

An improved taxonomic sampling is a necessary but not sufficient condition for resolving inter-families relationships in Caridean decapods

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Abstract During the past decade, a large number of multi-gene analyses aimed at resolving the phylogenetic relationships within Decapoda. However relationships among families, and even among sub-families, remain poorly defined. Most analyses used an incomplete and opportunistic sampling of species, but also an incomplete and opportunistic gene selection among those available for Decapoda. Here we test in the Caridea if improving the taxonomic coverage following the hierarchical scheme of the classification, as it is currently accepted, provides a

better phylogenetic resolution for the inter-families relationships. The rich collections of the Muséum National d'Histoire Naturelle de Paris are used for sampling as far as possible at least two species of two different genera for each family or subfamily. All potential markers are tested over this sampling. For some coding genes the amplification success varies greatly among taxa and the phylogenetic signal is highly saturated. This result probably explains the taxon-heterogeneity among previously published studies. The analysis is thus restricted to the genes homogeneously amplified over the whole sampling. Thanks to the taxonomic sampling scheme the monophyly of most families is confirmed. However the genes commonly used in Decapoda appear non-adapted for clarifying inter-families relationships, which remain poorly resolved. Genome-wide analyses, like transcriptome-based exon capture facilitated by the new generation sequencing methods might provide a sounder approach to resolve deep and rapid radiations like the Caridea.

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Introduction

During the last decade, many efforts have been employed to resolve, using multi-gene approaches, the deeper nodes in the phylogeny of Decapoda (e.g. Martin et al. 2010; Ah Yong et al. 2011; Tsang et al. 2014). However, the comparison among molecular studies gives a picture as contradictory as are the morphological studies. As pointed out for example by Shen et al. (2013) or Tsang et al. (2014) unbalanced taxon sampling is a major source of incongruence among multi-gene analyses. Moreover, most of the available datasets differ both in taxon sampling and in gene

sampling. As a consequence it is very difficult to combine and compare the results among studies. The objective of this paper is to test if the incongruence comes primarily from incomplete taxon and gene sampling or from a lack of phylogenetic signal in the genetic markers used in published datasets. For testing this hypothesis, we take advantage of the large Decapoda collection of the Muséum National d'Histoire Naturelle of Paris (MNHN) which benefit from 40 years of marine expeditions as part of the ongoing Tropical Deep-Sea Benthos program, led by the MNHN and the Institut de Recherche pour le Développement (IRD). This program has generated an important flow of new material from the Pacific and Indian Oceans that have been studied by an active network of taxonomists (Richer de Forges et al. 2013). This collection has also been shown to be an adequate source of specimens for molecular analysis and was used in large DNA-barcoding projects (Puillandre et al. 2012; Zuccon et al. 2012).

To perform such an analysis we selected the Caridea that, with more than 3,200 extant species, is after the Brachyura, the second most speciose infraorder of Decapoda (De Grave et al. 2009). Caridean shrimps are present worldwide, from tropical to polar regions, both in marine and freshwater environments. If the definition of Caridea is not questioned (e.g. De Grave et al. 2009; Bracken et al. 2010; Shen et al. 2013), the classification at the superfamily and family levels is far from being resolved. Several studies use a molecular phylogenetic approach to discuss the classification. However, the results remain unsatisfactory because none of the studies combined a wide taxonomic coverage of Caridean diversity and a phylogenetic signal in the sampled genetic markers that provides support for the family-level relationships. For example, the study of Bracken et al. (2009) includes 31 of the 38 currently accepted families, with 17 families represented by at least 2 species, but only 2 non-coding genes (mitochondrial 16S and nuclear 18S). Conversely, Li et al. (2011) use 5 nuclear genes (18S and 4 coding genes: NaK, PEPCCK, H3 and enolase) but only 20 Caridean families with only 10 of them represented by at least 2 species. The aim of the present study is to provide a multi-gene analysis at the family-level based on an improved taxonomic sampling of the Caridea. To enhance the genetic sampling we also tested all the genetic markers used in the recent Decapoda literature and explored the public genetic databases to identify potential new markers. The main sources of markers were the recent articles on Caridea (Bracken et al. 2009; Chan et al. 2010; Li et al. 2011; Kou et al. 2013) and Decapoda phylogeny (Toon et al. 2009). The taxonomic sampling follows the hierarchical scheme of the latest revisions of the classification of Decapoda (i.e. De Grave et al. 2009, 2014; De Grave and Fransen 2011; Short et al. 2013). The sampling is designed to test the monophyly at

the family level and to infer inter-familial relationships using within each family as far as possible at least two species from two distinct genera.

Materials and methods

The state-of-the-art of Caridean classification and taxon selection

Several classifications have been proposed for the Caridea. The classification used in WoRMS (World Register of Marine Species: www.marinespecies.org) is a synthesis of current proposals. This classification is primarily based on the classification proposed by De Grave et al. (2009) using a comparative morphology approach. The *Carideorum Catalogus* (De Grave and Fransen 2011) and recent molecular phylogenies (Page et al. 2008b; Bracken et al. 2010; Chan et al. 2010; De Grave et al. 2010, 2014; Short et al. 2013) provided five main modifications to the classification of De Grave et al. (2009). First, the Procarididae were excluded from the Caridea and included in a distinct infraorder, the Procarididea (Bracken et al. 2010). Second, the Opolophoridae were shown to be polyphyletic (Chan et al. 2010) and thus separated into two distinct families, the Acanthephyridae and the Opolophoridae s.s.. These two families were included in the same superfamily, the Opolophoroidea. Third, two genera, *Eugonatonotus* and *Galatheacaris*, were synonymized and placed in the Eugonatonotidae (De Grave et al. 2010). Fourth, the family Kakaducarididae was synonymized with Palaemonidae (Short et al. 2013). The fifth is a just published work (De Grave et al. 2014) separating Hippolytidae into five families, namely Merguiddae, Bythocarididae, Thoridae, Hippolytidae s.s. and Lysmatidae. In this revised classification, 14 superfamilies and 38 families are considered as valid (Table 1). Because the goal of the study was to test the monophyly of these families and the relationships among them, we tried to include in the sampling at least two genera, each represented by at least two species, for as many families as possible.

The MNHN collections were screened to select specimens based on their taxonomic identification but also based on the availability of field data (i.e. precise location, depth, habitat, etc.). To increase the success of the DNA sequencing, we also considered additional criteria such as the sampling dates and the apparent conservation state of the specimen. Specimens that might have been previously fixed in formaldehyde were tentatively excluded although this information is generally lacking. For the taxa not present in the MNHN collections or for which the sequencing success rate was too low, we supplemented our dataset with sequences from GenBank.

Table 1 Taxonomic sampling, in number of specimens per family, in the articles of Bracken et al. (2009), Li et al. (2011) and in this present study

Superfamily	Family	Bracken et al. (2009)		Li et al. (2011)		This study	
		Nb sp	Monophyly	Nb sp	Monophyly	Nb sp	Monophyly
Alpheoidea	Alpheidae	15	Yes	3	Yes	3	Yes
	Barbouriidae	–	–	1	?	2	Yes
	Bythocarididae	–	–	–	–	–	–
	Hippolytidae	8	No	1	?	6	No
	Lysmatidae	4	Yes	1	?	3	No
	Merguiidae	–	–	–	–	–	–
	Ogyrididae	2	Yes	–	–	–	–
	Thoridae	1	?	–	–	2	Yes
Atyoidea	Atyidae	10	Yes	2	Yes	8	Yes
Bresilioidea	Agostocarididae	1	?	–	–	–	–
	Alvinocarididae	4	Yes	1	?	8	Yes
	Bresiliidae	–	–	–	–	1	?
	Disciadidae	1	?	–	–	1	?
	Pseudochelidae	–	–	–	–	–	–
Campylonotoidea	Bathypalaemonellidae	1	?	1	?	2	Yes
	Campylonotidae	–	–	1	?	1	?
Crangonoidea	Crangonidae	3	Yes	2	Yes	4	Yes
	Glyphocrangonidae	2	?	2	Yes	2	Yes
Nematocarcinoidea	Eugonatonotidae	1	?	1	?	3	Yes
	Nematocarcinidae	3	Yes	3	Yes	5	Yes
	Rhynchocinetidae	1	?	4	Yes	2	Yes
	Xiphocarididae	1	?	–	–	1	?
Oplophoroidea	Acanthephyridae	5	Yes	–	–	2	Yes
	Oplophoridae	2	?	1	?	2	Yes
Palaemonoidea	Anchistioidea	1	?	–	–	2	Yes
	Desmocarididae	1	?	–	–	–	–
	Euryrhynchidae	1	?	–	–	–	–
	Gnathophyllidae	3	No	1	?	1	?
	Hymenoceridae	1	?	1	?	1	?
	Palaemonidae	18	No	2	No	13	No
	Typhlocarididae	1	?	–	–	–	–
Pandaloidea	Pandalidae	6	Yes	2	Yes	9	Yes
	Thalassocarididae	1	?	–	–	–	–
Pasiphaeoidea	Pasiphaeidae	5	No	2	Yes	4	Yes
Physetocaridoidea	Physetocarididae	–	–	–	–	–	–
Processoidea	Processidae	4	Yes	–	–	2	Yes
Psalidopodoidea	Psalidopodidae	1	?	–	–	1	?
Stylodactyloidea	Stylodactylidae	2	Yes	3	Yes	3	Yes

Family status according to each study is indicated as follow: Yes: monophyly, No: non-monophyly,?: monophyly non tested,–: no data

Whenever available, for each taxon we selected several specimens sampled in different collecting events (i.e. cruises or expeditions). This strategy allows us to check the reliability of the sequences obtained from the same taxon and easily detect potential contaminations, and also to identify within each taxon the sample providing the DNA extract of better quality (see also below).

Three species from other infraorders of Decapoda were selected in GenBank and used as outgroup: *Litopenaeus vannamei* (Dendrobrachiata), *Uroptychus parvulus* (Anomura, Pleocyemata) and *Cancer pagurus* (Brachyura, Pleocyemata). Because the Procarididea were considered Caridea by some authors, two species of Procarididae from GenBank were also added to the matrix to corroborate the

hypothesis that Procarididea is a distinct infraorder from the Caridea.

Genetic markers selection

The most documented genes in the public databases for the Caridea are the fragment of the COI mitochondrial gene used in DNA-barcoding projects and fragments of the 18S RNA and 28S RNA nuclear genes. The sequences available in GenBank (and BOLD for the COI) cover most of the Caridean families. We thus use this set of genetic markers to detect possible contamination of the DNA extracts and/or sequences resulting from potentially poor DNA quality in part of the museum specimens. These markers are used to select among the museum specimens those that will provide the better quality DNA extracts.

In a second step, six additional gene fragments (16S, EPRS, H3, NaK, PEPCK and TM9SF4), used in various phylogenetic studies in groups of Caridea and/or Decapoda (e.g. Bracken et al. 2009; Toon et al. 2009; Li et al. 2011), were tentatively amplified and sequenced. Because these genes are more difficult to amplify and sequence, contaminations at the PCR step were expected. However, their detection should be facilitated by the fact that potential contamination at the previous step (DNA extraction) would have been ruled out. Since nuclear coding genes are supposed to increase the resolution of the phylogeny at this scale (Tsang et al. 2008), GenBank was explored for additional coding gene markers. However, only few genomic data are available for Caridea and only one marker, previously not used in Caridean phylogeny, was identified as a new candidate gene: a fragment of a β -actine gene, for which six sequences for four distinct species (*Exopalaemon carinicauda*, *Macrobrachium amazonicum*, *Macrobrachium rosenbergii* and *Palaemonetes pugio*) were available in GenBank (ref: JX948081, JQ045354, AF221096, AY651918, AY626840, AY935989).

DNA amplification and sequencing

To extract DNA, a pleopod was used because these structures are generally uninformative in Caridean taxonomy (Chace 1992; Bracken et al. 2009). Pleopods III or IV were preferentially used since for many Carideans the second and sometime the first pleopods may provide diagnostic characters for males. Total genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen) or the NucleoSpin 96 Tissue kit (Macherey–Nagel) with the automated pipetting system epMotion 5075, according to the manufacturer instructions. The PCR reactions were performed in 20 μ L reaction volume, containing a final concentration of 1X reaction buffer, 3.4 mM MgCl₂, 0.26 mM dNTP, 0.3 mM of primers, 5 % DMSO and 1.2 units of Qiagen Taq polymerase, plus 1.5 μ L of DNA extract. The amplification thermal profiles consisted

of an initial denaturation for 5 min at 94 °C, followed by cycles of denaturation at 94 °C, annealing, extension at 72 °C and a final extension at 72 °C for 5 min. The number of cycles, duration for denaturation, annealing and extension steps, and annealing temperature for each fragment are provided in Table 3 (Supplementary material). All markers were amplified in a single fragment, except for the 18S RNA gene which was amplified in three overlapping fragments. The PCR products were visualised on a 2 % agarose gel stained with ethidium bromide and the positive PCR products were purified and sequenced in both directions using the Sanger method by an external sequencing facility (Génoscope, Evry, France). The sequence chromatograms were assembled using CodonCode Aligner (<http://www.codoncode.com>).

Curation and quality control of sequence data

The sequences obtained for the first set of markers (COI, 18S, 28S), well represented in sequence databases, were analyzed to detect contaminations at the DNA extraction step by using BLAST (Basic Local Alignment Search Tool) in GenBank (www.ncbi.nlm.nih.gov) or the identification tool in BOLD (for the COI gene) (www.barcodeoflife.org). If, by removing the contaminated DNAs, a taxon is removed from our dataset, a second sampling in the MNHN collection is performed to maintain the expected taxonomic coverage of the Caridea. Specimens from this second sampling are, as the first one, sequenced for the first set of genes to check the quality of the DNA extracts. Additional sampling is performed until enough specimens have been gathered such as to get as close as possible to the expected taxonomic coverage. Then, the other selected genes from the literature (16S, EPRS, H3, NaK, PEPCK, TM9SF4 and β -actine) are sequenced.

For each gene-fragment, the sequences were aligned using MUSCLE (Edgar 2004) implemented in MEGA 5.2 (Tamura et al. 2011), and the alignment accuracy was adjusted by eye. Some regions of the non-coding markers (16S, 18S and 28S) were extremely divergent and therefore difficult to align: GBLOCKS 0.91b (Castresana 2000) was used to omit poorly aligned positions. Single gene-fragment trees were then generated through a Neighbor-Joining (NJ) analysis under MEGA 5.2, with the Maximum Composite Likelihood method and a 100 bootstrap replicates to detect potential incongruences between trees that might indicate the presence of contaminated sequences. Notably, trees for the three fragments of the 18S gene were compared to avoid the creation of chimeric 18S sequence with contamination for one or two fragments of the marker only. For the 18S the final set of selected sequences has thus no supported incongruence (support >70 %) and thus, the fragments were assembled using CodonCode Aligner (www.codoncode.com).

Assembly of the taxa x genes matrix

Some taxa, because of a relatively high amplification success rate and low contamination rate, were comparatively over-represented in the matrix. To obtain a more balanced matrix for the phylogenetic analysis, only one specimen per species was retained, selecting the specimen for which the highest gene number and/or a species level identification was available. For this selection, a first analysis of the concatenated dataset is done using a NJ tree, with a bootstrap of 100 replicates. The matrix is then re-balanced at the family scale, with a selection of at least two genera per family or per well-supported group for non-monophyletic families, following the same criteria (highest number of genes and/or best identification). To limit artifacts resulting from missing data, only specimens for which at least half of the selected genes have been obtained were retained in the final analysis.

Phylogenetic analysis

As no well-supported incongruence between single gene NJ trees was observed, the retained gene-fragments were concatenated using CodonCode Aligner (<http://www.codoncode.com>). The dataset was partitioned by gene, and, for the coding genes, by codon position. We applied a GTR+G model for RAxML and GTR+I+G model for MrBayes. All Maximum Likelihood (ML) analysis and Bayesian Inference (BI) analyses were performed using the CIPRES Science Gateway (www.phylo.org/index.php/portal). The ML analysis was conducted under RaxML-HPC2 on XSEDE (v.8.0.9) (Stamatakis 2014). Confidence in the resulting topology was assessed using non-parametric bootstrap estimates (Felsenstein 1985) with 1,000 replicates. The BI analysis was done using Mr Bayes 3.2.2 on XSEDE (Ronquist and Huelsenbeck 2003), with the following parameters: 30,000,000 generations, 8 chains, 5 swaps, temperature of 0.02, tree sampling frequency of 10,000; all other parameters are set to default. The first 3,000,000 (10 %) generations were discarded as “burn-in”. Stability of each parameter (ESS values superior to 200) was checked with Tracer 1.5 (Rambaut and Drummond 2009).

Results

Taxonomic coverage

A total of 28 families (out of 38) from 13 superfamilies (out of 14) were included in our analysis (Table 1). The families Agostocarididae, Merguiidae, Physetocarididae and Pseudochelidae, each of them only comprising a single genus, were not represented in the MNHN collections,

neither available from our network of collaborators. For the families Bythocarididae (four genera), Desmocarididae (one genus), Euryrhyndidae (one genus), Gnathophyllidae (five genera), Ogyrididae (one genus), Thalassocarididae (two genera), Typhlocarididae (one genus) and Xiphocarididae (one genus), although some specimens were available we failed at obtaining at least half of the selected sequences. Sequences of Gnathophyllidae and Xiphocarididae were available in public databases to complement our dataset. Unfortunately, the sequences of Bythocarididae and Merguiidae from De Grave et al. (2014) do not cover the final set of genes selected for the analysis and were thus not included in the present dataset. Similarly for Desmocarididae, Euryrhyndidae and Thalassocarididae, sequences were available for only two genes (18S and 16S, Kou et al. 2013) and were thus not added in the analysis.

Data selection

Our working hypothesis was that the DNA degradation in collection specimens occurs gradually. Following this hypothesis the success rate of PCR amplification should decrease with the specimen age, and the collection date and the number of genes successfully amplified should be positively correlated. This correlation was statistically significant ($R = 0.525$; $n = 288$; $\alpha = 0.05$) over the whole dataset (Fig. 1). However, the distribution is not uniform over the entire range of dates. The graphic suggests a discontinuity around 1990. Indeed, when testing the correlation for specimens collected before 1990, there is no significant correlation between the date of collect and the amplification success ($R = -0.083$; $n = 101$; $\alpha = 0.05$), and the success rate is globally low (mean success rate before 1990: 41 %, and after 1990: 76 %). Conversely, for the specimens collected from 1990, the correlation is significantly positive ($R = 0.320$; $n = 185$; $\alpha = 0.05$). This analysis suggests that the degradation of DNA mainly occurs during the first 25 years after collection. An alternative hypothesis is that the fixation protocols in the field and later in the museum were improved during the last 25 years.

The recursive sampling in the MNHN collection resulted in 455 extracted specimens. Among those, 138 were discarded from the final dataset because neither the COI nor the 18S or the 28S sequences were obtained. As these three genes fragments are easy to amplify, a failure was supposed to result from low-quality DNA. For those specimens, we considered that the quality and/or the quantity of the DNA extracts were too low and the other markers were not tested. For each of these three genes, about 13 % of all sequences obtained were identified as resulting from contaminations. No evidence of intra-individual variability was observed neither for the 28S nor

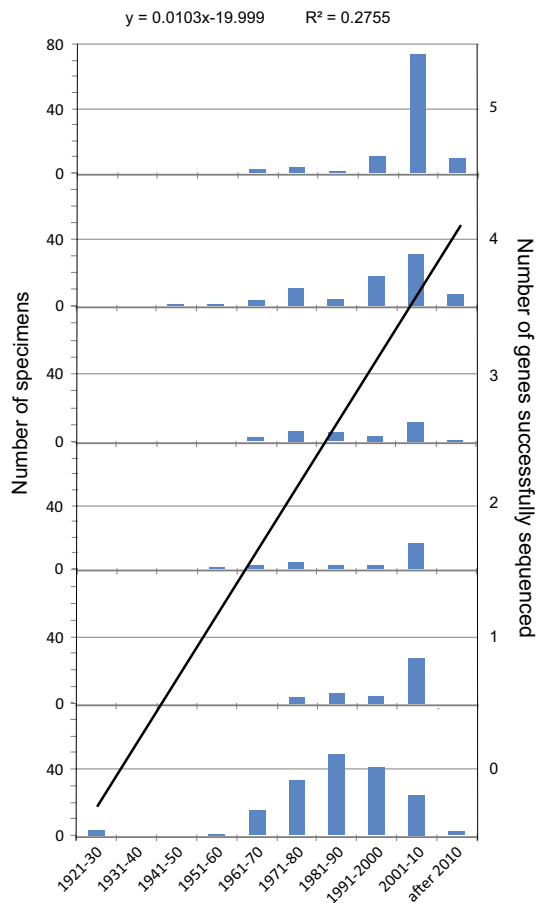


Fig. 1 Histograms of the number of specimens per date of collect, for which respectively the sequences of 0, 1, 2, 3, 4 or 5 genes were successfully obtained (i.e. number obtained after excluding the potentially contaminated sequences). Correlation between the date of collect of the specimens, and the number of validated sequences for the five selected genes

for the 18S fragments. The success rates for each gene are detailed in Table 2. Also the DNA extracts that provided contaminated sequences were considered of too low quality and were thus excluded from the subsequent analyses. Using these two criteria 179 DNA extracts were removed from the dataset.

For the 276 remaining DNA extracts, we amplified the 16S mitochondrial gene using the universal primers that are usually used for Decapoda. Using these primers about 31 % of the sequences matched with human 16S sequences in GenBank. This high rate of contamination is probably explained by the combination of the low quality and quantity of the DNA extracts with poorly specific primers. Therefore, we designed new primers, based on the comparison of available sequences of 16S for several Caridea and *Homo sapiens*, to amplify Caridea DNA preferentially to human DNA. As expected, the success rate of this new pair of primers was largely enhanced and we were able to

Table 2 Sequencing success rates for each gene, based on the number of sequences obtained post-detection of contaminations

Gene			Success (%)
18S	Nuclear	Non-coding	71
28S	Nuclear	Non-coding	60
COI	Mitochondrial	Coding	67
16S	Mitochondrial	Non-coding	54
16S (new primers)	Mitochondrial	Non-coding	78
β -actine	Nuclear	Coding	43
EPRS	Nuclear	Coding	0
H3	Nuclear	Coding	62
NaK	Nuclear	Coding	4
PEPCK	Nuclear	Coding	4
TM9SF4	Nuclear	Coding	0

The first three genes are the reference markers. The other genes are tested only for specimens successfully amplified for the references genes

obtain 90 % of the specimens for which a human sequence was amplified with the first pair of primers.

For some of the tested nuclear coding genes, the sequencing success was very low (Table 2). The analysis of the available sequences in GenBank shows that these genes are highly saturated on the 3rd position of the codon, potentially leading to important mismatches with the primers and possibly explaining the low success rate of PCR amplifications. Among the nuclear coding genes, only the H3 gene-fragment was successfully amplified and sequenced in more than half of the specimens. For the β -actine gene 43 % of the specimens were successfully amplified. Unfortunately the sequences revealed that several copies of the same size were present in the amplification, suggesting that the designed primers amplify not a single gene but several genes from a multigenic family.

At this taxonomic scale, and using specimens from museum collections, we were able to obtain reliable data for only five genes (16S, 18S, 28S, COI and H3) that are then retained for the final phylogenetic analysis. Only for 207 specimens at least three of these five genes were obtained, and among them, the taxonomic coverage was still very unbalanced because of a higher success in some groups. The over-represented taxa were thus subsampled, leading to a final selection of 70 specimens. Taxa with poor coverage in this dataset were re-equilibrated using sequences of 29 species from GenBank.

Phylogenetic analysis

For non-coding genes, GBlocks deleted a large proportion of the data, even with the least stringent conditions. For example, in the alignment of the 16S sequences, up to 41 % of the alignment obtained with MUSCLE was

deleted. The MUSCLE alignments were thus checked by eye using GBlocks results as a guide for suppressing poorly aligned parts. The NJ analysis of the single-gene dataset displayed no significant incongruence and the genes were concatenated in an alignment of 4,282 bp. No supported incongruence between the trees obtained with ML and BI analysis was detected. The BI tree was used to summarize the results (Fig. 2) because it displayed more supported nodes. Nodes with high support in ML (bootstrap >70) are also well supported in BI, but several nodes were only supported in BI and are labelled on Fig. 2 with little stars.

For some families only a single species was available in our analysis, preventing us to test properly the monophyly of Bresiliidae (two genera), Disciadiidae (one genus), Campylonotidae (one genus), Gnathophyllidae (five genera), Hymenoceridae (two genera), Psalidopodidae (one genus) and Xiphocarididae (one genus). However, none of the samples belonging to these families are recovered nested within any of the other family clades. Monophyly is well-supported (BI > 0.96 and/or ML > 70) for 19 families: Acanthephyridae (seven genera), Alpheidae (more than ten genera), Alvinocarididae (seven genera), Anchistoidae (one genus), Atyidae (more than ten genera), Barbouriidae (three genera), Bathypalaemonellidae (two genera), Crangonidae (more than ten genera), Eugonatonotidae (one genus), Glyphocrangonidae (one genus), Nematocarcinidae (four genera), Oplophoridae (three genera), Pandalidae (more than ten genera), Pasiphaeidae (seven genera), Processidae (five genera), Rhynchocinetidae (two genera), Stylodactylidae (five genera) Thoridae (eight genera). Three families are non-monophyletic: the Lysmatidae (five genera) Hippolytidae (more than ten genera) and the Palaemonidae (more than ten genera). Relationships among family-level clades are at best poorly resolved with the exception of the superfamily Palaemonoidea that is well-supported (BI: 1, ML: 100). Families are though distributed among two major multi-familial clades, with a high support in BI (BI: 0.99 both), with the exception of Bathypalaemonellidae, Disciadiidae and Rhynchocinetidae which have a basal position. The first multi-familial clade (Clade I) includes Atyidae, Psalidopodidae, Stylodactylidae and Xiphocarididae. All other families belong to the second clade (Clade II).

Discussion

The aim of the study was also to test if the relationships among families may be resolved using currently used markers sequenced over a dense taxonomic coverage. The availability of specimens in the MNHN collection allowed us to enhance the taxonomic coverage of Caridean families for a multigene phylogenetic analysis. However, some taxa

are still missing either because they were rare or not available at all in MNHN collections or because the DNA was too degraded in specimens available in MNHN collection. With 28 families analyzed over 38, among which 21 are represented by at least 2 species, our taxonomic coverage was comparable with that of Bracken et al. (2009). The number of genetic markers analysed was comparable with that of Li et al. (2011) but included both nuclear and mitochondrial markers corresponding either to coding or non-coding genes. Among the ten lacking families most include a very small number of species and/or are associated to poorly sampled habitats. For example, the family Desmocarididae only contains one genus and two species (*Desmocaridus bisliniata* and *D. trispinosa*) both living in estuaries in Africa. Also these families are rare and the scarce material available in the MNHN collections was generally collected before 1990, explaining the poor amplification success.

At the family rank, the inclusion within the same dataset of an adequate sampling of major families of Caridea allows us to support with high confidence the monophyly hypothesized in the literature for twelve families (Acanthephyridae, Alpheidae, Alvinocarididae, Atyidae, Crangonidae, Glyphocrangonidae, Nematocarcinidae, Pandalidae, Processidae, Rhynchocinetidae, Stylodactylidae and Thoridae). We also provide new supports for Bathypalaemonellidae and Oplophoridae but also for two monogeneric families Anchistoidae and Eugonatonotidae. The monophyly of Oplophoridae, that was suggested in the analysis of Bracken et al. (2009) and then explored in more detail in Chan et al. (2010), is here corroborated thanks to a better coverage of other Caridean families. We also confirm the results of Bracken et al. (2009); Li et al. (2011); Short et al. (2013), recovering Palaemonidae polyphyletic and supporting the synonymizing of Kakaducarididae with Palaemonidae. The species traditionally included in Palaemonidae form two well supported clades (noted A and B on Fig. 2), one comprising the genera *Macrobrachium*, *Cryphiops*, and *Leptopalaemon* (previously belonged to the now abandoned family Kakaducarididae), and a second clade for the genera *Leander*, *Palaemon* and *Periclimenes*, sister to the Anchistoididae, Gnathophyllidae and Hymenoceridae families. As Palaemonidae is a very large family containing more than 100 genera with members exhibiting very different morphology, in depth morphological comparison and analysis are necessary to re-build a natural classification system to reflect the relationships within the superfamily Palaemonoidea.

The status of Hippolytidae remains unresolved. Among the species sampled in this family, specimens attributed to the genera *Lysmata* and *Ligur* form a clade with the Barbouriidae species of the genus *Parhippolyte*, whereas the position of the eight other Hippolytid species remains



◀ **Fig. 2** Caridea phylogeny: IB topology, with posterior probability shown when >0.94. *asterisk* nodes only supported in BI. All other nodes have similar support both in ML and in BI (bootstrap >70 in ML and posterior probability >0.96 in BI)

unresolved. These results are congruent with the study of Li et al. (2011) in which *Lysmata* is closely related to *Janicea antiguesis* (Barbouriidae). A very recent paper (De Grave et al. 2014) explored the status of Hippolytidae using several genes and a larger taxonomic coverage within clade B. This study confirmed that Hippolytidae as defined in De Grave and Fransen (2011) are not monophyletic. The strongly supported close relationships of *Eualus gaimardii* and *Lebbeus polaris* revealed by the present study agrees with the resurrection of the family Thoridae by De Grave et al. (2014). Although Barbouriidae is confirmed to be closely related to *Lysmata* spp. and *Ligur ensiferus*—two genera attributed to the family Lysmatidae recently resurrected by De Grave et al. (2014)—the present result revealed that *Ligur ensiferus* is sister to Barbouriidae with very strong support.

The Pasiphaeidae (with seven genera) are found monophyletic. However, since we were not able to obtain sequences for the second genus sampled in the MNHN collection (*Leptocheila*), this result does not challenge the results of Bracken et al. (2009). Similarly, Barbouriidae (with four genera) is recovered monophyletic but since only two species of the same genus are included this result remains to be confirmed with additional sampling of species from other genera.

The relationships among families are generally poorly resolved even so two major clades are supported. A first clade includes four families (Clade I: Atyidae, Psalidopodidae, Stylodactylidae and Xiphocarididae). Within clade I, two sister lineages are supported: Psalidopodidae and Stylodactylidae on one hand and Atyidae and Xiphocarididae on the other. The close relationship between the two latter families was already pointed out by Page et al. (2008a) and Bracken et al. (2009). Clade II is more diverse and includes 23 major lineages, most of them corresponding to traditionally defined families. However, the deeper nodes are not supported. Clade II includes the Palaemonoidea which is the only well-supported superfamily in the Caridea. Within this superfamily two clades may be distinguished. First a well-supported clade (BI: 1, ML: 98) includes *Macrobrachium*, *Cryphiops* and *Lep-topalaemon* (currently all attributed to Palaemonidae). Anchistioididae, Gnathophyllidae, Hymenoceridae and all other Palaemonidae genera form a second clade. Within the latter clade, Gnathophyllidae and Hymenoceridae are closely related. However, the sampling within these two families is still too restricted to validate their close relationships.

The five latest major taxonomic revisions within the Caridean classification are almost all corroborated. (1) Although we do not test the position of the Procarididae among other infraorder of Decapoda, the position of the two Procarididae included in the dataset as sister-group of the Caridea is congruent with their reclassification as a distinct infraorder. This node is well-supported (BI: 1, ML: 100) and the branch lengths do not suggest that this position results from a reconstruction artifact. Indeed branch lengths for the two Procarididae are close to that of other included outgroups, notably that of *C. pagurus*. (2) The Acanthephyridae and the Opolophoridae are two distinct lineages with high support (BI: 1, ML: 100 for both). However, the relationship between these two families is not resolved and thus the definition of the superfamily Opolophoroidea cannot be rejected. (3) Within the family Eugonatonotidae, the recent synonymization of *Galatheacarididae* with *Eugonatonotus* is supported (De Grave et al. 2010). (4) The recent synonymization of Kakaducarididae with Palaemonidae (Short et al. 2013) is also well supported. (5) Only the very recent separation of Hippolytidae by De Grave et al. (2014) is not completely supported. Although the present result also reveals that Lysmatidae is close to Barbouriidae, the former is showed to be polyphyletic. The monophyly of the resurrected Thoridae is strongly supported but Hippolytidae s.s. may still be polyphyletic.

Our analysis shows that additional data are needed to revise some families, notably Palaemonidae, Hippolytidae s.l. and Pasiphaeidae. However such a revision needs additional taxonomic sampling. Although not fully resolved at the deeper nodes, the phylogenetic hypothesis presented here may provide a guide for the taxonomic sampling of future family-level revisions and notably for the selection of adequate outgroups.

One of our working hypotheses was that higher taxonomic coverage will improve the phylogenetic resolution of multi-gene analyses. This approach allowed us to corroborate the proposed monophyly of some families but did not provide significant resolution for the deeper nodes of the Caridea tree. The poor success of amplification of nuclear coding genes points to the limits of PCR amplification associated with Sanger sequencing in multigene phylogenetic analyses. A phylogenomic analysis based on the generation of a large amount of through next-generation sequencing might provide the data needed for a better resolution between the oldest Caridean lineages. However, at present the genomic data available for the Caridea are rather scarce. No Caridean genome has been sequenced and only few transcriptome datasets are available for two Palaemonidae species, *M. rosenbergii* (Mohd-Shamsudin et al. 2013) and *M. nipponense* (Ma et al. 2012; Jin et al. 2013). The most documented type of genomic data remains

the mitogenomes that are available for 11 Caridean species (Miller et al. 2005; Ivey and Santos 2007; Shen et al. 2009; Qian et al. 2011; Kim et al. 2013; Yang et al. 2012, 2013). Indeed, sequencing complete mitochondrial genomes is easier and more affordable than sequencing complete genomes or transcriptomes. However, mitogenomics will provide the reconstruction of mitochondrion genealogies that might be only partly correlated to organisms' genealogy. The sequencing of complete genome for comparative studies is hampered by the very large and variable genome size within Caridea (Rees et al. 2008). Moreover, the analysis of the variation of the genome size across populations revealed unexpected intraspecific variation in the Alvinocarid shrimp *Mirocaris fortunata* (Bonnivard et al. 2009). In this context and with the objective of resolving deeper phylogenetic nodes, the sequencing of transcriptomes might be a way to define loci that are widely shared and not too variable to provide the adequate phylogenetic signal at this phylogenetic depth.

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Conflict of interest None.

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