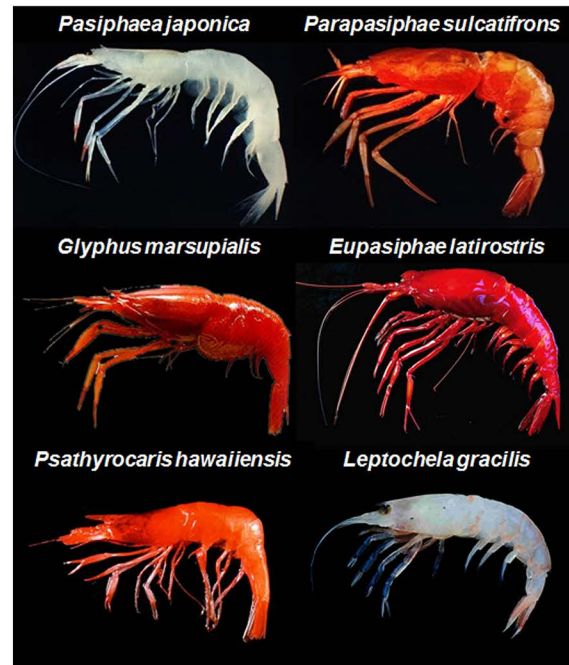
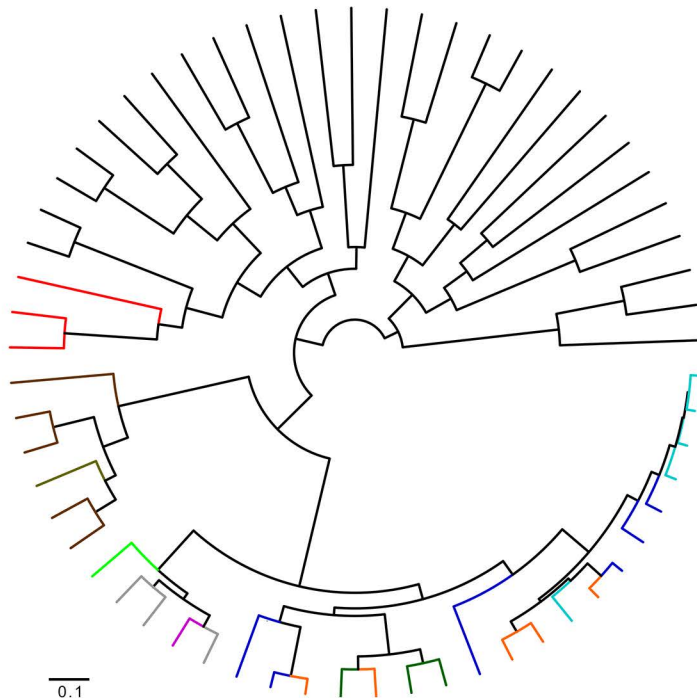


- "*Pasiphaea alcocki*" group
- "*Pasiphaea cristata*" group
- "*Pasiphaea sivado*" group
- Other *Pasiphaea*
- *Eupasiphae*
- *Parapasiphae*
- *Glyphus*
- *Leptochela* (*Leptochela*)
- *Leptochela* (*Proboloura*)
- *Psathyrocaris*
- Other carideans



**Highlights**

- The first molecular phylogeny of the caridean family Pasiphaeidae is herein constructed.
- Genetic data are generated for two mitochondrial and four nuclear markers.
- The monophyly of Pasiphaeidae is challenged.
- Systematic incongruence of the current classification is revealed.

**Molecular phylogeny of Pasiphaeidae (Crustacea, Decapoda, Caridea) reveals systematic incongruence of the current classification**

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## Graphical Abstract

See attachment.

## Highlights

- The first molecular phylogeny of the caridean family Pasiphaeidae is herein constructed.
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## Abstract

Caridean shrimps constitute one of the most diverse groups of decapod crustaceans, but relationships within this infraorder remain poorly understood. One of the systematically controversial families in Caridea is the predominantly pelagic Pasiphaeidae, which contains 101 species belonging to seven genera. Pasiphaeidae species possess high diversity in morphology and inhabit shallow to very deep waters (> 4000 m). The present work presents the first molecular phylogeny of this family, based on a combined dataset of six mitochondrial and nuclear gene markers (12S rDNA, 16S rDNA, histone 3, sodium–potassium ATPase  $\alpha$ -subunit, enolase and ATP synthase  $\beta$ -subunit) from 33 species belonged to six genera of Pasiphaeidae with 19 species from 12 other caridean families as outgroup taxa. Maximum likelihood and Bayesian inference analyses conducted on the concatenated dataset of 2265 bp suggest the family Pasiphaeidae is not monophyletic, with *Psathyrocaris* more closely related to other carideans than to the other five pasiphaeid genera included in this analysis. *Leptochela* occupies a sister position to the remaining genera and is genetically quite distant from them. At the generic level, the analysis supports the monophyly of *Pasiphaea*, *Leptochela* and *Psathyrocaris*, while *Eupasiphae* is paraphyletic, closely related to *Parapasiphae* and *Glyphus*. The present molecular phylogenetic study strongly suggests that some characters used in grouping the species of Pasiphaeidae may not be synapomorphic and the classification within the family needs to be revised.

**Keywords:** Decapoda; Caridea; Pasiphaeidae; Phylogenetics; Protein-coding genes

## 1. Introduction

Caridean shrimps (Infraorder Caridea) constitute the second most speciose group of decapod crustaceans, with 36 families and over 3400 species currently recognized (De Grave et al., 2015; De Grave and Fransen, 2011; De Grave et al., 2014; Short et al., 2013). Pasiphaeidae is a cosmopolitan family, members of which constitute an important component of the mesopelagic and bathypelagic planktonic communities, including the polar regions (Komai et al., 2012). Most pasiphaeids are pelagic and carry out Diel Vertical Migration (DVM) movements, feeding frequently on planktonic species at night (Cartes, 1993). Some species are very abundant and even exploited commercially (Motoh, 1999). As currently recognized, Pasiphaeidae contains seven genera with 101 species (De Grave and Fransen, 2011; Komai and Chan, 2012; Komai et al., 2012). Members of the family are largely characterized by the comb-like fingers of the chela of the first two pairs of pereopods, as well as joined incisor and molar processes on the mandible and well-developed exopods on the pereopods (Chace, 1992; Holthuis, 1993). Due to the combination of the latter two characters, the family has previously been considered to be a relatively basal group within Caridea (Chace, 1992; Thompson, 1967), although such a position is not supported in the phylogenetic analysis of Li et al. (2011). Rooted in the work of Spence Bate (1888) and Ortmann (1890), the pasiphaeid genera are currently treated as a single family Pasiphaeidae, previously placed in its own superfamily (see Chace, 1992; De Grave et al., 2009; Holthuis, 1955, 1993; Martin and Davis, 2001; Thompson, 1967). Although Paulson (1875) erected a separate family, Leptochelidae for the genus *Leptochela*, this has largely been ignored due to the similarity in mouthparts between *Leptochela* and the other pasiphaeid genera (see Chace, 1976). In marked contrast to the widely accepted classification, Christoffersen (1986) on the basis of a morphological cladistic analysis, placed the family Pasiphaeidae as well as seven other families in a superfamily Atyoidea, which he believed to be the basal group amongst his eight redefined caridean superfamilies (sensu Christoffersen, 1990).

Pasiphaeid shrimps are currently separated into seven genera (*Alainopasiphaea*, *Eupasiphae*, *Glyphus*, *Leptochela*, *Parapasiphae*, *Pasiphaea* and *Psathyrocaris*) based on characters such as the presence or absence of a mandibular palp, the number of arthrobranches

on the third maxilliped and the relative length of the pleopodal exopods (schematically represented in Fig. 1). The current classification scheme thus remains largely based on adult external morphological features following Chace (1992) and Holthuis (1993). This approach may not be accurate enough to elucidate the systematic status and phylogenetic relationships among genera (Fransen and De Grave, 2009) and does not take account of, for example, discrete inter-generic differences in the morphology of the foregut ossicles (see Felgenhauer and Abele, 1983, 1989) or their general ecologies. Accordingly, the current morphologically recognized genera need to be scrutinized by molecular phylogenetics. One such genus is *Leptochela*, currently comprises 15 species (De Grave and Fransen, 2011), divided into two subgenera *Leptochela* and *Proboloura* (Chace, 1976). Additionally, within the heterogeneous genus *Pasiphaea*, Hayashi (1999, 2004, 2006a, b), informally utilized several species groups, viz., “*P. alcocki*” group, “*P. cristata*” group and “*P. sivado*” group, but their validity and relationships have been challenged (see Komai et al., 2012).

To address such latent systematic controversies, molecular phylogenetics has been long recognized to be a powerful approach, including for Caridea and Decapoda in general. In the last decade, several caridean families have been analyzed in a broad scale molecular framework, followed by focused systematic revisions. For example, extensive systematic re-arrangements were done in Palaemonidae by De Grave et al. (2015), following the foundation work by Mitsuhashi et al. (2007), Kou et al. (2013), Gan et al. (2015) and Kou et al. (2015). To date, however, such a comprehensive body of work is not available for Pasiphaeidae, though the early analysis by Bracken et al. (2009) did include two genera from the family (*Pasiphaea* and *Leptochela*), which indicated the potential polyphyly of the family.

In the present study we provide a phylogenetic foundation for Pasiphaeidae based on the analysis of two mitochondrial genes: 12S rDNA and 16S rDNA, and four nuclear protein-coding genes: histone 3 (H3), sodium–potassium ATPase  $\alpha$ -subunit (NaK), enolase, and ATP synthase  $\beta$ -subunit (*atp $\beta$* ) to infer the systematic position of Pasiphaeidae within Caridea, as well as examine the relationships among and within pasiphaeid genera. While the first three nuclear markers are commonly used in phylogenetic studies of crustaceans (e.g. Aznar-Cormano et al., 2015; Li et al., 2011; Tsang et al., 2011; Tsang et al., 2008), ATP synthase  $\beta$ -subunit (*atp $\beta$* ) has been deployed in phylogenetic analyses of plants (Hoot et al.,

1995; Savolainen et al., 2000) and molluscs (Sharma et al., 2012), but not yet in crustaceans. We herein adopted this marker for decapod phylogenetics for the first time.

## 2. Materials and methods

### 2.1. Taxon sampling

Of the 101 species currently assigned to the family Pasiphaeidae (De Grave and Fransen, 2011; Komai and Chan, 2012; Komai et al., 2012), 33 species from six genera were included in the present study (Table 1), including respective type species of all 6 genera. *Alainopasiphaea* was the only genus of the family, which could not be included. This genus was separated from the “*Pasiphaea sivado*” group by the simpler branchial formula, notably the absence of arthrobranchs (Hayashi, 1999). Given the close morphological similarity between both genera, a sister position *Alainopasiphaea* - *Pasiphaea* could be expected, although perhaps alternatively the genus may not be valid and both known species of *Alainopasiphaea* may represent modified *Pasiphaea* species. Amongst the taxa analyzed, a total of 19 species of *Pasiphaea* were included, across the three species groups of Hayashi (1999, 2004, 2006a, b) (Table 1). To elucidate the systematic status of Pasiphaeidae within Caridea, all caridean species which had sequences available for at least four of the six markers, either downloaded from GenBank (largely generated by our team’s previous work, e.g. De Grave et al., 2014; Li et al., 2011) or newly generated herein were included (Table 1). In total, 28 species from 16 other caridean families were included for the subsequent analyses.

### 2.2. DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from pleopod muscle or eggs (10-15 mg) using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). Partial segments of six genes, 12S rDNA, 16S rDNA, H3, NaK, enolase, and *atpβ* were amplified from the diluted DNA. While universal primer sets were used to amplify the segments of the first five genes (Table 2), the

*atpβ* segment was amplified by nested PCR, with primers newly designed based on available sequences of other decapods in GenBank (Liang et al., 2010; Martinez-Cruz et al., 2011). PCR amplifications were conducted in 50-μL reaction mixture, containing 1-5 μL of template DNA, 1x PCR reaction buffer, 3 mM MgCl<sub>2</sub>, 200 nM of each primer, 200 μM dNTPs and 1.5 U of *Taq* polymerase (Takara, Japan). The PCR cycling profile was as follows: 3 min at 95°C for initial denaturation, then 40 cycles of 30 sec at 95°C, 30 sec at 48°C-55°C (see Table 2) and 1 min at 72°C, and 3 min at 72°C for final extension. After purification with Millipore MultiScreen 96-Well Filter Plates (Millipore, USA), PCR products were sequenced with the same primer sets of amplification on an ABI 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), following the standard sequencing protocol. The sequencing data were checked and confirmed by eye using SeqTrace (Stucky, 2012).

### 2.3. Data analyses

The sequences generated were blasted (Basic Local Alignment Search Tool, BLAST, National Center for Biotechnology Information, NCBI) against GenBank to avoid potential contamination. The nucleotide sequences were aligned using the MUSCLE program (Edgar, 2004) as implemented in MEGA 6.0 (Tamura et al., 2013). Alignments of the four nuclear protein-coding genes were further translated into their corresponding amino acid sequences to avoid indels or stop codons. Pairwise comparisons were examined using Kimura 2-parameter (K2P) distance (Kimura, 1980) as implemented in MEGA 6.0. Since many regions in the 12S and 16S rDNA datasets were ambiguous and difficult to align, GBlocks v0.91b (Castresana, 2000) was used to delete poorly aligned positions (parameters optimized for 12S/16S: minimum number of sequences for a conserved position = 24/31; minimum number of sequences for a flanking position = 39/51; maximum number of contiguous non-conserved positions = 8/8; minimum length of a block = 5/5; allowed gap positions = half/half).

The six-marker (with *atpβ*) and the five-marker (without *atpβ*) datasets were analyzed herein to evaluate this new marker. Both datasets were initially partitioned by markers, with the nuclear protein-coding markers further separated into three codon positions. The best partitioning strategy of the concatenated datasets and the best-fit model of DNA substitution



for each partition were then determined by PartitionFinder v1.1.1 (Lanfear et al., 2012), according to the Bayesian information criteria (BIC; Schwarz, 1978). Consequently, the six-gene dataset was divided into seven partitions with respective best-fit models of DNA substitution (Table 3), while the five-gene one divided into six partitions (Supplementary Table S1). The datasets were analyzed under maximum likelihood (ML) (Felsenstein, 1981) as implemented in RAxML v8.2.4 program (Stamatakis, 2014) and also Bayesian inference (BI) as implemented in BEAST v1.8.1 (Drummond et al., 2012) with computations performed on the computer cluster of the Cyberinfrastructure for Phylogenetic Research Project (CIPRES) at the San Diego Supercomputer Center. In the ML analysis, the model GTRGAMMAI was used for each partition with all free parameters estimated by RAxML. Confidence in the resulting topology was assessed using non-parametric bootstrap estimates (Felsenstein, 1985) with 1,000 replicates. In the BI analysis, Metropolis-coupled Monte Carlo (MCMC) Markov chains ran for 10,000,000 generations with model parameters estimated during the analysis. Chains were sampled every 1000 generations, and the trees before convergence were discarded as burn-in to ensure that the analysis had stabilized, which was determined by TRACER v1.6 (Rambaut et al., 2014). A maximum clade credibility tree of the remaining trees was constructed to assess clade support from posterior probability estimation. Each individual gene dataset was analyzed by ML and BI as outlined above. Each analysis was run three times to evaluate the consistency among runs.

Alternative systematic hypotheses were statistically examined in six-gene dataset using Bayes factor and the approximately unbiased (AU) test (Shimodaira, 2002). The null hypothesis ( $H_0$ ) was that there was no difference between trees. To analyze Bayes factor, constrained trees were obtained by constraining the topology in BEAUti v1.8.1 before Bayesian analyses. Harmonic means of different tree-likelihood values were obtained by sampling the posterior distribution after burn-in removed (calculated by TRACER v1.6). The Bayes factor was calculated as twice the difference in the harmonic means of  $\ln L$  scores between the unconstrained versus the constrained trees (Brandley et al., 2005; Nylander et al., 2004). Based on the criteria of Kass and Raftery (1995), values  $> 10$  indicate very strong evidence against  $H_0$ . To conduct the AU test, constrained trees were inferred by RAxML with topology constraints. Subsequently, the per site log Likelihoods for trees set were computed

in RAxML and the confidence values of  $H_0$  were estimated by CONSEL (Shimodaira and Hasegawa, 2001) with default setting. Six different hypotheses were tested in this study: (A) monophyly of the family Pasiphaeidae; (B) monophyly of the genus *Eupasiphae*; (C) monophyly of the subgenus *Leptochela* (*Leptochela*); (D) monophyly of the “*Pasiphaea alcocki*” species group; (E) monophyly of the “*Pasiphaea cristata*” species group; and (F) monophyly of the “*Pasiphaea sivado*” species group.

### 3. Results

In the present study, 47 12S rDNA sequences, 61 16S rDNA sequences, 61 H3 sequences, 60 NaK sequences, 52 enolase sequences and 33 *atpβ* sequences were used, of which 215 were newly generated sequences. The six-gene dataset comprised 2335 bp after excision of the poorly aligned rDNA positions by Gblocks; alignment gaps were designated as ‘-’ and missing data assigned as ‘N’ in all analyses. Individual gene trees from the six markers and the trees from the combined datasets were not mutually contradictory in the ML and BI analyses (ML bootstrap (BP) > 70 or BI posterior probability (PP) > 0.95), while the combined datasets show better resolution in higher relationships. Additionally, the topology of the trees from the six-marker dataset (Figs. 2, 3) and five-marker dataset (Supplementary Figs. S1, S2) does not differ significantly, justifying the applicability of *atpβ* in caridean phylogenetics. Thus, the ML and BI trees of the six-marker dataset were presented herein with ML BP and BI PP indicated on corresponding nodes (Figs. 2, 3).

At family level, Pasiphaeidae as currently defined was not monophyletic in our trees (Figs. 2, 3). The monophyly of Pasiphaeidae was rejected by Bayes factor (BF = 68.406, Table 4) but not by the AU test (P = 0.342, Table 4). Members of *Psathyrocaris* did not cluster with the remaining pasiphaeid genera, but instead formed a clade with eight of the other caridean families included in the analysis. However, this relationship was exclusively supported in the BI analysis although with high support (PP = 0.97). Within this clade, the *Psathyrocaris* - Alvinocarididae subclade (BP = 85%; PP = 1.0) was nested with other carideans, with these two taxa being reciprocally monophyletic with strong support (BP > 90%; PP > 0.95).

The other five pasiphaeid genera included in the analysis were separated into two highly supported clades (BP = 100%; PP = 1.0). One clade solely consisted of *Leptochela* species, whilst the other clade consisted of the included species of *Eupasiphae*, *Glyphus*, *Parapasiphae* and *Pasiphaea*. The phylogenetic relationships within each of these clades were well resolved in our analysis. The monophyly of *Pasiphaea* was strongly supported (BP = 98%; PP = 1.0); although in contrast *Eupasiphae* resolved as paraphyletic, with *Parapasiphae sulcatifrons* nested within (PP = 0.57). In the ML tree, *Glyphus marsupialis* was also nested within *Eupasiphae*, clustered with *Eupasiphae gilesii* and *P. sulcatifrons*, but with low support (BP = 64%). Monophyly of the genus *Eupasiphae* was rejected by Bayes factor (BF = 26.666, Table 4), but not by the AU test (P = 0.228, Table 4). Within *Pasiphaea*, the monophyly of the three species groups (“*P. alcocki*” group, “*P. cristata*” group and “*P. sivado*” group, see Table 1 for species composition) was rejected by both Bayes factor and the AU test (BF > 10, P < 0.05, Table 4). Nevertheless, the species of the *P. sivado* group did form a monophyletic group, albeit it with the closely-related *P. orientalis* included (BP = 100%; PP = 1.0). Within *Leptochela*, the subgenus *Leptochela* (*Leptochela*) was found to be paraphyletic due to the inclusion of *L. soelae*, the representative of the subgenus *Leptochela* (*Proboloura*) included in our analysis; logically monophyly of the subgenus *Leptochela* (*Leptochela*) was not supported (BF = 63.684, P < 0.05, Table 4).

The monophyly of all other families, which had more than one representative in the present analysis (i.e. AcanthePHYridae, Alpheidae, Alvinocarididae, Atyidae, Bathypalaemonellidae, Crangonidae, Glyphocrangonidae, Oplophoridae, Palaemonidae and Pandalidae), was highly supported in both trees (BP > 85%; PP = 1.0) except the paraphyletic position of AcanthePHYridae. The clade comprised of Alpheidae, Barbouriidae, Crangonidae, Glyphocrangonidae, Hippolytidae, Lysmatidae, Palaemonidae and Pandalidae (BP = 70%; PP = 0.64) was sister to the clade (BP = 70%; PP = 0.98) comprised of all other caridean taxa, including Pasiphaeidae.

#### 4. Discussion

The present molecular study seemingly supports the separation of *Psathyrocaris* into its

own family, as all three included members of the genus form a clade with Alvinocarididae rather than pasiphaeid genera. Certainly, the genetic distances between *Psathyrocaris* and “Pasiphaeidae” or the closely related family Alvinocarididae, respectively do fall within the range of inter-family rather than intra-family distances, especially in the conserved nuclear protein-coding genes (Table 5). However, the monophyly of Pasiphaeidae as currently defined, with *Psathyrocaris* included, was only rejected by Bayes factor but not the AU test. Although we recognize that future studies may indeed lend more support to the family level distinctness of *Psathyrocaris*, potentially necessitating the erection of a new family, we herein maintain the genus within Pasiphaeidae given the current level of equivocal support.

So far, there have been very few morphological studies on this strange, deep-water genus, hampered by its fragile body (Lin and Chan, 2001). In his key to pasiphaeid genera, Holthuis (1993) separates *Psathyrocaris* from the other genera by having a denticulate rostrum and distinct pleopods comprising a filiform exopod and a much shorter endopod. Furthermore, the genus possesses other distinct characters within the family, such as the non-reduced second maxillae and first maxilliped, presence of an exopod on the second maxilliped, and elongated exopods on the ambulatory pereopods (Holthuis, 1951). The evolutionary relevance of these morphological differences remains currently unknown.

In the present analysis, an affinity between *Psathyrocaris* and the deep-sea family Alvinocarididae is suggested, although morphologically this appears to be potentially spurious as their morphologies are rather divergent. Nevertheless, this affinity between *Psathyrocaris* and Alvinocarididae might be resolved with further morphological and molecular data sets, especially as potential family-level characters (e.g. P1-CP brush, foregut structure and larval characters) are scantily documented in both families.

Based on a broad analysis across many caridean families using mitochondrial 16S and nuclear 18S rDNA, Bracken et al. (2009) recovered Pasiphaeidae as polyphyletic and argued for the separation of *Leptochela* from Pasiphaeidae despite the unifying presence of pectinate chela and highly similar mouthparts. In the present six-marker trees, *Leptochela* does cluster with four other Pasiphaeidae genera. However, the genetic distance between the two clades is high, comparable to the divergence values between many caridean families (Table 5). For example, the mean K2P distance between these two lineages varies from 0.141 in *atpβ* to

0.286 in 12S rDNA, much higher than intra-clade distances (0.022-0.21; 0.071-0.119), and clearly within the range among the other caridean families herein examined (0.108-0.367). Nevertheless, as with *Psathyrocaris* discussed above, the monophyletic status of Pasiphaeidae was not rejected by the AU test, only by Bayes factor and we thus refrain herein from recognizing a further caridean family, Leptochelidae.

To work towards a falsification of the Pasiphaeidae concept as currently defined, it is highly recommended to conduct a comprehensive morphological and developmental analysis of all genera, which could usefully target a number of avenues, although much of this information is currently lacking for *Psathyrocaris*. (1) The caridean lobe is an external rounded projection on the basal part of the exopod of the first maxilliped, being a specific but variable feature within carideans (Felgenhauer and Abele, 1983). The differential degree of development in the hippolytid genera *Latreutes* and *Bythocaris* for example concurs with the recent resurrection of Bythocarididae (see De Grave et al., 2014). Among pasiphaeids the structure of the first maxilliped, not only the caridean lobe, differ considerably between *Pasiphaea* (see Holthuis, 1951) and *Leptochela* (see Chace, 1976). (2) The first pereopod carpo-propodal (P1-CP) antennal flagellar grooming brush, a unique specialization of some carideans used to groom the antennal flagellum, was thought to be phylogenetically informative at family level (De Grave and Goulding, 2011). For example, based on the considerable differences in P1-CP brush structure, Lysmatidae can be separated from Hippolytidae (De Grave et al., 2014). Among pasiphaeids, *Pasiphaea* species possess a rather distinct linear carpal brush with 13–14 elongated, serrulate setae at the basal part of the propodus. In contrast, *Leptochela* completely lacks the P1-CP brush (De Grave and Goulding, 2011). (3) Foregut ossicles have been suggested to be a useful phylogenetic character at family level (Felgenhauer and Abele, 1983, 1985, 1989), as indeed with Brachyura and Anomura (Brösing et al., 2007; Reimann et al., 2011). Among the pasiphaeids, *Leptochela* possesses a primitive well-developed gastric mill with a median tooth (Felgenhauer and Abele, 1983), whilst in contrast *Pasiphaea* has a less chitinized foregut without distinct ossicles nor a median tooth (Felgenhauer and Abele, 1989). (4) Hernández-Ávila et al. (2015) summarized selected larval features for a range of carideans, including pasiphaeids, generally finding intra-familial consistency. However, amongst pasiphaeids, the larval development

differs radically with *Leptochela* exhibiting extended development with five stages (Gurney, 1942) versus abbreviated larval development in *Pasiphaea* and *Parapasiphae* (Gurney, 1942; Hayashi and Miyake, 1969; Hernández-Ávila et al., 2015; Williamson, 1960).

The present study also rejects the reciprocal monophyly of the subgenera *L. (Leptochela)*, and *L. (Proboloura)*. Chace (1976) established the subgenus *L. (Proboloura)* for *L. (P.) carinata*, a species which differed from all other *Leptochela* then known, by the presence of a dorsal, moveable lappet on the sixth abdominal somite and the arrangement of the dorsal telson spines. Hanamura (1987) later added an Australian species, *L. (P.) soelae* to this subgenus, which exhibited those same features. The position of *L. (P.) soelae* in the present analysis clearly demonstrates that those characters are only species-level characteristics and no higher systematic importance should be accorded to them. We herein thus propose to no longer recognize the subgenus *Proboloura*.

The four remaining genera, *Pasiphaea*, *Eupasiphae*, *Glyphus* and *Parapasiphae* form a single clade, with *Pasiphaea* occupying a sister position to the other genera. These four genera show considerable similarity in caridean lobe shape (Crosnier, 1988; Holthuis, 1951; Tirmizi, 1969), P1-CP brush (De Grave and Goulding, 2011), larval characters (Gurney, 1942), body size as well as general ecological niches, i.e. meso- to bathy-pelagic. *Pasiphaea* is however morphologically rather distinct in the absence of a mandibular palp (Holthuis, 1993), a more reduced branchial formula (Thompson, 1967), and the absence of lateral carina on the carapace. In the *Eupasiphae* - *Glyphus* - *Parapasiphae* clade, the monophyly of *Eupasiphae* is rejected by Bayes factor but not by the AU test. Therefore, these three genera are kept separate for the time being, as congruence with any morphological evidence has not been established yet.

Within *Pasiphaea*, the most speciose genus of the family, three informal species groups were established by Hayashi (1999, 2004, 2006a, b) based on the posterior margin of the telson, spinulation pattern of the first pereopod and the number of pleurobranchs on the eighth thoracomere. However, none of these groups are shown to be monophyletic in the present analysis. The analysis does however indicate a close relationship between the “*P. sivado*” group and *P. orientalis*, a fact to which Komai et al. (2012) already alluded. On the other hand, species of the “*P. alcocki*” and “*P. cristata*” groups are scattered throughout the

*Pasiphaea* clade, suggesting that their common characters are not synapomorphic traits. It thus seems logical to henceforth no longer use these species group designations.

Our analysis clearly demonstrates considerable incongruence of the current classification of Pasiphaeidae. Although at present we do not recognize novel families for some genera nor suggest the synonymization of certain genera, it is evident that further studies may corroborate these findings and indeed it may become necessary to introduce big systematic changes in the family.

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## Figure Captions

**Figure 1.** Morphological classification schematic of pasiphaeid genera (inferred from Holthuis, 1993; Hayashi, 1999). Genera are indicated in italic with species number (in parentheses, inferred from De Grave and Fransen, 2011; Komai and Chan, 2012; Komai et al., 2012). Classified keys and corresponding character states are indicated as numbers and letters above branches, with detailed descriptions below.

**Figure 2.** Phylogenetic tree resolved by maximum likelihood analysis of DNA sequences based on the combined dataset of six genes. Bootstrap values (based on 1000 pseudoreplicates) from maximum likelihood analysis are indicated on each branch. Only values  $> 50\%$  are shown. Solid bars denote the current family classification, whilst species in different color indicate different genera/subgenera/species groups. Species with asterisk suffix represent type species in corresponding pasiphaeid genera.

**Figure 3.** Phylogenetic tree resolved by Bayesian inference analysis of DNA sequences based on the combined dataset of six genes. Bayesian posterior probabilities are indicated on each branch. Only posterior probabilities  $> 0.5$  are shown. Solid bars denote the current family classification, whilst species in different color indicate different genera/subgenera/species groups. Species with asterisk suffix represent type species in corresponding pasiphaeid genera.

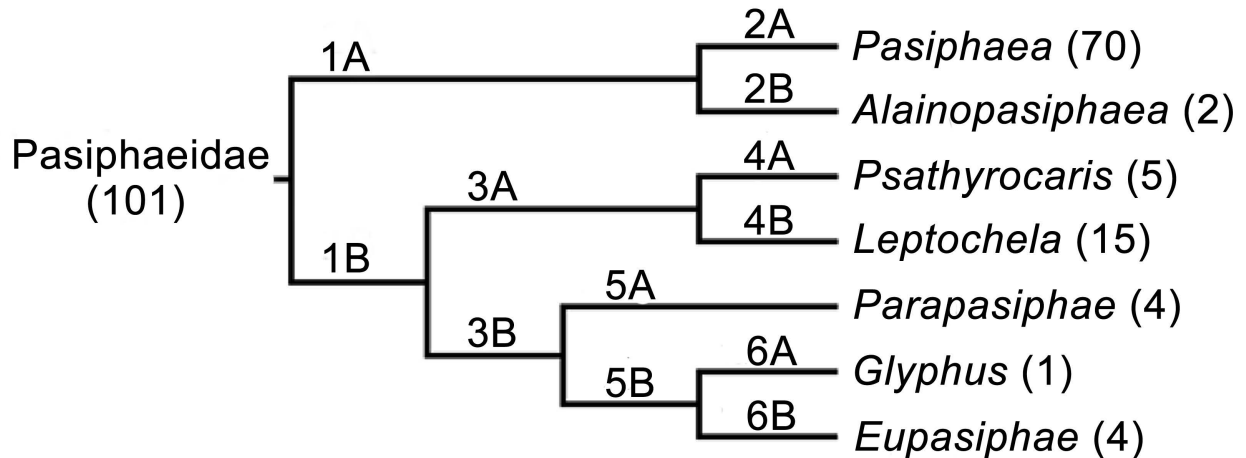
## Supporting Information

### Figure S1.

Phylogenetic tree resolved by maximum likelihood analysis of DNA sequences based on the combined dataset of five genes (without *atpβ*). Bootstrap values (based on 1000 pseudoreplicates) from maximum likelihood analysis are indicated on each branch. Only values > 50% are shown. Solid bars denote the current family classification, whilst species in different color indicate different genera/subgenera/species groups. Species with asterisk suffix represent type species in corresponding pasiphaeid genera.

### Figure S2.

Phylogenetic tree resolved by Bayesian inference analysis of DNA sequences based on the combined dataset of five genes (without *atpβ*). Bayesian posterior probabilities are indicated on each branch. Only posterior probabilities > 0.5 are shown. Solid bars denote the current family classification, whilst species in different color indicate different genera/subgenera/species groups. Species with asterisk suffix represent type species in corresponding pasiphaeid genera.



1A: mandible without a palp, rostrum formed by an erect postfrontal spine

1B: mandibular palp present, rostrum a normal forwards directed prolongation of the carapace

2A: arthrobranch present on pereopods

2B: arthrobranch absent on pereopods

3A: fourth pereopod longer than fifth, though sometimes shorter than third

3B: fourth pereopod distinctly shorter than either third or fifth

4A: third and fourth pereopods slender, of about equal length and not shorter than first, pleopods with the exopod very long and narrow, the endopod much shorter, rostrum dorsally with teeth

4B: fourth pereopod shorter than third, both much shorter than first, pleopods with exo- and endopod short and about equal in length, rostrum dorsally without teeth

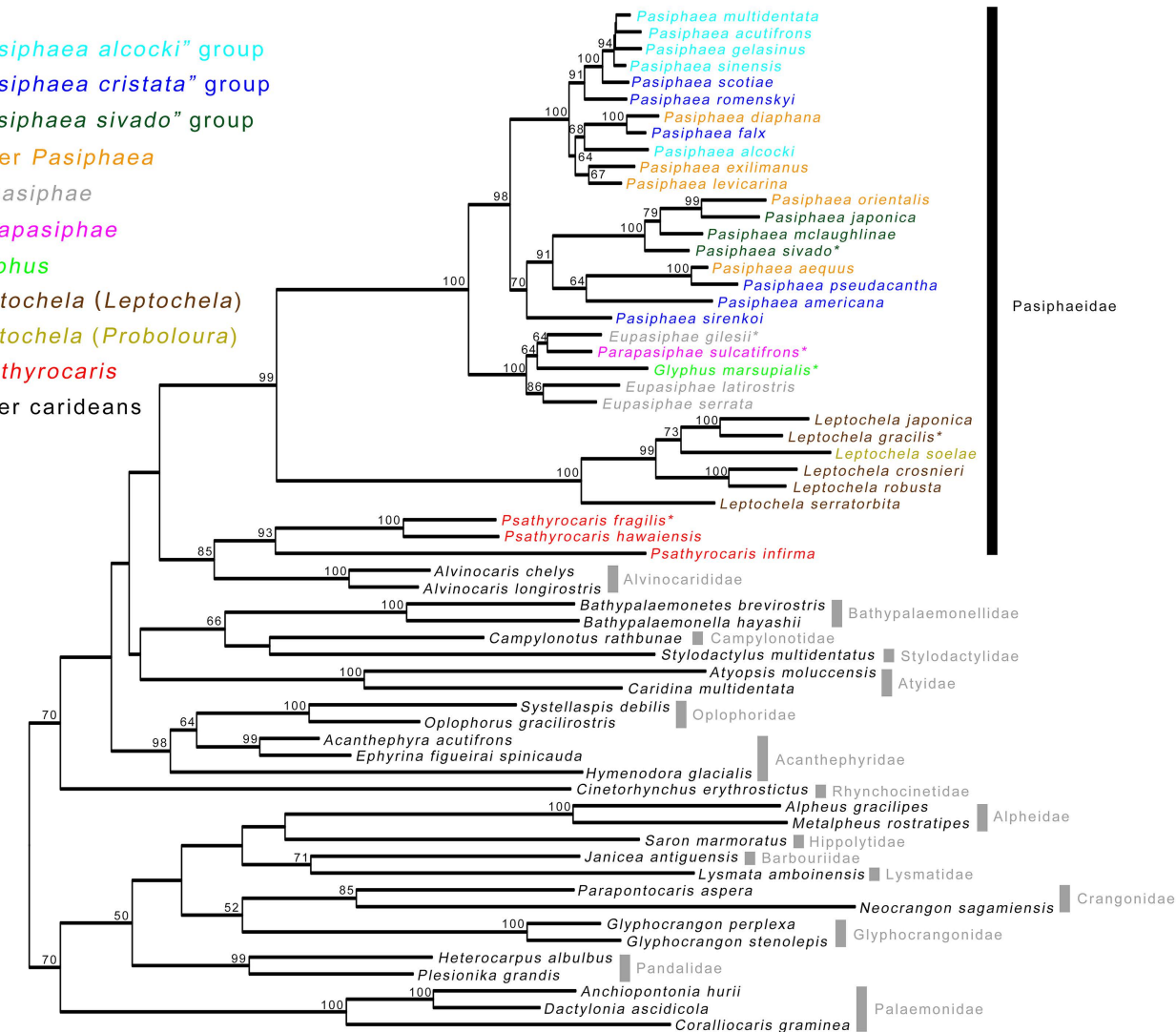
5A: antennal and branchiostegal spines absent, dorsal margin of carapace usually without teeth

5B: antennal and branchiostegal spines present, dorsal margin of carapace with teeth

6A: third maxilliped with one arthrobranch

6B: third maxilliped with two arthrobranches

- “*Pasiphaea alcocki*” group
- “*Pasiphaea cristata*” group
- “*Pasiphaea sivado*” group
- Other *Pasiphaea*
- *Eupasiphae*
- *Parapasiphae*
- *Glyphus*
- *Leptochela* (*Leptochela*)
- *Leptochela* (*Proboloura*)
- *Psathyrocaris*
- Other carideans





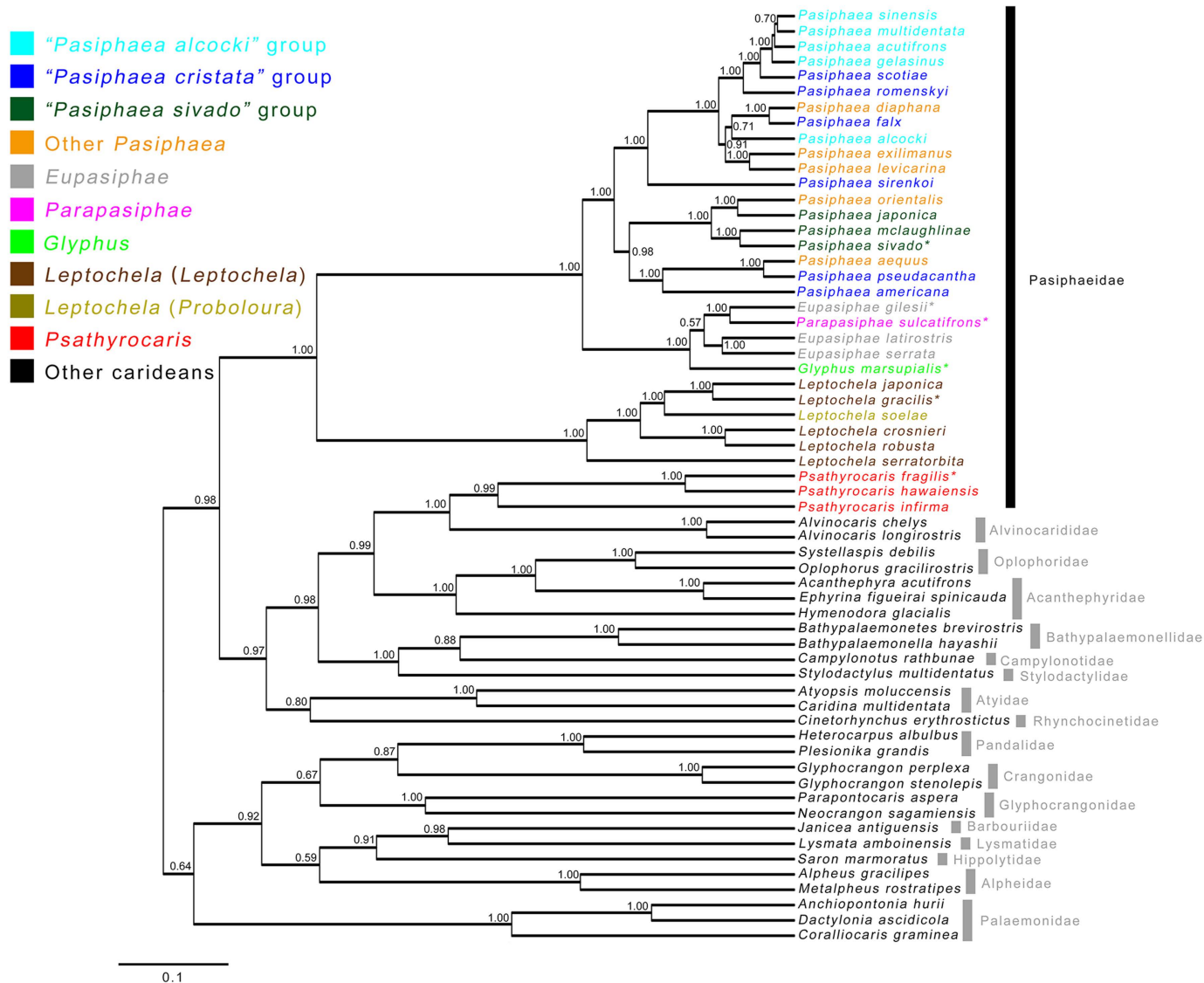


Table 1

Classification, sampling localities and GenBank accession numbers of species used in the present study.

Family	Species	Species group/  Subgenus	Voucher ID	Sampling locality	12S	16S	H3	NaK	enolase	atp8
Pasiphaeidae	<i>Eupasiphae gilesii</i> *		OUMNH.ZC.2011-07-023	Indian Ocean	MF279458	MF279497	MF279387	MF279423	MF279355	MF279321
	<i>Eupasiphae latirostris</i>		NTOU M02027	Taiwan	MF279459	MF279498	MF279388	MF279424	MF279356	MF279322
	<i>Eupasiphae serrata</i>		NTOU M02028	Taiwan	MF279460	MF279499	MF279389	MF279425	MF279357	MF279323
	<i>Glyphus marsupialis</i> *		MNHN IU-2014-6331	Papua New Guinea	MF279461	MF279500	MF279390	MF279426	MF279358	MF279324
	<i>Leptochela crosnieri</i>	<i>Leptochela</i>	OUMNH.ZC.2013-05-067	Australia	MF279462	MF279501	MF279391	MF279427	MF279359	MF279325
	<i>Leptochela gracilis</i> *	<i>Leptochela</i>	NTOU M02029	Taiwan	MF279463	MF279502	MF279392	MF279428	–	MF279326
	<i>Leptochela japonica</i>	<i>Leptochela</i>	NTOU M02030	Taiwan	MF279464	MF279503	MF279393	MF279429	MF279360	MF279327
	<i>Leptochela robusta</i>	<i>Leptochela</i>	NMV JS8410	Australia	MF279465	MF279504	MF279394	MF279430	–	MF279328
	<i>Leptochela serratorbita</i>	<i>Leptochela</i>	OUMNH.ZC.2009-18-006	Panama	–	MF279505	MF279395	MF279431	MF279361	MF279329
	<i>Leptochela soelae</i>	<i>Proboloura</i>	MNHN IU-2014-6332	Solomon Islands	–	MF279506	MF279396	MF279432	–	MF279330
	<i>Parapasiphae sulcatifrons</i> *		NTOU M02031	Taiwan	MF279466	MF279507	MF279397	MF279433	MF279362	MF279331
	<i>Pasiphaea acutifrons</i>	<i>alcocki</i> group	OUMNH.ZC.2007-21-004	South Georgia	MF279467	MF279508	MF279398	MF279434	MF279363	MF279332
	<i>Pasiphaea aequus</i>		NTOUM 02032	Taiwan	MF279468	MF279509	MF279399	MF279435	MF279364	MF279333
	<i>Pasiphaea alcocki</i>	<i>alcocki</i> group	NTOU M01089	Taiwan	MF279469	MF279510	MF279400	MF279436	MF279365	MF279334
	<i>Pasiphaea americana</i>	<i>cristata</i> group	OUMNH.ZC.2014-01-056	Mexico	MF279470	MF279511	MF279401	MF279437	MF279366	MF279335
	<i>Pasiphaea diaphana</i>		OUMNH.ZC.2011-07-004	Indian Ocean	MF279471	MF279512	MF279402	MF279438	MF279367	MF279336
	<i>Pasiphaea exilimanus</i>		NTOU M01074	Taiwan	MF279472	MF279513	MF279403	–	–	MF279337
	<i>Pasiphaea falk</i>	<i>cristata</i> group	NTOU M01067	Taiwan	MF279473	MF279514	MF279404	MF279439	MF279368	MF279338
	<i>Pasiphaea gelasinus</i>	<i>alcocki</i> group	OUMNH.ZC.2011-07-024	Indian Ocean	MF279474	MF279515	MF279405	MF279440	MF279369	MF279339
	<i>Pasiphaea japonica</i>	<i>sivado</i> group	NTOU M01046	Taiwan	MF279475	MF279516	MF279406	MF279441	MF279370	MF279340
	<i>Pasiphaea levicarinata</i>		NTOU M01103	Taiwan	MF279476	MF279517	MF279407	MF279442	MF279371	MF279341
	<i>Pasiphaea mclaughlinae</i>	<i>sivado</i> group	NTOU M01057	Taiwan	MF279477	MF279518	MF279408	MF279443	MF279372	MF279342
	<i>Pasiphaea multidentata</i>	<i>alcocki</i> group	OUMNH.ZC.2004-21-009	Norway	MF279478	MF279519	MF279409	MF279444	MF279373	MF279343
	<i>Pasiphaea orientalis</i>		NTOU M00108	Taiwan	MF279479	MF279520	MF279410	MF279445	MF279374	–
	<i>Pasiphaea pseudacantha</i>	<i>cristata</i> group	NMV JS9333	Australia	MF279480	MF279521	MF279411	MF279446	MF279375	–
	<i>Pasiphaea romenskyi</i>	<i>cristata</i> group	OUMNH.ZC.2011-07-115	Indian Ocean	MF279481	MF279522	MF279412	MF279447	MF279376	MF279344
	<i>Pasiphaea scotiae</i>	<i>cristata</i> group	OUMNH.ZC.2007-21-009	South Georgia	MF279482	MF279523	MF279413	MF279448	MF279377	MF279345
	<i>Pasiphaea sinensis</i>	<i>alcocki</i> group	NTOU M02033	East China Sea	MF279483	MF279524	MF279414	MF279449	MF279378	MF279346
	<i>Pasiphaea sirenkoi</i>	<i>cristata</i> group	NTOU M01068	Taiwan	MF279484	MF279525	MF279415	MF279450	MF279379	MF279347
	<i>Pasiphaea sivado</i> *	<i>sivado</i> group	OUMNH.ZC.2004-21-033	Norway	MF279485	MF279526	MF279416	MF279451	MF279380	MF279348
	<i>Psathyrocaris fragilis</i> *		NTOU M02034	Taiwan	MF279486	MF279527	MF279417	MF279452	MF279381	MF279349
	<i>Psathyrocaris hawaiiensis</i>		NTOU M02035	Taiwan	MF279487	MF279528	MF279418	MF279453	MF279382	MF279350
	<i>Psathyrocaris infirma</i>		NTOU M02036	Pratas	MF279488	MF279529	MF279419	MF279454	MF279383	–
Acanthephyridae	<i>Acanthephyra acutifrons</i>		HBG 1265	Gulf of Mexico	<a href="#">KP075952</a>	<a href="#">KP075877</a>	<a href="#">KP076083</a>	<a href="#">KP076036</a>	–	–
	<i>Ephyrina figueirai spinicauda</i>		NTOU M01847	Taiwan	<a href="#">KP075966</a>	<a href="#">KP075911</a>	<a href="#">KP076105</a>	<a href="#">KP076038</a>	–	–
	<i>Hymenodora glacialis</i>		HBG 96	North Atlantic	<a href="#">KP076019</a>	<a href="#">KP075909</a>	<a href="#">KP076134</a>	<a href="#">KP076048</a>	–	–
Alpheidae	<i>Alpheus gracilipes</i>		OUMNH.ZC.2011-05-021	Moorea	–	<a href="#">KF023114</a>	<a href="#">JF346335</a>	<a href="#">JF346371</a>	<a href="#">JF346299</a>	–
	<i>Metapheus rostratipes</i>		OUMNH.ZC.2011-05-028	Moorea	–	<a href="#">KF023115</a>	<a href="#">JF346336</a>	<a href="#">JF346372</a>	<a href="#">JF346300</a>	–

Alvinocarididae	<i>Alvinocaris longirostris</i>	NTOU M01014	Okinawa	MF279489	MF279530	<u>JF346315</u>	<u>JF346351</u>	<u>JF346279</u>	—
	<i>Alvinocaris chelys</i>	NTOU M01671	Taiwan	MF279490	MF279531	MF279420	MF279455	MF279384	MF279352
Atyidae	<i>Atyopsis moluccensis</i>	NTOU M00728	Hong Kong	MF279491	<u>KF023110</u>	<u>JF346322</u>	<u>JF346358</u>	<u>JF346286</u>	—
	<i>Caridina multidentata</i>	NTOU M01002	Taiwan	—	<u>KF023111</u>	<u>JF346303</u>	<u>JF346339</u>	<u>JF346267</u>	—
Barbouriidae	<i>Janicea antiquensis</i>	OUMNH.ZC.2004-15-002	Cape Verde	—	<u>KF023112</u>	<u>JF346333</u>	<u>JF346369</u>	<u>JF346297</u>	—
Bathypalaemonellidae	<i>Bathypalaemonetes brevirostris</i>	NTOU M01007	Taiwan	MF279492	MF279532	<u>JF346306</u>	<u>JF346342</u>	<u>JF346270</u>	—
	<i>Bathypalaemonella hayashii</i>	NTOU M02037	Taiwan	MF279493	MF279533	MF279421	MF279456	MF279385	MF279353
Campylonotidae	<i>Campylonotus rathbunae</i>	NMV J54342	Australia	MF279494	MF279534	<u>JF346314</u>	<u>JF346350</u>	<u>JF346278</u>	—
Crangonidae	<i>Parapontocaris aspera</i>	NTOU M01012	Taiwan	—	<u>KF023107</u>	<u>JF346302</u>	<u>JF346338</u>	<u>JF346266</u>	—
	<i>Neocrangon sagamiensis</i>	NTOU M01011	Taiwan	—	<u>KF023106</u>	<u>JF346301</u>	<u>JF346337</u>	<u>JF346265</u>	—
Glyphocrangonidae	<i>Glyphocrangon perplexa</i>	NTOU M00706	Taiwan	—	<u>KF023104</u>	<u>JF346325</u>	<u>JF346361</u>	<u>JF346289</u>	—
	<i>Glyphocrangon stenolepis</i>	NTOU M01013	Taiwan	—	<u>KF023103</u>	<u>JF346308</u>	<u>JF346344</u>	<u>JF346272</u>	—
Hippolytidae	<i>Saron marmoratus</i>	NTOU M01009	Taiwan	—	<u>KF023102</u>	<u>JF346312</u>	<u>JF346348</u>	<u>JF346276</u>	—
Lysmatidae	<i>Lysmata amboinensis</i>	MSLKHC-CA23Lyamb	Hong Kong	—	<u>KF023091</u>	<u>JF346318</u>	<u>JF346354</u>	<u>JF346282</u>	—
Oplophoridae	<i>Systellapsis debilis</i>	HBG 910	Gulf of Mexico	<u>KP076010</u>	<u>KP075871</u>	<u>KP076056</u>	<u>KP076042</u>	—	—
	<i>Oplophorus gracilirostris</i>	HBG 907	Gulf of Mexico	<u>KP075983</u>	<u>KP075918</u>	<u>KP076069</u>	<u>KP076044</u>	—	—
Palaemonidae	<i>Anchiopontonia hurii</i>	NTOU M01830	Okinawa, Japan	<u>KJ019536</u>	<u>KF738358</u>	<u>KF738309</u>	<u>KF738338</u>	<u>KF738295</u>	—
	<i>Coralliocaris graminea</i>	NTOU M00935	Taiwan, China	<u>KJ019548</u>	<u>KF738361</u>	<u>KF738313</u>	<u>KF738343</u>	<u>KF738298</u>	—
	<i>Dactylosia ascidicola</i>	NTOU M01549	Taiwan	<u>KJ019563</u>	<u>KF738363</u>	<u>KF738317</u>	<u>KF738345</u>	<u>KF738300</u>	—
Pandalidae	<i>Heterocarpus albulus</i>	NTOU M00707	Taiwan	—	<u>KF023105</u>	<u>JF346327</u>	<u>JF346363</u>	<u>JF346291</u>	—
	<i>Plesionika grandis</i>	NTOU M01010	Taiwan	—	<u>KF023113</u>	<u>JF346329</u>	<u>JF346365</u>	<u>JF346293</u>	—
Rhynchocinetidae	<i>Cinetorhynchus erythrostictus</i>	NTOU M00690	Taiwan	MF279495	MF279535	<u>JF346309</u>	<u>JF346345</u>	<u>JF346273</u>	—
Stylodactylidae	<i>Stylodactylus multidentatus</i>	NTOU M02038	Taiwan	MF279496	MF279536	MF279422	MF279457	MF279386	MF279354

Classification follows De Grave and Fransen (2011) and Komai et al. (2012), with modifications by De Grave et al. (2014) and De Grave et al. (2015). Species with asterisk suffix represent type species; “—” represents missing data; underlined accession numbers represent sequences obtain from GenBank. Voucher specimens are deposited in NTOU (National Taiwan Ocean University, Keelung), OUMNH-ZC (Oxford University Museum of Natural History, Zoological Collection, UK), MNHN (Muséum national d’Histoire naturelle, Paris), NMV (Museum Victoria, Melbourne) and MSLKHC (Marine Science Laboratory, The Chinese University of Hong Kong).

**Table 2**

Primer information used for PCR amplification. T, annealing temperature; L, lengths of PCR products.

Gene/Primer	Sequence (5'-3')	T (°C)	L (bp)	References
<i>12S</i>		48	~550	
12S-FB	GTG CCA GCA GCT GCG GTT A			Tsang et al. (2009)
12S-R2	CCT ACT TTG TTA CGA CTT ATC TC			Tsang et al. (2009)
<i>16S</i>		48	~550	
16S-AR	CGC CTG TTT ATC AAA AAC AT			Simons et al. (1994)
16S-1472	AGA TAG AAA CCA ACC TGG			Crandall and Fitzpatrick (1996)
<i>H3</i>		53	~380	
H3-AF	ATG GCT CGT ACC AAG CAG ACV GC			Colgan et al. (1998)
H3-AR	ATA TCC TTR GGC ATR ATR GTG AC			Colgan et al. (1998)
<i>NaK</i>		55	~550	
NaK-N79	GAY AAR WCC TCY GAA GGT TGG AA			De Grave et al. (2014)
NaK-N610	RGG AGG ATC RAT CAT RGA CAT			De Grave et al. (2014)
<i>enolase</i>		53	~400	
Enol-EA2	AGT TGG CTA TGC AGG ART TYA TGA T			Tsang et al. (2011)
Enol-ES2	ACC TGG TCG AAT GGR TCY TC			Tsang et al. (2011)
<i>atpβ</i>				
atpβ-F1	GCY GGW GTM GGC AAG ACT	53	~500	This study
atpβ-R1	ATA CCC ARC TCG GCA ATA			This study
atpβ-F2	AAG GCT CAY GGT GGT TAY TC	55	~450	This study
atpβ-R2	TAC CCA RCT CGG CAA TAC			This study

**Table 3**

Best partitioning scheme and best-fit substitution models of the six-gene dataset selected by PartitionFinder.

1, first codon; 2, second codon; 3, third codon.

Partition	Model
<i>12S, 16S</i>	GTR + I + G
<i>H3 1, atpβ 1</i>	SYM+I
<i>H3 2, atpβ 2</i>	K80
<i>H3 3</i>	GTR + I + G
<i>enolase 1, NaK 1</i>	GTR + I + G
<i>enolase 2, NaK 2</i>	GTR + I + G
<i>enolase 3, NaK 3, atpβ 3</i>	GTR+G

**Table 4**

Bayes factor testing and the AU test of phylogenetic hypotheses. In Bayes factor testing, the higher the value of Bayes factor implies stronger support against the monophyly of a particular group. In the AU test, P-Value < 0.05 indicates significant support against the monophyly of a particular group.

Hypotheses	Bayes factor testing			AU test
	Harmonic mean	Bayes factor (BF)	Evidence	P-Value
Unconstrained tree	-32462.533			
Monophyletic Pasiphaeidae	-32496.737	68.406	Very strong against	0.342
Monophyletic <i>Eupasiphae</i>	-32475.866	26.666	Very strong against	0.228
Monophyletic <i>Leptochela</i> ( <i>Leptochela</i> )	-32494.375	63.684	Very strong against	0.037
Monophyletic “ <i>Pasiphaea alcocki</i> ” species group	-32530.604	136.142	Very strong against	< 0.001
Monophyletic “ <i>Pasiphaea cristata</i> ” species group	-33122.315	1319.564	Very strong against	< 0.001
Monophyletic “ <i>Pasiphaea sivado</i> ” species group	-32485.578	46.088	Very strong against	0.047

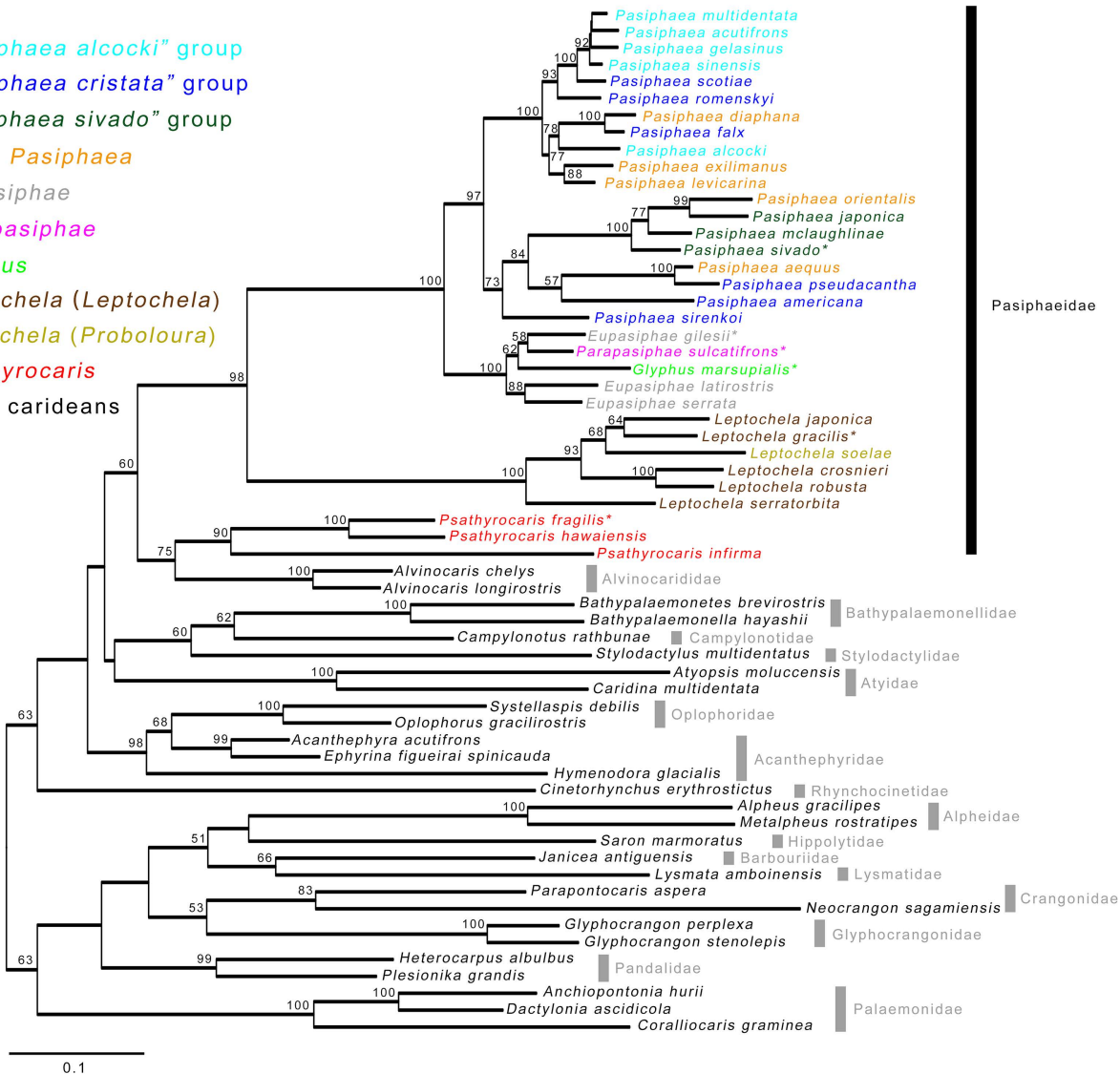
**Table 5**

Summary of pairwise distance of 12S, 16S, H3, NaK, enolase and *atpβ*, showing number of pairwise comparisons (in parentheses), the average and range (in square brackets) of K2P distance in different groups.

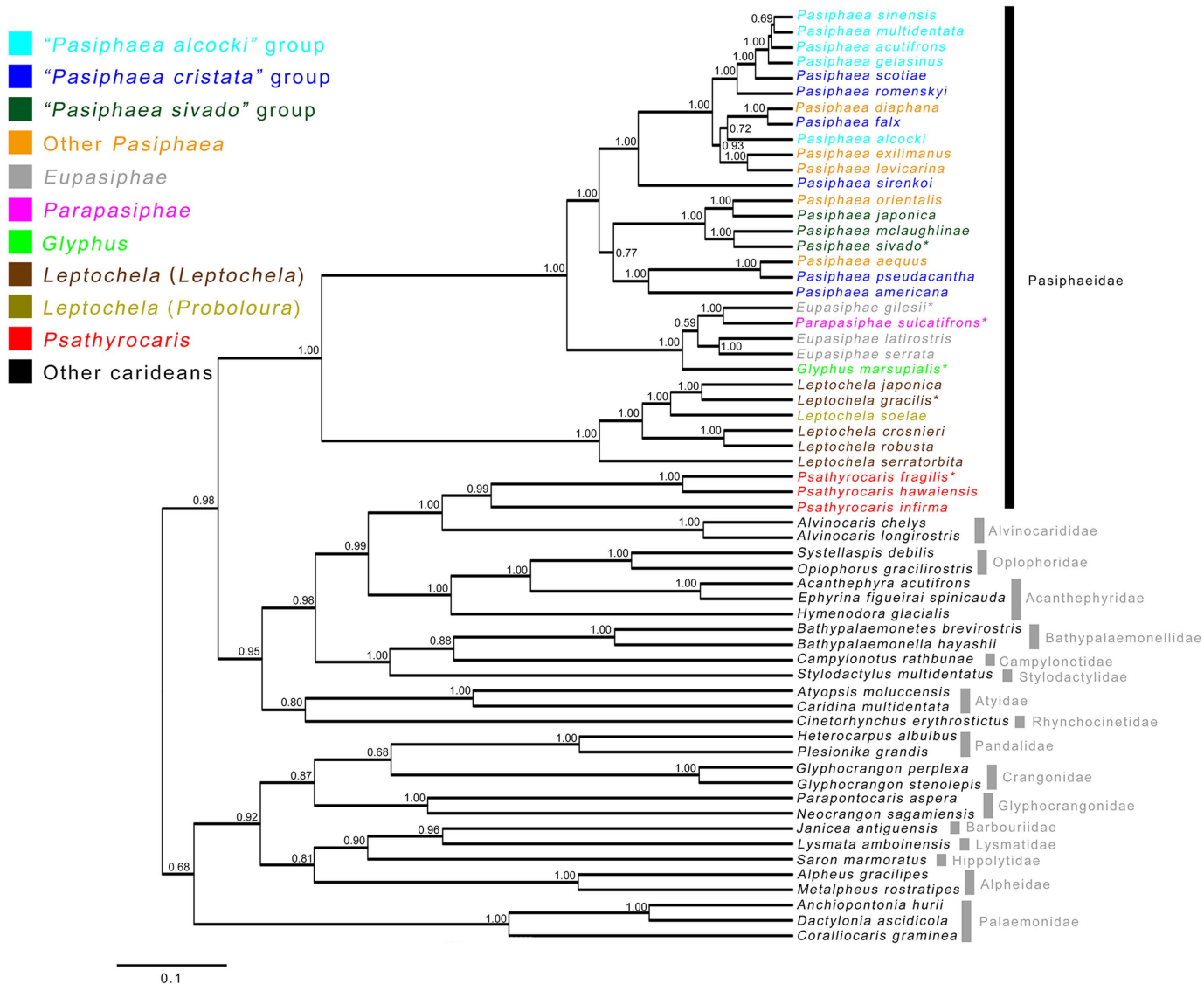
	12S	16S	H3	NaK	enolase	<i>atpβ</i>
Within mainstream Pasiphaeidae clade*	0.21 (276) [0.04-0.33]	0.197 (276) [0.027-0.290]	0.046 (276) [0-0.102]	0.041 (253) [0.002-0.076]	0.048 (253) [0.005-0.086]	0.022 (231) [0-0.046]
Within <i>Leptochela</i>	0.119 (6) [0.1-0.133]	0.119 (15) [0.065-0.165]	0.078 (15) [0.035-0.106]	0.088 (15) [0.031-0.119]	0.088 (3) [0.087-0.09]	0.071 (15) [0.023-0.111]
Within <i>Psathyrocaris</i>	0.193 (3) [0.155-0.219]	0.174 (3) [0.114-0.223]	0.1 (3) [0.034-0.134]	0.116 (3) [0.055-0.15]	0.127 (3) [0.033-0.178]	0.041 (1) [–]
Within Alvinocarididae	0.097 (1) [–]	0.098 (1) [–]	0.061 (1) [–]	0.033 (1) [–]	0.042 (1) [–]	–
Mainstream Pasiphaeidae clade vs. <i>Leptochela</i>	0.286 (96) [0.236-0.366]	0.265 (144) [0.223-0.334]	0.148 (144) [0.12-0.181]	0.186 (138) [0.166-0.219]	0.22 (69) [0.19-0.263]	0.141 (132) [0.099-0.192]
Mainstream Pasiphaeidae clade vs. <i>Psathyrocaris</i>	0.296 (72) [0.204-0.38]	0.282 (72) [0.196-0.326]	0.112 (72) [0.088-0.149]	0.151 (69) [0.135-0.177]	0.237 (69) [0.214-0.261]	0.114 (44) [0.1-0.124]
<i>Leptochela</i> vs. <i>Psathyrocaris</i>	0.231 (12) [0.203-0.27]	0.249 (18) [0.221-0.275]	0.154 (18) [0.12-0.188]	0.209 (18) [0.187-0.241]	0.26 (9) [0.242-0.279]	0.17 (12) [0.136-0.209]
<i>Psathyrocaris</i> vs. Alvinocarididae	0.224 (6) [0.205-0.244]	0.214 (6) [0.194-0.238]	0.117 (6) [0.091-0.157]	0.105 (6) [0.081-0.143]	0.193 (6) [0.161-0.232]	0.093 (2) [0.086-0.101]
Between caridean families excluding Pasiphaeidae	0.367 (111) [0.18-0.549]	0.307 (364) [0.166-0.482]	0.155 (364) [0.057-0.222]	0.173 (364) [0.074-0.342]	0.197 (243) [0.124-0.301]	0.108 (3) [0.096-0.118]

\* include all Pasiphaeidae species except *Leptochela* and *Psathyrocaris*.

- “*Pasiphaea alcocki*” group
- “*Pasiphaea cristata*” group
- “*Pasiphaea sivado*” group
- Other *Pasiphaea*
- *Eupasiphae*
- *Parapasiphae*
- *Glyphus*
- *Leptochela* (*Leptochela*)
- *Leptochela* (*Proboloura*)
- *Psathyrocaris*
- Other carideans







**Table S1**

Best partitioning scheme and best-fit substitution models of the five-gene dataset selected by PartitionFinder.

1, first codon; 2, second codon; 3, third codon.

Partition	Model
<i>12S, 16S</i>	GTR + I + G
<i>H3 1, enolase 1, NaK 1</i>	GTR + I + G
<i>H3 2</i>	K80
<i>H3 3</i>	GTR + I + G
<i>enolase 2, NaK 2</i>	GTR + I + G
<i>enolase 3, NaK 3</i>	GTR + I +G