossils in particular, should be re-assessed.

While this effectively rules out any definite conclusion on the age of atyids, the molecular clock data nevertheless support the assumption of an ancient origin of this freshwater taxon (Hedgpeth, 1968; Martin and Wicksten, 2004). The timing of diversification within Atyidae also varies considerably in accord with the uncertainties pertaining to the age of the entire group, but it seems likely that the split of *Atya* and *Caridella* groups occurred more recently that the split of *Paratya* and *Typhlatya* groups (but see Fig. 6D).

4.4. Atyid biogeography

Large scale distributions of atyids at the generic level and above are limited by ocean boundaries (Fig. 2). This reflects the high dispersal potential of those atyid taxa with a high number of small eggs and amphidromous larvae. Long-distance dispersal with ocean currents has been suggested before for some atyid taxa (Page et al., 2005, 2008a) and is supported by our data. In contrast, the landlocked clades are barred from oceanic dispersal and are thus endemic to continuous land areas of varying extent (continental scale – e.g., Africa, Australia – to small islands – e.g., Peleng near Sulawesi). However, in atyid shrimps, endemism is not necessarily correlated with a large egg size – although this is the case in most genera – and thus with direct larval development (i.e., they lack planktonic larval stages; see Lai and Shy, 2009). One exception is the genus *Limnocaridina*, which produces rather small eggs despite their landlocked occurrence in Lake Tanganyika (Mashiko et al., 1991).

Atyids appear to have radiated primarily in the Indo-Pacific, with a basal split between an East Pacific clade (*Syncaris*) and an

Table 4
New classification of Atyidae.

Suprageneric taxon	Genus
Atyinae	
Incertae sedis (not sequenced)	*Archaeatya*
	Atydina
	Jolivetya
	Limnocaridella
	Mancicaris
	Puteonator
	Typhlocaridina
	Typhlopatsa
Atya group	*Atya*
	Atyoida
	Atyopsis
	Australatya
	Micratya
	Jonga
	Potimirim
Caridella group	*Atyella*
	Caridella
	Caridina
	Caridinides
	Caridinopsis
	Edoneus
	Lancaris
	Limnocaridina
	Marosina
	Neocaridina
	Paracaridina
	Parisia
	Pycneus
	Pycnisia
	Sinodina
Paratya group	*Atyaephyra*
	Dugastella
	Gallocaris
	Palaemonias
	Paratya
	Troglocaris
Typhlatya group	*Antecaridina*
	Halocaridina
	Halocaridinides
	Stygiocaris
	Typhlatya
Syncaridinae	*Syncaris*

Indo-West Pacific clade (all other atyids; Fig. 2). The basal position of *Syncaris*, which occurs in a few coastal streams in northern California and has never been found at elevations higher than 125 m (Martin and Wicksten, 2004), is rather intriguing. *Syncaris pacifica* is the only extant representative of the genus, as one other species, *Syncaris pasadenae* from southern California, is presumed to be extinct (Hedgpeth, 1968). Despite its lowland occurrence close to the sea, it cannot tolerate brackish or marine water and the large egg size indicates a life cycle in complete freshwater (Born, 1968). Martin and Wicksten (2004) discuss *Syncaris* as relics of a Mesozoic biota or of ancient ecosystems in California together with the giant sequoia (coast redwood) of the Sierra Nevada. They argue that fossils of marine animals such as plesiosaurs are indicators of shallow marine areas present during the Mesozoic in California, which enabled the ancestors of *Syncaris* to disperse by sea during that time before they possibly became adapted to a life in pure freshwater (see also discussion in Hedgpeth, 1968). Generally, the Atyidae are considered an ancient group of shrimps that invaded freshwater habitats much earlier than the other large freshwater family Palaemonidae (Hedgpeth, 1968). Martin and Wicksten (2004) discuss a move from marine to freshwater habitats in atyids during the Jurassic. Fossil atyids have been described from Lower Cretaceous freshwater deposits in Brazil (Beuren, 1950) and Spain (the exclusively fossil *Delclosia* Rabadá, 1993), and Tertiary deposits in

France (Oligocene; Glaessner, 1969) and Brazil (Beuren, 1950). The placement of *Syncaris* at the root of the Atyidae is certainly consistent with the hypothesis of Martin and Wicksten (2004), as are our molecular clock data (Fig. 6).

Within the Indo-West Pacific clade, the Atlantic has secondarily been colonized at least three times independently (possibly more often, if poorly supported clades should prove to be non-monophyletic). In the two Atlantic clades which comprise taxa from both sides of the ocean, the separation between West and East Atlantic taxa has apparently occurred at two very different time levels: in the *Paratya* group, *Dugastella* in the Mediterranean and (cavernicolous) *Palaemonias* in North America are separated by a deep genetic split, suggesting either ancient transoceanic dispersal or vicariance. For *Palaemonias*, widespread extinction is likely in either scenario. While the large range of divergence time estimates and varying tree topologies in our molecular clock analyses (Fig. 6) prevent a rigorous test of a trans-Atlantic vicariance hypothesis here, the resulting minimum age of 50 my for the *Palaemonias* lineage underlines the antiquity of this North American clade. In contrast, in the *Atya* group *Atya* species from West Africa and South America have closely related haplotypes, which points towards recent gene flow between both regions, implying transoceanic dispersal as discussed by Page et al. (2008a). Some *Atya* species also occur in Pacific drainages (see Page et al., 2008a), like *Atya margaritacea*, which shares a haplotype with the Caribbean *Atya scabra*. Again, this rather indicates recent dispersal in this amphidromous group (or alternatively issues with current species level taxonomy).

Landlocked clades have arisen in all major lineages of atyids, particularly in the *Caridella* group as defined here. With the exception of *Limnocaridina* in Lake Tanganyika (see above), all landlocked clades comprise genera or species with a low number of large eggs and a complete freshwater life cycle (see also introduction). The transition from a diadromous life cycle to abbreviated larval development and being landlocked is thus a frequent event in atyids, making it likely that few changes in developmental pathways are underlying this phenomenon. An ancestral reconstruction of egg size evolution (not shown), however, fails to identify unambiguously the ancestral state for atyids in general and the *Caridella* group. The lack of support for most internal nodes within the latter clade in our analyses and the incomplete taxon sampling in this most speciose group of atyids are probable reasons for this somewhat unexpected result. Nevertheless, this highlights the frequency of the origin of landlocked taxa in atyids.

These landlocked clades are of particular evolutionary interest, since they include virtually all endemic radiations of atyids, e.g., in Lake Tanganyika (Fryer, 2006) or the ancient lakes of Sulawesi (von Rintelen and Cai, 2009; von Rintelen et al., 2010). The most speciose landlocked clade of atyids is most likely the East Asian clade comprising species currently assigned to *Caridina, Neocaridina, Paracaridina* and *Sinodina* from China and Japan. According to De Grave et al. (2008), 136 atyid species have been found in China so far and new ones are being described regularly, e.g., three new species of stygobitic atyids (YC, unpubl. data). If all these species fall into the endemic East Asian clade identified here for the first time, this group would contain more than a third of all atyids. The origin of this group might be linked to a vicariance event potentially driven by the uplift of the Himalayas and a subsequent recent radiation of this monophyletic landlocked atyid group. However, this hypothesis needs testing with more reliable diversification time estimates.

The disjunct distribution of several other genera (typically the anchialine cave dwellers) suggests that the present day distribution often represents relict populations and that vicariance scenarios are likely, such as in the genera *Stygiocaris* and *Typhlatya* (Page et al., 2008b).

4.5. Evolution of cavernicolous atyids

Our data reveal multiple cave invasions in atyid freshwater shrimps in all major clades except *Syncaris*. Our study recovered up to nine cave colonizations (Fig. 3–5). While the lack of nodal support for sister group relationships between cave-dwelling and epigean taxa is compatible with fewer colonization events, the distribution of cavernicolous taxa nevertheless supports an their independent origin). Considering the higher number of described cave dwellers within the family, which our study did not include (compare Table 1), the number of cave invasions is likely to be significantly higher. The frequent independent evolution of cave dwellers on all continents (regarding a cave species from Madagascar as a representative of Africa) suggests that the transition to a life in the dark is developmentally equally easy as transition to a complete freshwater life cycle. The degree of troglomorphy, i.e., the morphological adaptation to cave habitats, varies considerably in atyids, ranging from a (occasionally only moderate) reduction in eye size (in some species of *Atya* and *Caridina*) to complete loss of eyes and pigmentation (e.g., *Marosina*, *Parisia*). The majority of cave-dwelling atyids are landlocked taxa, suggesting that a complete freshwater life cycle is a prerequisite for troglobitism. The anchialine cave dwellers, which form a monophyletic group in our phylogeny, are a notable exception. The brackish anchialine caves are a rather special habitat, though, and incompatible with a full-scale adaptation to freshwater. Anchialine cave dwellers have probably also originated more than once in atyids, as some taxa that would presumably cluster with the *Caridina*-like clade could not be sequenced for this study, for example *Caridina rubella* Fujino and Shokita, 1975. The *Typhlatya* group, which are entirely anchialine, are also remarkably old, having split from the *Paratya* group in the Cretaceous to early Cenozoic at the very latest, depending on the calibration scheme (Fig. 6). Generally, while most cave lineages are at least between 10 and 25 my old (tmrca with epigean sister group), the earliest intra-cave speciation occurred in the Plio-Pleistocene under all but one calibration scheme. This time lag between the origin of a cavernicolous lineage and demonstrated occurrence in caves is probably due to a combination of poor sampling of epigean relatives and extinction. In *Palaemonias*, where a lineage of Cretaceous or early Cenozoic origin apparently only diversified in the Plio-Pleistocene, extinction seems the most probably explanation.

Most landlocked cavernicolous taxa have epigean sister groups, but the limited species level sampling of our study makes it difficult to make statements on the evolution of cave dwellers with respect to the reproductive mode of their sister group. For both *Edoneus* and *Marosina*, e.g., our trees reveal a widely distributed taxon with diadromous larvae as sister group (albeit without nodal support for the former taxon), which would imply the evolution of a complete freshwater life cycle, a stygobitic mode of life, and troglomorphy within the cave colonizing lineages. It will be interesting to investigate the time frame for these significant changes in morphology and life history.

4.6. Concluding remarks

Our study is the first large-scale approach to atyid systematics using multiple markers and a comprehensive genus level sampling with a wide geographic coverage including epigean as well as subterranean representatives. A major result from our data is the need for a revision of atyid taxonomy at all levels. Here we propose a new classification at the suprageneric level and indicate problematic taxa at the generic level for further taxonomic study. A reassessment of atyid morphology seems advisable in order to provide for the non-molecular diagnosability of subclades within atyids. A more comprehensive taxon sampling will be needed to resolve issues at the generic level and below, for example within the genera *Caridina*, *Parisia*, *Atya* and *Typhlatya*. Atyids are certainly an old freshwater lineage, but more precise age estimates will require a new look for and at atyid fossils. The biogeographic patterns shown here are the result of a mixture of long-distance dispersal in taxa with amphidromous larvae and vicariance in these and particularly the landlocked groups. The latter especially provide suitable model systems for biogeographic studies at a regional scale. Finally, the high frequency of the evolution of landlocked life cycles and troglobitism suggests a high potential for atyids to serve as models in evo-devo research.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2011.12.015.

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