



# Molecular and morphological phylogeny of hooded shrimps, genera *Betaeus* and *Betaeopsis* (Decapoda, Alpheidae): Testing the center of origin biogeographic model and evolution of life history traits

Arthur Anker<sup>a,1</sup>, J. Antonio Baeza<sup>b,c,d,\*</sup>

<sup>a</sup>Instituto de Ciências do Mar (Labomar), Universidade Federal do Ceará (UFC), Fortaleza, Ceará, Brazil

<sup>b</sup>Department of Biological Sciences, Old Dominion University, Norfolk, VA 23435, USA

<sup>c</sup>Smithsonian Marine Station at Fort Pierce, 701 Seaway Drive, Fort Pierce, FL 34949, USA

<sup>d</sup>Departamento de Biología Marina, Facultad de Ciencias del Mar, Universidad Católica del Norte, Larrondo 1281, Coquimbo, Chile

## ARTICLE INFO

### Article history:

Received 3 October 2011

Revised 23 April 2012

Accepted 24 April 2012

Available online 8 May 2012

### Keywords:

Cladistics

Sociobiology

Character mapping

Biogeography

Symbiosis

Center of origin

## ABSTRACT

The phylogenetic relationships of the alpheid shrimp genera *Betaeus* (Dana, 1852) (15 species) and *Betaeopsis* (Yaldwyn, 1971) (three species), collectively known as hooded shrimps, are analyzed with morphological, molecular (16S and H3) and combined “total evidence” (morphology + DNA) datasets. The tree topology resulting from morphological and combined analyses places *Betaeus jucundus* as sister to all the remaining species of *Betaeus* and *Betaeopsis*, rendering *Betaeus* paraphyletic. On the other hand, *Betaeopsis* is recovered as monophyletic. *Betaeus australis* is positioned as sister to the remaining species of *Betaeus s. str.* (excluding *B. jucundus*), which is composed of three well-supported and resolved clades. Mapping of biogeographic traits on the combined tree suggests at least two possible historic scenarios. In the first scenario, the North-East Pacific harboring the highest diversity of hooded shrimps (seven species of *Betaeus*), acted as the “center of origin”, where species appeared, matured and eventually migrated toward peripheral regions. In the second scenario, *Betaeus + Betaeopsis* originated in the southern Indo-West Pacific and subsequently colonized the North-East Pacific, where a major radiation involving dispersal/vicariance events took place. The mapping of life history traits (symbiosis vs. free living and gregariousness vs. single/pair living) in the combined tree suggests (1) that different types of symbioses with dissimilar host organisms (sea urchins, abalones, other decapods, spoon worms) evolved independently more than once in the group (in *B. jucundus* and in various lineages of *Betaeus s. str.*), and (2) that gregariousness was ancestral in the *Betaeus s. str. – Betaeopsis* clade and later shifted toward single/pair living in several lineages.

Published by Elsevier Inc.

## 1. Introduction

Within the crustacean order Decapoda, caridean shrimps exhibit a remarkable morphological, ecological, and behavioral diversity (Bauer, 2004). Recent studies on the Caridea, especially those combining molecular and/or morphological approaches with behavior and/or ecology, have revealed many unusual traits and the conditions favoring their evolution. Examples include the multiple origins of eusociality in the alpheid genus *Synalpheus* Bate, 1888 (Duffy, 1996; Morrison et al., 2004), the evolution of a powerful snapping claw in concert with peculiar eye-protecting structures (carapacial orbital hoods) in the family Alpheidae (Anker

et al., 2006), and the evolution of extreme female-biased sex allocation and social monogamy in sequential-simultaneous hermaphrodites in the hippolytid genus *Lysmata* Risso, 1816 (Bauer, 2004; Baeza, 2010). Our knowledge of the evolutionary history and ecological diversity of caridean shrimps has increased substantially over the past decades. Nonetheless, the behavior and auto-ecology of numerous species and the phylogenetic relationships of many groups remain largely unknown.

Within the family Alpheidae, one of the most species-rich shrimp clades (De Grave et al., 2009), the genus *Betaeus* (Dana, 1852) represents a model group to study evolutionary and biogeographic processes in the marine environment. For instance, it is possible to explore the evolution of some life history traits (social monogamy, symbiotic relationships) of these shrimps and the conditions favoring them. The 16 currently recognized species of *Betaeus* are collectively known as “hooded shrimps” or “visored shrimps” (Butler, 1980; Jensen, 1995). Hooded shrimps are characterized by the broadly rounded frontal margin of the carapace,

\* Corresponding author at: Smithsonian Marine Station at Fort Pierce, 701 Seaway Drive, Fort Pierce, FL 34949, USA.

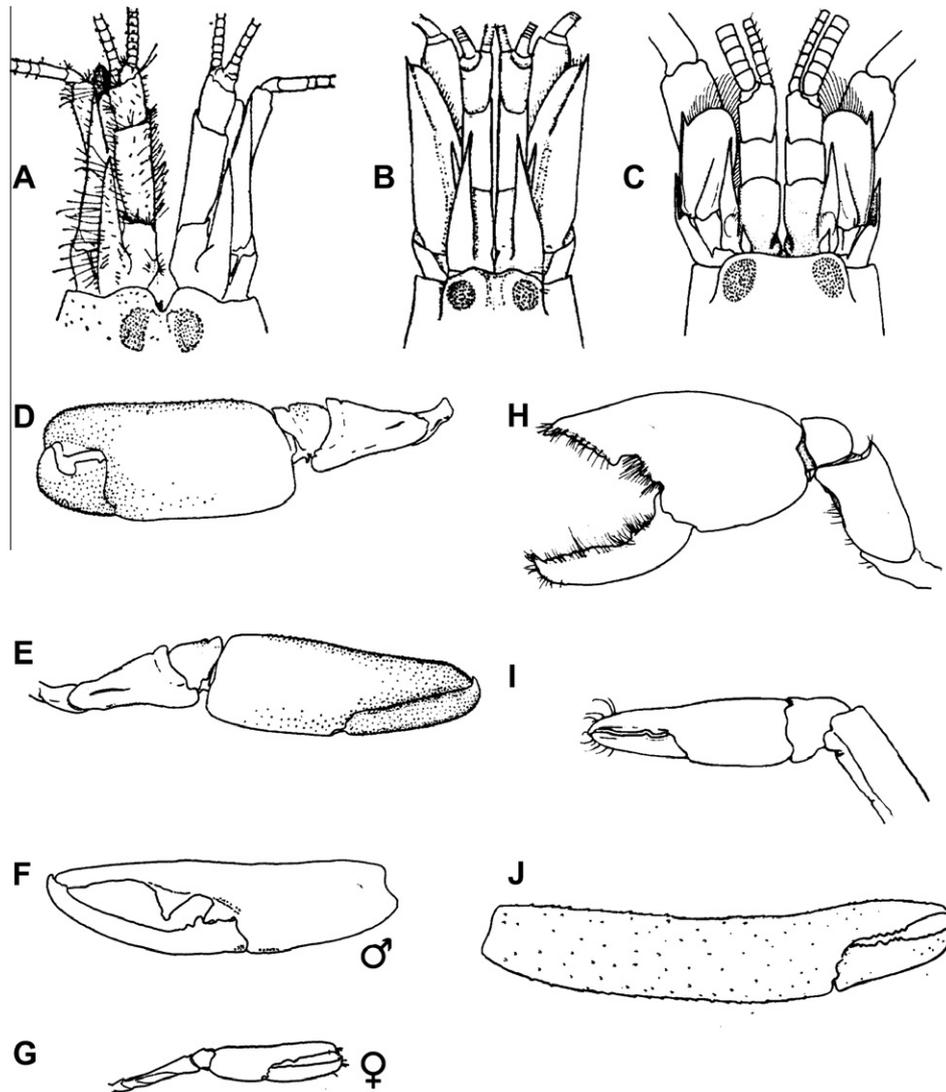
E-mail addresses: [arthuranker7@yahoo.com](mailto:arthuranker7@yahoo.com) (A. Anker), [baezaa@si.edu](mailto:baezaa@si.edu) (J. A. Baeza).

<sup>1</sup> Equal authorship.

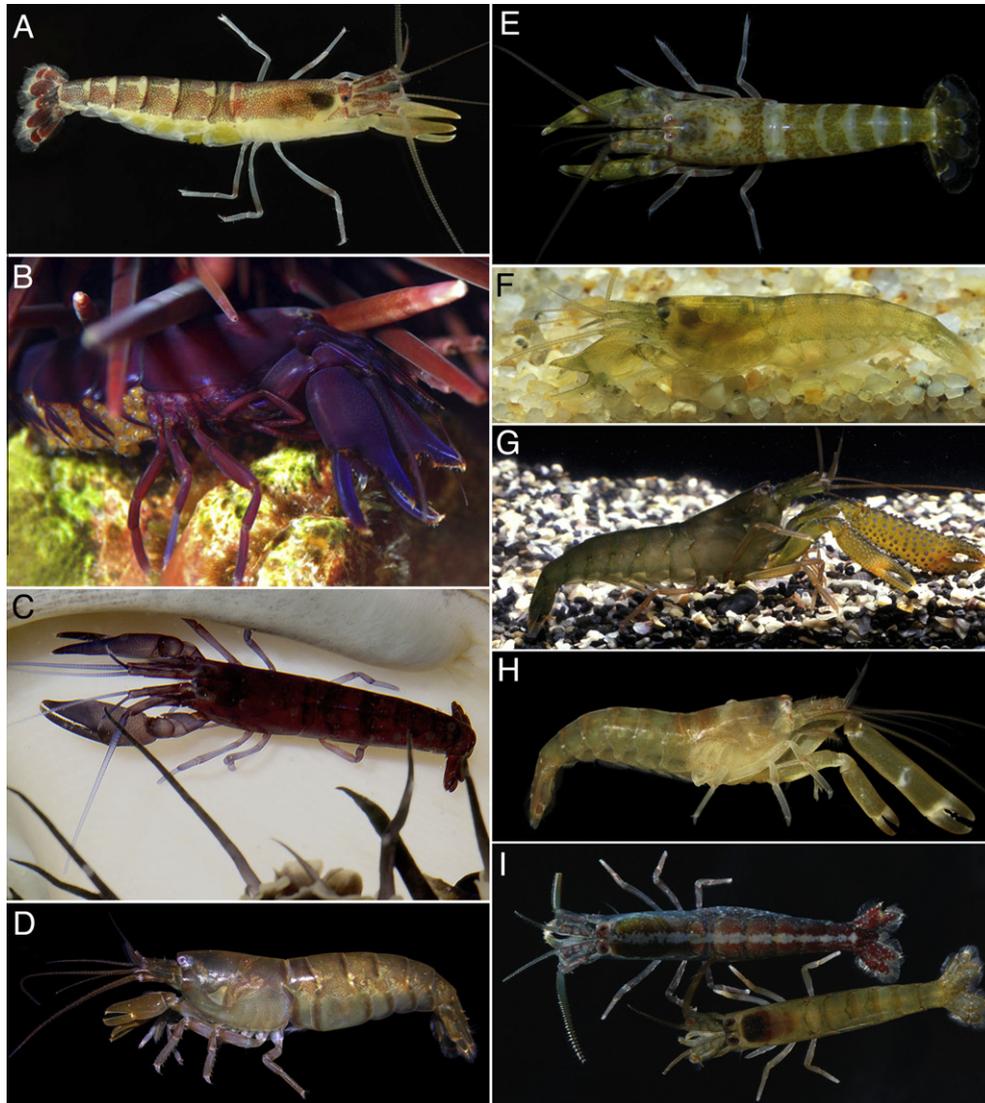
which conceals the eyes in dorsal and lateral views (Fig. 1A and B). This configuration is reminiscent of a cap visor or a hood, hence their common name. The chelipeds (=enlarged, claw-bearing first pereopods) of *Betaeus* are always carried extended frontwards, with the dactylus in ventral position (Figs. 1D–J and 2). Another important morphological feature of *Betaeus* is the presence of a subtriangular articulated plate on the sixth somite of the pleon (see for example Hart, 1964; Nomura and Komai, 2000). Hooded shrimps do not possess a snapping mechanism on their cheliped fingers and are therefore unable to snap as do the true snapping shrimps (species of *Alpheus* Fabricius, 1798 and *Synalpheus*). Nevertheless, the chelipeds of *Betaeus* are quite diverse in their general shape and degree of asymmetry (Figs. 1D–J and 2) and may also display age-related polymorphism and sexual dimorphism (Hart, 1964; Miya, 1972; see also Fig. 1F and G). Hooded shrimps also exhibit a wide variety of color patterns (Fig. 2).

The phylogenetic position of *Betaeus* within the Alpheidae remains unsettled. Coutière (1899) considered *Betaeus* as possibly “derived” from *Arete* Stimpson, 1860, a genus much more closely related to *Athanas* Leach, 1814 than to *Betaeus* (see for example

Banner and Banner, 1960; Anker and Jeng, 2007). Anker et al. (2006) provided a cladistic analysis of morphological characters of all alpheid genera known at that time. A close relationship between *Betaeus* and *Arete* was not supported in their analysis. On the other hand, the genus *Betaeopsis* Yaldwyn, 1971 (Figs. 1C and 2I) was found embedded within *Betaeus*, albeit with a relatively low statistical support (Anker et al., 2006). *Betaeopsis* (hereafter abbreviated as *Bp.* to distinguish it from *Betaeus*, abbreviated as *B.*) was originally separated from *Betaeus* (Yaldwyn, 1971) based on the absence of an articulated plate on the sixth abdominal somite and the reduced number of mastigobranchs and setobranchs. Subsequently, Anker and Jeng (2002) redefined *Betaeopsis*, recognizing three species within the genus. These authors also added two other characters to distinguish *Betaeopsis* from *Betaeus*, namely the presence of a pair of peculiar hook-like spiniform setae on each uropodal endopod and an arrow-shaped, anteriorly projecting process on each eyestalk (visible in Fig. 1C). The close relationship between *Betaeus* and *Betaeopsis* and the possible paraphyly of *Betaeus* suggested by the inter-generic phylogeny of Anker et al. (2006) requires confirmation. One of the major goals



**Fig. 1.** Some morphological characters of hooded shrimps from the genera *Betaeus* (*B.*) and *Betaeopsis* (*Bp.*): A, B, C – anterior margin of carapace and frontal appendages of *B. setosus* (A), *B. granulimanus* (B), and *Bp. acanthops* (C); D–J – chelipeds of five species of *Betaeus*: D, E, major and minor cheliped, respectively, of large adult male of *B. emarginatus*, showing heterochely/asymmetry; F, G, male and female chelipeds, respectively, of *B. longidactylus*, showing sexual dimorphism; H, cheliped of *B. harfordi* with a short, smooth, rounded palm; I, feebly enlarged cheliped of *B. jucundus*; J, elongate major chela of large adult male of *B. lilianae*. Not to scale. Figures adapted from: A, F, G, Hart, 1964; B, Kim, 1977; C, Bruce and Iliffe, 1991; D, E, Holthuis, 1952; H, I, Anker, 2001; J, Boschi, 1966.



**Fig. 2.** Habitus (view of the entire animal) and color patterns of hooded shrimps from the genera *Betaeus* (*B.*) and *Betaeopsis* (*Bp.*): A – *B. harrimani*, ovigerous female, found associated with a mud-shrimp burrow, Friday Harbor, Washington, USA [FLMNH]; B – *B. macginitieae*, ovigerous female *in situ*, under the sea urchin *Strongylocentrotus franciscanus*, Catalina Island, California, USA; C – *B. harfordi*, *in situ*, male, inside the mantle cavity of abalone *Haliotis* sp., Santa Barbara, California; D – *B. emarginatus*, female, Coquimbo, Chile [OUMNH]; E – *B. truncatus*, male, free-living under rocks, Coquimbo, Chile [OUMNH]; F – *B. gelasinifer*, male, free-living under rocks, central Japan; G – *B. granulimanus*, large male, free-living under rocks, central Japan; H – *B. lilliana*, large male, free-living in tide pools, Mar del Plata, Argentina [OUMNH]; I – *Bp. aequimanus*, two individuals showing variation in the color pattern, free-living among kelp holdfasts and rocks, Leigh, New Zealand [OUMNH]. Photographic credits: A, Gustav Paulay; B, Kevin Lee; C, Larry Jon Friesen; D, E, Arthur Anker; F, G, Madoka Itoh; H, Antonio Baeza; I, Richard Taylor.

of the present study is to conduct a more refined phylogenetic analysis of nearly all members of *Betaeus* and *Betaeopsis* to test whether the genus *Betaeus* is monophyletic or paraphyletic.

Clarification of phylogenetic relationships of *Betaeus* and *Betaeopsis* may also elucidate some biogeographical processes in this group of marine crustaceans. *Betaeus* is the only genus in the family Alpheidae that includes species restricted to cool-temperate and cold-water regions, with a few species penetrating into subtropical waters. In contrast, most other alpheid genera (e.g., *Alpheus*, *Synalpheus*, *Athanas*, etc.) have a predominantly tropical-subtropical distribution; only a few species extend into temperate and cool-temperate waters of the world's oceans, both northwards and southwards.

The 16 species of *Betaeus* are confined to six well-defined biogeographical regions: (1) North-East Pacific (=NEP), with seven species ranging from southern Alaska to Mexico; (2) North-West Pacific (=NWP), with four species ranging from the Russian Far East and Japan to Taiwan; (3) South-East Pacific (=SEP), with two

species ranging from southern Peru to Cape Horn; (4) South-West Pacific (=SWP), with one species restricted to south-eastern and southern Australia; (5) South-West Atlantic (=SWA), with two species ranging from Uruguay to southern Argentina and Falkland Islands (one of them also occurring in the SEP); (6) South-East Atlantic (=SEA), with one species restricted to the Cape Province of South Africa (see Table 1). The three species of *Betaeopsis* have different geographical ranges that do not overlap with those of *Betaeus*: one species is confined to the coasts of New Zealand and adjacent islands (part of the SWP), whilst two species are known from scattered localities in the tropical Indo-West Pacific (=IWP) (Anker and Jeng, 2002; see also Table 1).

The coasts of southern California (part of the NEP) harbor the highest diversity of hooded shrimps, with six species of *Betaeus*. It is often assumed that the area with the highest extant concentration of diversity within a clade may be its locus of origin. This “Center of Origin” model (hereafter CofO model) postulates that species first evolve within a geographical center of distribution. As these

**Table 1**  
Currently known species of the *Betaeus* Dana, 1852 and *Betaeopsis* Yaldwyn, 1971. The last column indicates if the species was included (yes) or not (no) in the morphological and DNA analyses; for specimen details and GenBank accession numbers see Table 2. Abbreviations: NEP – North-East Pacific; NWP – North-West Pacific; SEA – South-East Atlantic; SEP – South-East Pacific; SWA – South-West Atlantic; SWP – South-West Pacific; IWP – Indo-West Pacific.

Taxon	Distribution	Ecology	Main references	Morphology/ DNA analysis
<i>Betaeus</i> Dana, 1852				
<i>B. australis</i> Stimpson, 1860	SWP: New South Wales, Victoria, South Australia	Free-living, gregarious	Banner and Banner, 1973	yes/yes
<i>B. emarginatus</i> (H. Milne-Edwards, 1837)	SEP: S Peru, Chile	Free-living, gregarious	Holthuis, 1952; A. Baeza, pers. obs.	yes/yes
<i>B. ensenadensis</i> Glassell, 1938	NEP: S California to Baja California	Associated with burrows of <i>Upogebia pugettensis</i> (Dana) and <i>Neotrypaea californiensis</i> (Dana)	Glassell, 1938; Hart, 1964	yes/yes
<i>B. gelasinifer</i> Nomura and Komai, 2000	NWP: Japan, Korea	Free-living	Nomura and Komai, 2000; Yang et al., 2007	yes/no
<i>B. gracilis</i> Hart, 1964	NEP: California	Free-living	Hart, 1964	yes/yes*
<i>B. granulimanus</i> Yokoya, 1927	NWP: Japan, Korea, China, Hong Kong, Taiwan	Free-living, apparently gregarious	Miya, 1972	yes/yes
<i>B. harfordi</i> (Kingsley, 1878)	NEP: California to N Mexico	Obligate associate of <i>Haliotis</i> spp.	Hart, 1964; Chace and Abbott, 1980	yes/yes
<i>B. harrimani</i> Rathbun, 1904	NEP: S Alaska to California	Usually associated with burrows of <i>Upogebia pugettensis</i> (Dana) and <i>Neotrypaea californiensis</i> (Dana); occasionally free-living	Hart, 1964; Butler, 1980; Jensen, 1995	yes/yes
<i>B. jucundus</i> Barnard, 1946	SEA: Cape Province of South Africa	Associated with burrows of <i>Callinectes kraussi</i> (Stebbing) and <i>Upogebia africana</i> (Ortmann)	Barnard, 1950; Branch et al., 1994	yes/no
<i>B. levifrons</i> Vinogradov, 1950	NWP: Russian Far East	Associated with burrows of <i>Upogebia major</i> (De Haan)	Vinogradov, 1950; Marin, 2010	yes/yes
<i>B. liliana</i> Boschi, 1966	SWA: Uruguay, Argentina	Free-living or shelter-sharing with <i>Platyxanthus patagonicus</i> A. Milne-Edwards, gregarious	Boschi, 1966; Baeza et al., 2010	yes/yes
<i>B. longidactylus</i> Lockington, 1877	NEP: California to Mexico	Usually free-living; sometimes in burrows of <i>Upogebia pugettensis</i> (Dana) and <i>Urechis caupo</i> Fisher & MacGinitie	Hart, 1964; Chace and Abbott, 1980; Jensen, 1995	yes/yes
<i>B. macginitieae</i> Hart, 1964	NEP: California	Obligate associate of <i>Strongylocentrotus</i> spp.	Hart, 1964; Jensen, 1995	yes/yes
<i>B. pingi</i> Yu, 1930	NWP: China	Free-living	Yu, 1930	no/no**
<i>B. setosus</i> Hart, 1964	NEP: British Columbia to California	Usually shelter-sharing with <i>Pachycheles rudis</i> Stimpson	Hart, 1964; Butler, 1980; Jensen, 1995	yes/yes
<i>B. truncatus</i> Dana, 1852	SEP: Peru, Chile SWA: S Argentina, Falkland Is.	Free-living, gregarious	Holthuis, 1952; A. Baeza, pers. obs.	yes/yes
<i>Betaeopsis</i> Yaldwyn, 1971				
<i>B. acanthops</i> (Bruce and Iliffe, 1991)	IWP: Samoa	Free-living, in coastal lava tubes	Bruce and Iliffe, 1991; Anker and Jeng, 2002	yes/no
<i>B. aequimanus</i> (Dana, 1852)	SWP: New Zealand, Chatham Is.	Free-living, gregarious	Anker and Jeng, 2002; A. Anker, pers. obs.	yes/yes
<i>B. indicus</i> (de Man, 1910)	IWP: Red Sea, Indonesia, Philippines, Taiwan, NW Australia	Free-living	Anker and Jeng, 2002	yes/no

\* The specimen examined and sequenced differs slightly from Hart's (1964) description.

\*\* *Betaeus pingi* appears to be a junior synonym of *B. granulimanus* (Anker, 2001; see also Miya, 1972); this taxon is treated here under *B. granulimanus*.

species become older, their geographic ranges expand while they spread out toward peripheral zones where some of them eventually become extinct (Briggs, 1974, 2000, 2003). This model makes specific predictions about the relationship between geographic location, geographic range, and age of species within a monophyletic clade. Young species with small geographic ranges should be theoretically found in the presumed center of origin (e.g., NEP in *Betaeus* – *Betaeopsis*), while older lineages with wider geographic ranges should be theoretically found in regions farther away from the center given their older evolutionary history (Briggs, 2000, 2003; Barber and Bellwood, 2005). Phylogenetic, phylogeographic and biogeographic analyses of some marine groups have provided only partial support for the CoFO hypothesis, for example in some Indo-Pacific and Caribbean reef fishes (Bernardi et al., 2004; Rocha et al., 2008; Barber and Bellwood, 2005). In the present study, we will examine whether or not the NEP represents the center of origin for the genera *Betaeus* and *Betaeopsis*.

Hooded shrimps constitute an ecologically diverse group, with species differing in their levels of social organization (gregarious vs. pair living or solitary species) and life habits (free living vs.

symbiotic species) (see Table 1). Social monogamy is by far the most typical social organization among alpheid shrimps (e.g., Mathews, 2003; Rahman et al., 2003). However, within *Betaeus*, only some species are socially monogamous (Hart, 1964); others typically live in small groups or aggregations (Baeza et al., 2010) or mostly solitarily (Jensen, 1986, 1995). Most hooded shrimps are free living, dwelling under rocks or among kelp holdfasts in the intertidal zone or in subtidal lava tube caves (Holthuis, 1952; Hart, 1964; Antezana et al., 1965; Banner and Banner, 1973; Bruce and Iliffe, 1991; Jensen, 1995; Anker and Jeng, 2002). Several species of *Betaeus* use galleries built and inhabited by larger burrowing animals, such as mud-shrimps (Upogebiidae), ghost-shrimps (Callinassidae), or spoon worms (Urechidae), as permanent or occasional shelters (Hart, 1964; Branch et al., 1994; Table 1). Two species of *Betaeus* live symbiotically among the spines of sea urchins (Fig. 2B) or in the mantle cavity of abalones (Fig. 2C). Lastly, two species of *Betaeus* are known to share shelters, e.g. empty barnacle shells, with porcelain and stone crabs (Porcellanidae, Xanthidae) (Hart, 1964; Jensen, 1995; Baeza et al., 2010; Table 1). The social and ecological diversity of hooded shrimps makes

them ideal candidates to explore the role of environmental conditions on the evolution of social systems and symbiotic habits.

The aim of the present study is to provide the first phylogenetic hypothesis of hooded shrimps, i.e. the presumed monophyletic clade comprising *Betaeus* and *Betaeopsis* (*sensu* Anker et al., 2006), using both morphological and molecular characters. The molecular analysis is based on two markers, one nuclear gene (Histone H3) and one mitochondrial gene (16S rRNA). The resulting phylogenetic frame will be used to (1) test the monophyly of the genus *Betaeus*; (2) examine whether the CofO model explains the diversification process in this clade; and (3) shed some light on the evolution of symbiosis and social systems in these shrimps, based on published accounts and our own unpublished and published field observations (e.g. Baeza et al., 2010).

## 2. Material and methods

### 2.1. Taxon sampling

Samples were either collected by the authors in the field in Chile (AA, AB), California (AB), Argentina (AB), Canada (AA), New Zealand (AA), or obtained from various colleagues (see Acknowledgments). Out of 19 species currently recognized in the genera *Betaeus* (16 species) and *Betaeopsis* (three species), 18 species were included as ingroup terminals in the cladistic analysis of morphological characters and 13 species were included in the molecular analyses (see Table 2 for details, including voucher specimens and GenBank access information).

The only taxon that was included in neither the morphological nor in the molecular analysis was *B. pingi*, a nominal species that is

most probably a junior synonym of the morphologically variable *B. granulimanus* (Anker, 2001; see also Miya, 1972). One of us (AA) examined the holotype and the only known specimen of *B. pingi*, which was collected in the 1920s and deposited in the Muséum national d'Histoire naturelle in Paris (MNHN). As no major morphological differences between *B. granulimanus* and *B. pingi* were observed, we decided to treat *B. pingi* under *B. granulimanus*.

Two species of *Betaeus*, namely *B. gelasinifer* and *B. jucundus*, were included in the cladistic analysis of morphological characters, but not in the DNA analyses. The type series of *B. gelasinifer* from Japan was initially fixed in formalin (T. Komai, pers. comm.). We obtained several specimens from Korea collected in 1999–2006 and reported by Yang et al. (2007). No DNA sequences were obtained from these specimens, possibly due to their preservation. As for *B. jucundus*, two specimens of this species collected in the late 1990s and deposited in the MNHN were examined and subsampled. Unfortunately, no targeted DNA sequences could be obtained from these samples, even after multiple attempts. We were also unable to obtain fresh specimens of *B. jucundus* from South Africa. This species appears to be uncommon and difficult to collect (C. Griffith, pers. comm.).

Only the type species of *Betaeopsis* (i.e., *Bp. aequimanus*) was included in both morphological and molecular analyses. The remaining two species, *Bp. indicus* and *Bp. acanthops*, are almost identical morphologically and both share a great number of morphological characters with *Bp. aequimanus* (Anker and Jeng, 2002). Therefore, the absence of *Bp. indicus* and *Bp. acanthops* from the molecular dataset is not seen as critical.

For *B. gracilis*, which was included in both morphological and molecular analyses, the morphological characters were scored using both the excellent description and detailed illustrations of

**Table 2**

*Betaeus* and *Betaeopsis* species and other alpheid shrimps used for the molecular and morphological phylogenetic reconstruction. Deposition institution abbreviations: FLMNH, Florida Museum of Natural History, University of Florida, Gainesville, USA; IRBK, Invertebrate Resource Bank of Korea, Seoul National University, South Korea; MNHN, Muséum national d'Histoire naturelle, Paris, France; NTM, Museum and Art Gallery of the Northern Territory, Darwin, Australia; NTOU, National Taiwan Ocean University, Keelung, Taiwan; OUMNH, Oxford University Museum of Natural History, Oxford, UK; USNM, National Museum of Natural History, Smithsonian Institution, Washington DC, USA.

Taxon	Collection locality	Voucher (deposition institution*)	GenBank access Number 16S	GenBank access Number H3
<i>Betaeus australis</i>	Sydney, Australia	MNHN-Na 13629	JX010757	JX010762
<i>Betaeus emarginatus</i>	Coquimbo, Chile	OUMNH.ZC.2012-01-0028	JX010755	JX010763
	Coquimbo, Chile	OUMNH.ZC.2012-01-0029	—	—
<i>Betaeus ensenadensis</i>	Ensenada, Baja California	OUMNH.ZC.2012-01-0030	JX010759	JX010764
<i>Betaeus gelasinifer</i>	Shizuoka, Japan	MNHN-Na 13605	—	—
	Pohang, South Korea	IRBK 003761	—	—
<i>Betaeus cf. gracilis</i>	Año Nuevo Point, California	OUMNH.ZC.2012-01-0031	JX010747	JX010765
<i>Betaeus granulimanus</i>	Boso Peninsula, Japan	OUMNH.ZC.2003-08-002	JX010754	JX010766
	Hong Kong	USNM 173779	—	—
<i>Betaeus harfordi</i>	La Jolla, California	MNHN-IU- 2010-4212	JX010751	JX010767
	La Jolla, California	OUMNH.ZC.2012-01-0032	—	—
<i>Betaeus harrimani</i>	Bamfield, BC, Canada	MNHN-IU- 2010-4211	JX010749	JX010768
	Bamfield, BC, Canada	OUMNH.ZC.2012-01-0034	—	—
<i>Betaeus jucundus</i>	Cape Province, South Africa	USNM 283253	—	—
	Cape Province, South Africa	MNHN-Na 15569	—	—
<i>Betaeus levifrons</i>	Peter the Great Bay, Russia	NTOU M 00955	JX010748	JX010769
<i>Betaeus liliana</i>	Mar del Plata, Argentina	OUMNH.ZC.2012-01-0035	JX010756	JX010770
<i>Betaeus longidactylus</i>	La Jolla, California	OUMNH.ZC.2012-01-0037	JX010752	JX010771
<i>Betaeus macginitiae</i>	La Jolla, California	MNHN-IU- 2010-4210	JX010758	JX010772
	La Jolla, California	OUMNH.ZC.2012-01-0039	—	—
<i>Betaeus pingi</i>	"Amoy", China	MNHN-Na 2010-5555	—	—
<i>Betaeus setosus</i>	California	— (not available)	JX010750	JX010773
	Oregon	FLMNH UF	—	—
<i>Betaeus truncatus</i>	Coquimbo, Chile	OUMNH.ZC.2012-01-0040	JX010753	—
<i>Betaeopsis acanthops</i>	Upolu, Samoa	NTM Cr007421	—	—
<i>Betaeopsis aequimanus</i>	Wellington, New Zealand	OUMNH.ZC. 2012-01-0026	JX010760	JX010774
	Leigh, New Zealand	OUMNH.ZC. 2012-01-0023	—	—
<i>Betaeopsis indicus</i>	Ch'uan-fan-shih, Taiwan	USNM 213508	—	—
<i>Alpheopsis yaldwyni</i>	Moorea, French Polynesia	FLMNH UF 15470	—	—
<i>Athanas nitescens</i>	São Tomé	OUMNH.ZC. 2011-03-0102	—	—
<i>Metabetaeus minutus</i>	Ryukyu Islands, Japan	FLMNH UF 23251	FJ943435	FJ943455
<i>Nennalpheus sibogae</i>	Mayotte, SW Indian Ocean	FLMNH UF 13629	JX010761	JX010775

Hart (1964) and an imperfect specimen from California identified as *B. cf. gracilis* (due to some minor discrepancies with the original description).

## 2.2. Outgroups

Several species from different alpheid genera were used as outgroup terminals. *Alpheopsis yaldwyni* Banner and Banner, 1973, *Athanas nitescens* Leach, 1814, and *Metabetaeus minutus* (Whitellegge, 1897) were used as outgroups in the morphological analyses. *Metabetaeus minutus* and *Nennalpheus sibiogae* (De Man, 1910) were used as outgroups in the molecular (H3 + 16S) analyses. All outgroup taxa were used in the final ‘total evidence’ analyses (see below).

## 2.3. Analysis of morphological data

Specimens were examined under the dissecting microscope and characters from each specimen were scored by the same researcher (AA). A morphological data matrix was constructed in Mesquite 2.73 (Maddison and Maddison, 2010). Characters from live color patterns were included (where known) and treated the same way as morphological characters. Characters were neither weighted nor polarized; missing data (or inapplicable states for some characters) were scored as a ‘?’

Phylogenetic analyses conducted with the morphological dataset were Bayesian inference (BI) and Maximum Parsimony (MP) in MrBayes (Huelsenbeck and Ronquist, 2001) and PAUP\* (Swofford, 2002), respectively. The MP analysis was performed as a heuristic search with a starting tree obtained via stepwise addition, random addition of sequences, random replicates, and tree bisection and reconnection (TBR) branch swapping. All other parameters used were those of the default option in PAUP\*. For BI, unique random starting trees were used in the Metropolis-coupled Markov Monte Carlo Chain (MCMC) (Huelsenbeck and Ronquist, 2001); this analysis was performed for 6,000,000 generations. Every 100th tree was sampled from the MCMC analysis obtaining a total of 60,000 trees and a consensus tree with the 50% majority rule was calculated for the last 59,000 sampled trees. The robustness of the MP tree topology was assessed by bootstrap reiterations of the observed data 2000 times. Support for nodes in the BI tree topology was obtained by posterior probability values.

## 2.4. DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from pleopods or abdominal muscle tissue using the QIAGEN® DNeasy® Blood and Tissue Kit following the manufacturer’s protocol. The polymerase chain reaction (PCR) was used to amplify target regions of one nuclear gene (H3 [328 bp – Colgan et al., 1998]) and one mitochondrial gene (16S [557 bp] – Schubart et al., 2000). For amplification of the 16S and H3 gene segments, we used the primers 16L2 (5′-TGC CTG TTT ATC AAA AAC AT-3′), and 1472 (5′-AGA TAG AAA CCA ACC TGG-3′) (Schubart et al., 2000; Baeza et al., 2009) and H3AF (5′-ATG GCT CGT ACC AAG CAG ACV GC-3′) and H3AR (5′-ATA TCC TTR GCC ATR ATR GTG AC-3′) (Colgan et al., 1998), respectively.

Standard PCR 25 µl reactions (2.5 µl of 10× Taq buffer, 2 µl of 50 mM MgCl<sub>2</sub>, 2.5 µl of 10 mM dNTPs, 2.5 µl each of the two primers (10 mM), 0.625 U Taq, 1.25 µl of 20 mM BSI and 8.625 µl double distilled water) were performed on a Peltier Thermal Cycler (DYAD®) under the following conditions: initial denaturation at 95 °C for 5 min followed by 30–40 cycles (depending on the gene) of 95 °C for 45 s, 48–57 °C (depending on the species) for 1 min, and 72 °C for 1 min, followed by chain extension at 72 °C for 10 min. PCR products were purified with ExoSapIT (a mixture of exonuclease and shrimp alkali phosphatase, Amersham Pharma-

cia) and then sent for sequencing with the ABI Big Dye Terminator Mix (Applied Biosystems) to the Laboratory of Analytical Biology of the National Museum of Natural History (LAB – NMNH, Maryland), which is equipped with an ABI Prism 3730xl Genetic Analyzer (Applied Biosystems automated sequencer). All sequences were confirmed by sequencing both strands and a consensus sequence for the two strands was obtained using the software Sequencer 4.5 (Gene Codes Corp.).

## 2.5. Sequence alignment and analysis

Alignment of each set of sequences was conducted using Multiple Sequence Comparison by Log-Expectation in the software MUSCLE (Edgar, 2004). The alignment of the H3 gene fragment had no indels and was unambiguous. In contrast, the aligned sequences of the 16S gene fragment did contain several indels. Therefore, we identified positions that were highly divergent and poorly aligned in the 16S gene segments using the defaults settings in the software GBlocks v0.91b (Castresana, 2000), omitting them from the analyses. After highly divergent positions were pruned, the 16S dataset consisted of 509 bp. This pruned 16S gene fragment alignment is available at [www.treebase.org](http://www.treebase.org) (<http://purl.org/phylo/treebase/phylogs/study/TB2:S12658>).

Alignments were concatenated into a single dataset consisting of 16 sequences and 837 bp. However, we partitioned the dataset into two different segments, each with a different model of evolution. Missing data were designated as a ‘?’ in the alignment. Selection of an optimal model of base substitution for each gene was conducted with Modeltest v3.7 (Posada and Crandall, 1998) in PAUP\*. For the H3 gene fragment, the optimal model found by Modeltest (selected with hierarchical Likelihood Ratio Tests [hLRTs]) was a TrNef+I+G evolutionary model (–lnL = 1777.4392). The calculated parameters were as follow: assumed nucleotide frequencies  $A = G = T = C$ ; substitution rate matrix with A–C substitution = 1.0, A–G = 1.9631, A–T = 1.0, C–G = 1.0, C–T = 4.4733, G–T = 1.0; rates for variable sites assumed to follow a gamma distribution (G) with shape parameter = 1.5134 and a proportion of invariable sites (I) = 0.6086. For the 16S gene fragment, the optimal model found by Modeltest (selected with hLRTs) was a TVM+I+G evolutionary model (–lnL = 5565.1890). The calculated parameters were as follow: assumed nucleotide frequencies  $A = 0.3254$ ,  $G = 0.2022$ ,  $T = 0.3857$ ,  $C = 0.0868$ ; substitution rate matrix with A–C substitution = 0.6006, A–G = 7.7004, A–T = 1.3959, C–G = 0.4081, C–T = 7.7004, G–T = 1.0; rates for variable sites assumed to follow a gamma distribution (G) with shape parameter = 0.7469 and a proportion of invariable sites (I) = 0.3724. These models were implemented in MrBayes (for Bayesian inference analysis) and Treefinder (for Maximum Likelihood analysis – Jobb et al., 2004).

Phylogenetic analyses conducted herein were Maximum Likelihood (ML, in Treefinder), Bayesian inference (BI, in MrBayes), and Maximum Parsimony (MP, in PAUP\*). The MP analysis was performed as heuristic search with a starting tree obtained via stepwise addition, random addition of sequences, random replicates, and TBR (Tree-bisection–reconnection) branch swapping. All other parameters used were those of the default option in PAUP\*. For BI, unique random starting trees were used in the Metropolis-coupled Markov Monte Carlo Chain (MCMC) (Huelsenbeck and Ronquist, 2001). The analysis was performed for 6,000,000 generations. Every 100th tree was sampled from the MCMC analysis obtaining a total of 60,000 trees and a consensus tree with the 50% majority rule was calculated for the last 59,000 sampled trees. The robustness of the ML and MP tree topologies was assessed by bootstrap reiterations of the observed data 1000 and 2000 times, respectively. Support for nodes in the BI tree topology was obtained by posterior probability values.

## 2.6. Combined analysis of morphological and molecular data

The molecular and morphological datasets were combined to conduct final morphological plus molecular “total evidence” analyses (Grant and Kluge, 2003).

Total evidence analyses enhance the detection of real phylogenetic groups if there is no or minimal heterogeneity among different (e.g., molecular and morphological) datasets (de Queiroz et al., 1995). Our phylogenetic analyses using only morphological and only molecular datasets demonstrated minimal heterogeneity (see results). Thus, we argue that a “total evidence” analysis has the ability to more accurately reflect phylogenetic relationships (see de Queiroz et al., 1995). However, in combined analyses, the molecular data most frequently constitute a much greater proportion of characters than the morphological data. This asymmetrical contribution of molecular traits might overwhelm the phylogenetic signal from morphological data (Wahlberg et al., 2005). Therefore we conducted two different total evidence analyses to explore whether phylogenetic signal from morphological data was indeed overwhelmed by the molecular data. The first “total evidence” phylogenetic analysis included a total of 15 species (13 species of *Betaeus*, 1 species of *Betaeopsis*, and 1 species of *Metabetaeus* used as outgroup) for which both morphological and molecular data were available. In the second “total evidence” analysis, a total of 21 species (15 species of *Betaeus*, 3 species of *Betaeopsis*, and 3 species used as outgroups) were considered, for which morphological data was available. However, for six species used in this second analysis no molecular data was available (see above and Tables 1 and 2 for details). Total evidence analyses with incomplete datasets have been used before to infer the phylogeny of many other clades of marine and terrestrial vertebrates and invertebrates, including marine decapods, e.g., in other snapping shrimps (Duffy et al., 2000) and spider crabs (Hultgren et al., 2009).

The total evidence phylogenetic analyses conducted with the two combined datasets (15 Species and All Species dataset) were Bayesian inference in MrBayes. For each BI analysis, we partitioned the datasets into three different segments (morphology, 16S and H3). The segments corresponding to the two different gene fragments (16S and H3) have each a different model of evolution previously found using Modeltest (see above). Morphological characters have a third model of evolution (analog to a Jukes–Cantor model but with variable number of states) with states for each character considered unordered and equally weighted (Huelsenbeck and Ronquist, 2001). As above, for each BI analysis, unique random starting trees were used in the Metropolis–coupled Markov Monte Carlo Chain (MCMC) (Huelsenbeck and Ronquist, 2001). The analysis was performed for 6,000,000 generations. Every 100th tree was sampled from the MCMC analysis obtaining a total of 60,000 trees and a consensus tree with the 50% majority rule was calculated for the last 59,000 sampled trees. Support for nodes in the BI tree topology was obtained by posterior probability values.

## 2.7. Hypothesis testing

Two main hypotheses were examined in this study: (1) the monophyly of the genus *Betaeus*; and (2) the Center of Origin (CofO) model as a possible explanation for the diversification/biogeographic processes in *Betaeus*.

To test the first hypothesis, constrained trees (in which the monophyly of the genus *Betaeus* was enforced) were obtained in MrBayes with the command constraint. MCMC searches were run and the harmonic mean of tree-likelihood values was obtained by sampling the post burn-in, posterior distribution as above. Next, Bayes factors were used to evaluate whether or not there was evidence against monophyly of *Betaeus* (unconstrained vs. constrained trees) according to the criteria of Kass and Raftery

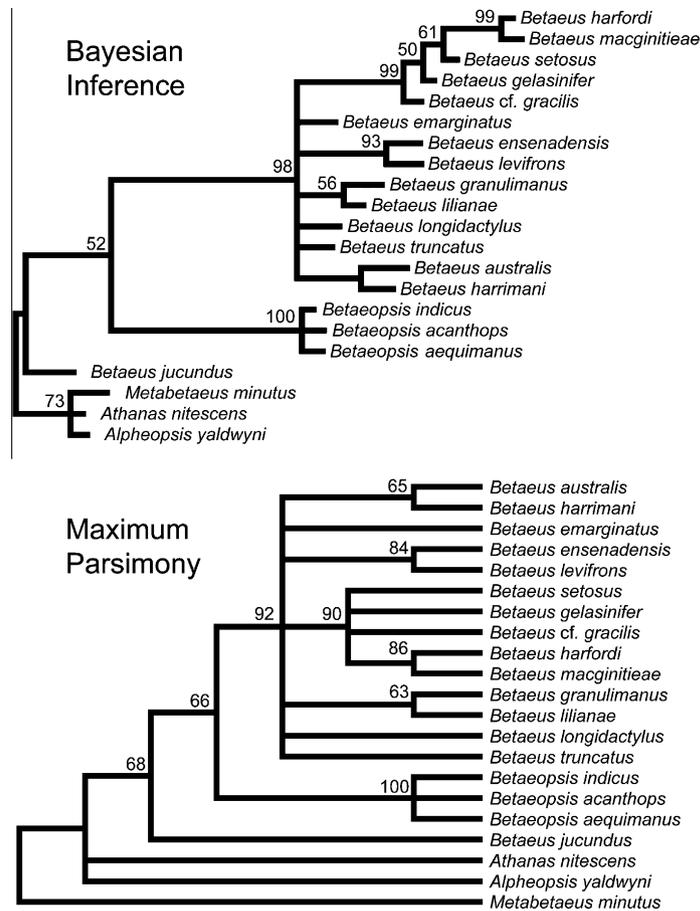
(1995). Bayes factors compare the total harmonic mean of the marginal likelihood of unconstrained vs. monophyly-constrained models. The higher the value of the Bayes factor statistic implies stronger support against the monophyly of a particular group (Kass and Raftery, 1995). Specifically, a value for the test statistic  $2\log_e(B_{10})$  between 0 and 2 indicates no evidence against  $H_0$ ; values from 2 to 6 indicate positive evidence against  $H_0$ ; values from 6 to 10 indicate strong evidence against  $H_0$ ; and values >10 indicate very strong evidence against  $H_0$  (Kass and Raftery, 1995; Nylander et al., 2004). The hypothesis above was tested using the phylogenetic tree resulting from the total evidence analysis (All Species dataset, using Bayesian inference) because this tree was the most resolved and had the greatest support values for the observed monophyletic clades compared to trees obtained with other datasets and analyses (see Section 3).

To examine the second hypothesis, we determined whether or not the more derived species of *Betaeus* (e.g., positioned further away from the root in the total evidence phylogenetic tree) were those inhabiting the NEP (e.g., the putative Center of Origin) and whether or not the less derived species (e.g., positioned closer to the root in the total evidence phylogenetic tree) were those inhabiting regions farther away from the NEP. Also, we tested for a statistically significant positive correlation between the age of a species and its geographic range, as predicted by the CofO model (Briggs, 2000, 2003; Barber and Bellwood, 2005). For this purpose, the approximate age of each species was calculated in relative terms, as the estimated branch length of the most recent node that connects that particular species to any other taxon or clade using a linearized molecular tree computed with the software MEGA 5. Given that, almost invariably, *Betaeus* and *Betaeopsis* are intertidal and/or shallow subtidal species, the geographical range of each taxon was calculated as kilometers of linear coast using the ‘path ruler’ tool in Google Earth (<http://earth.google.com/>). This analysis was conducted using a total of 12 species for which both geographic range and age were available and reliable (see Table 2 for geographic ranges of *Betaeus* and *Betaeopsis*).

## 3. Results

### 3.1. Morphological analysis

The final morphological data matrix comprised 39 characters, all of them parsimony informative, for a total of 21 terminals, including 15 species of *Betaeus* and three species of *Betaeopsis* (ingroup) and one representative each of *Alpheopsis*, *Athanas* and *Metabetaeus* (outgroups) (for details see Appendixes A and B). The MP and BI analyses of this dataset each produced a single most parsimonious tree. In general, both MP and BI trees were not well resolved and had rather low bootstrap support values (Fig. 3A). The monophyly of *Betaeus* is not supported both by MP and BI given the position of *B. jucundus*, which was recovered as sister to all the remaining species of *Betaeus* plus the three species of *Betaeopsis*, albeit with moderate support and credibility values (Fig. 3A). On the other hand, both MP and BI strongly support the monophyly of *Betaeopsis*, which was expected given the close morphological resemblance of *Bp. acanthops*, *Bp. aequimanus* and *Bp. indicus* (Anker and Jeng, 2002). The topology of the MP and BI trees indicates a sister position of *Betaeopsis* relative to *Betaeus* (*B. jucundus* excluded), however, with low support and credibility values. With the exception of *B. jucundus*, all the remaining species of *Betaeus* formed a well-supported monophyletic group (MP bootstrap and BI credibility values of 92 and 98, respectively). Furthermore, BI, but not MP, supported two clades of geminate species (i.e., weakly differentiated species that have evolved from a common ancestor – Jordan, 1908) of *Betaeus* (*B. harfordi* – *B. macginitieae* and *B. ensenadensis* – *B. levifrons*) and



**Fig. 3.** Morphological (A) and molecular (B) phylogenies of *Betaeus* and *Betaeopsis*. A. Tree topology that resulted from Bayesian Inference analysis of morphological characters of *Betaeus* (15 taxa), *Betaeopsis* (3 taxa), and outgroups (*Athanas*, *Alpheopsis*, *Metabetaeus*, each with 1 taxon). Numbers above or below the branches represent the posterior probabilities from the Bayesian inference (BI) analysis in MrBayes and bootstrap values obtained from Maximum Parsimony (MP) analysis in PAUP\* (BI/MP). For definition of characters and their states see Appendix A; for character matrix see Appendix B. B. Tree topology that resulted from the combined analysis of 16S and H3 gene fragments of *Betaeus* (13 taxa), *Betaeopsis* (1 taxon) and outgroups (*Nennalpheus*, *Metabetaeus*, each with 1 taxon), under Maximum Likelihood (ML). Numbers above or below the branches represent the posterior probabilities from the Bayesian inference (BI) analysis in MrBayes and bootstrap values obtained either from ML or Maximum Parsimony (MP) analyses in PAUP\* (ML/BI/MP). The general topology of the trees obtained from MP and BI analyses was the same.

a larger monophyletic clade comprising *B. harfordi*, *B. macginitieae*, *B. setosus*, *B. gelasinifer* and *B. cf. gracilis* (Fig. 3A). Other sister taxa recovered were *B. harrimani* – *B. australis* and *B. granulimanus* – *B. lilianae*, both with a weak statistical support.

### 3.2. Molecular analysis

A total of 211 positions were found to be parsimony informative out of 837 homologous alignment positions, for 16 terminals, including 13 species of *Betaeus* and one species of *Betaeopsis* (in-group) and one representative of each *Nennalpheus* and *Metabetaeus* (outgroups). All molecular phylogenetic trees obtained with the different inference methods (MP, ML, and BI) resulted in the same general topology (Fig. 3B). In general, the molecular trees were considerably more resolved than the morphological trees. However, and importantly, most of the clades that were recovered and well resolved by the morphological analysis were also supported by the molecular analysis (see below).

For the pool of taxa used herein, the molecular analysis places *Betaeopsis* (represented by *Bp. aequimanus*) as a sister group to *Betaeus* (excluding *B. jucundus* and *B. gelasinifer* from which sequences were not available, see above). The monophyly of *Betaeus* (13 species, not including *B. jucundus* and *B. gelasinifer*) is well supported by a high posterior probability obtained from the BI analysis and bootstrap support values from the ML and MP analyses.

The 13 species of *Betaeus* can be subdivided into three well-supported monophyletic clades, each containing at least three taxa, and one lineage containing a single taxon (Fig. 3B). The first clade (=Clade I), also resolved by the morphological analysis, is composed of two pairs of sister taxa: *B. gracilis* – *B. setosus* and *B. macginitieae* – *B. harfordi*. The second clade (=Clade II) is composed of two other pairs of sister taxa: *B. ensenadensis* – *B. levifrons* (also supported by the morphological dataset) and *B. harrimani* – *B. longidactylus*. Within this second clade, *B. emarginatus* is positioned as sister to all of the remaining species, although this position is not well supported by the ML and MP analyses. The third clade (=Clade III) is composed of *B. granulimanus*, *B. lilianae* and *B. truncatus*. This clade was not resolved by the morphological analysis. Although, the tree topology in Fig. 3B suggests that *B. lilianae* and *B. granulimanus* are sister taxa, their geminate status is not well supported by the analysis of the H3 + 16S gene segments. Finally, *B. australis* is always recovered as sister to all the species comprising Clade I and Clade II. The above implies that Clade III is also sister to a larger clade comprising Clades I + II and *B. australis* (Fig. 3B).

### 3.3. Combined morphological + molecular analysis

The two different total evidence phylogenetic analyses, i.e. the 15 species analysis (Fig. 4A) and the 21 species (“All Species”) analysis (Fig. 4B), resulted in the same tree topology, with the different

retrieved monophyletic clades having very similar support/credibility values. In the All Species analysis, *B. jucundus* is once again recovered as a taxon sister to all the remaining species of *Betaeus* (Clades I, II, III + *B. australis*) and *Betaeopsis* (Clade IV). Also, the Bayes factor analysis revealed no support for the monophyly of *Betaeus*. Comparison of the unconstrained tree (harmonic mean =  $-5233.94$ ) vs. the tree wherein *Betaeus* was imposed as a monophyletic clade, indicated support for the unconstrained tree (harmonic mean =  $-5229.02$ ,  $2\ln(B_{10}) = 3.19$ ). Based on these results, the genus *Betaeus* is paraphyletic either by including the genus *Betaeopsis* or by excluding *B. jucundus* that is recovered as sister to *Betaeopsis* plus the rest of *Betaeus* (hereafter *Betaeus s. str.*).

With the exclusion of *B. jucundus*, *Betaeopsis* is sister to *Betaeus s. str.* Within *Betaeus s. str.*, *B. australis* is sister to the species comprising the three clades recovered before by the molecular analyses. Indeed, the three clades recovered by the molecular analyses are also recovered in the two combined analyses: Clade I, II and III. Importantly, *B. gelasinifer* is placed in Clade I with a strong support, where it forms a smaller clade together with *B. gracilis* and *B. setosus* (Fig. 4). The combined analyses also support the geminate position of *B. granulimanus* and *B. lilianae* within Clade III. Finally, the credibility values of BI moderately support the sister position of Clades I and Clade II (including *B. emarginatus*).

In the two different total evidence phylogenetic analyses, Clades I and II, composed mostly of species from the NEP, constitute the most derived clades within *Betaeus + Betaeopsis* (compared to Clade IV, *B. australis*, and *B. jucundus* that inhabit geographical zones farther away from the NEP). Furthermore, Clade I, comprising almost exclusively species from the NEP, is more derived than species from Clade III. The latter clade is mostly composed of

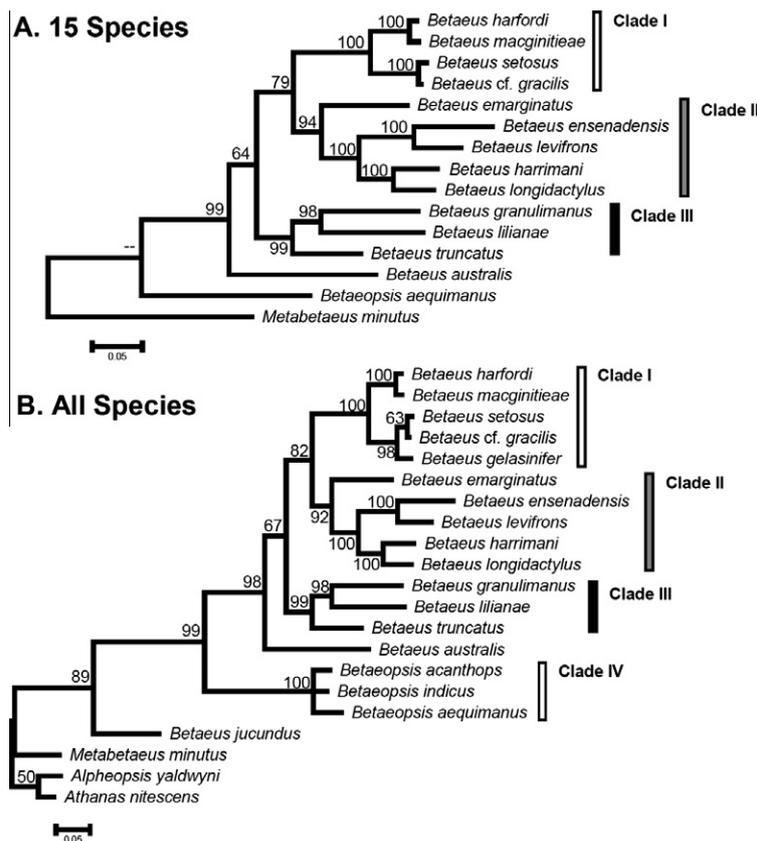
species from the southern hemisphere. No statistically significant correlation between age and geographic range was found ( $F = 3.46$ , d.f. = 1, 10,  $P = 0.0633$ ).

#### 4. Discussion

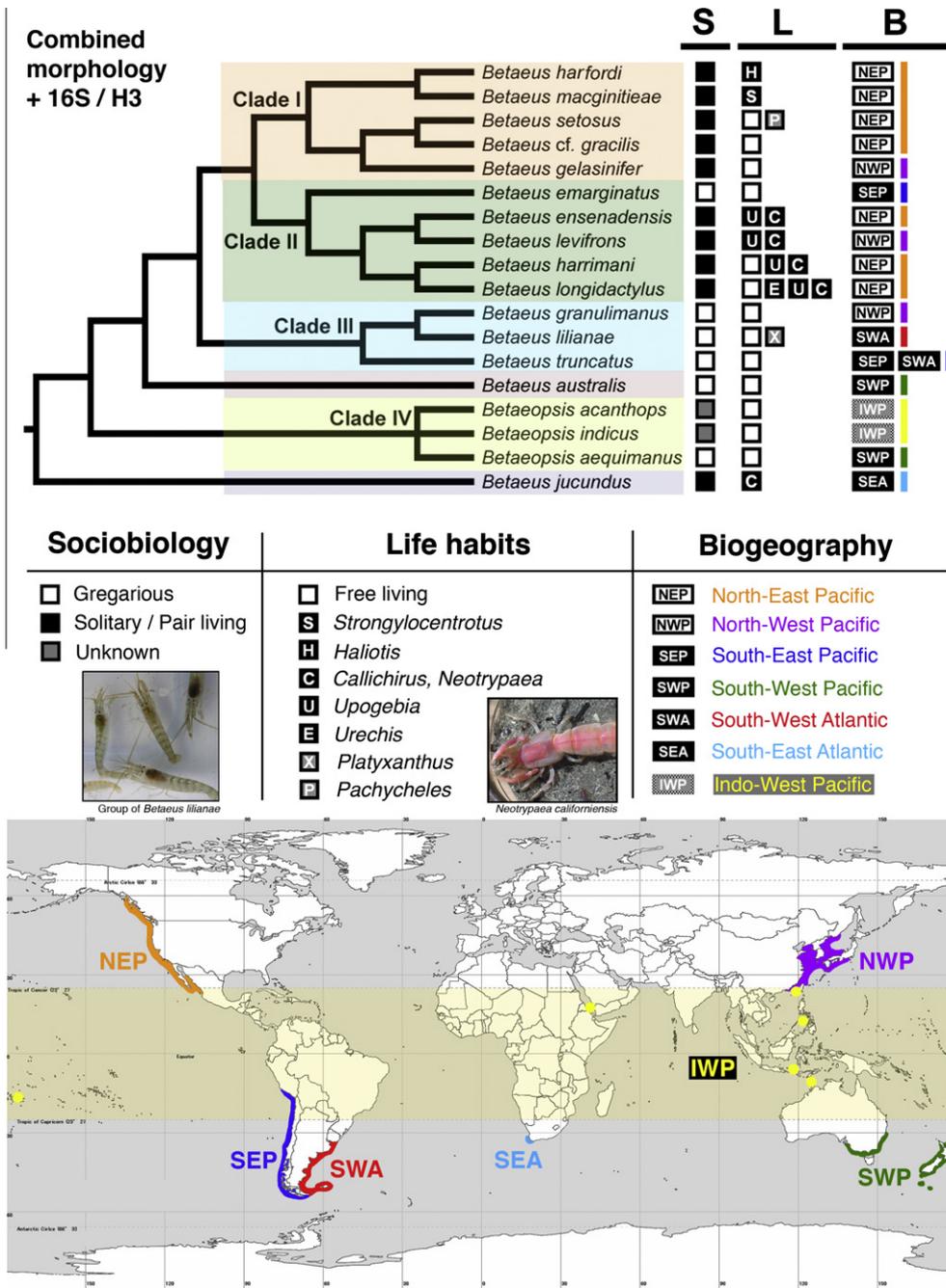
The phylogenetic tree of the *Betaeus + Betaeopsis* clade (Figs. 4 and 5) that resulted from the combined analysis of 39 morphological characters and two molecular marker, one mitochondrial gene (16S) and one nuclear gene (H3), appears to be sufficiently robust for the first tentative interpretations of the morphological and biogeographic patterns observed in this interesting shrimp lineage. Some clades are particularly well supported by either morphology or DNA, or both; this is, for instance, the case of Clades I, II and IV. Some uncertainties remain though, especially about the position of *B. jucundus* that appears to be sister to *Betaeopsis + Betaeus s. str.*, the position of *B. emarginatus* within Clade II, and the internal arrangement within Clade III (see below).

##### 4.1. Morphology

The exclusively North Pacific Clade I (Fig. 5) includes five species, four in the NEP (*B. harfordi*, *B. macginitieae*, *B. gracilis*, *B. setosus*) and one in the NWP (*B. gelasinifer*). All five species are characterized by (1) the relatively short, more or less rounded, sub-symmetrical chelae, (2) the stout and distinctly biunguiculate dactyli on the third, fourth and fifth pereopods, and (3) the very short first carpal article in the second pereopod (Hart, 1964; Nomura and Komai, 2000; Fig. 1H). In addition, most species also present a



**Fig. 4.** Combined phylogeny *Betaeus* and *Betaeopsis* based on morphological characters and 16S and H3 gene fragments. A. Tree topology that resulted from Bayesian Inference analysis of 15 species for which both morphological and molecular data were available: *Betaeus* (13 species), *Betaeopsis* (1 taxon) and outgroup (*Metabetaeus*, 1 taxon). B. Tree topology that resulted from Bayesian Inference analysis of 21 species (All Species analysis) of *Betaeus* (15 taxa), *Betaeopsis* (3 taxa) and outgroups (*Metabetaeus*, *Alpheopsis*, *Athanas*, each with 1 taxon), with no molecular data available for four ingroup taxa (*B. gelasinifer*, *B. jucundus*, *Bp. indicus*, *Bp. acanthops*). Numbers above or below the branches represent the posterior probabilities from the Bayesian inference (BI) analysis in MrBayes.



**Fig. 5.** Sociobiology (S), Life habits (L) and Biogeography (B) mapped on the tree resulting from the combined morphology + DNA analysis of *Betaeus* and *Betaeopsis* (for details see Fig. 4). **Sociobiology:** solitary + monogamous species [black squares], gregarious species [white squares], social habits unknown [gray squares]. **Life habits:** free-living species, e.g., under rocks and among kelp (white squares), symbiotic species living with echinoids (*Strongylocentrotus*) [S], abalones (*Haliotis*) [H], porcelain crabs (*Pachycheles*) [P], stone crabs (*Platyxanthus*) [X], mud shrimps (*Upogebia*) [U], ghost shrimps (*Neotrypaea*, *Callinectes*) [C], and echinurans (*Urechis*) [E]. **Biogeography:** NEP: *B. ensenadensis*, *B. gracilis*, *B. harfordi*, *B. harrimani*, *B. longidactylus*, *B. macginitieae*, *B. setosus*; NWP: *B. gelasinifer*, *B. granulimanus* (incl. *B. pingi*), *B. levifrons*; SEP: *B. emarginatus*, *B. truncatus*; SWP: *B. australis* [S Australia] *Bp. aequimanus* [New Zealand]; SWA: *B. lilianae*, *B. truncatus*; SEA: *B. jucundus*; IWP: *Bp. acanthops* [Samoa], *Bp. indicus* [Red Sea to Australia and Taiwan]. The map below shows collective distributions of species for each biogeographic region. For further details see text. Photographic credits: J.A. Baeza, A. Anker.

medially emarginated orbital hood (Fig. 1A). Within *Betaeus s. str.*, the short, rounded chelae and the biunguiculate dactyli of the walking legs are synapomorphies of Clade I. Interestingly, two types of unique color patterns exist in Clade I. The two sisters *B. macginitieae* and *B. harfordi* share a uniform dark-purple body color (Fig. 2B and C). The remaining three species, *B. setosus*, *B. gelasinifer* and *B. gracilis*, are semitransparent greenish-yellowish (Fig. 2F; see also photographs in Jensen, 1995; Nomura and Komai, 2000).

The North and South Pacific Clade II (Fig. 5) includes five species, three in the NEP (*B. harrimani*, *B. longidactylus*, *B. ensenadensis*), one

in the NWP (*B. levifrons*), and one in the SEP (*B. emarginatus*). The first four species are more closely related, forming a well-supported monophyletic clade. They share (1) a finely toothed transverse suture on the uropodal exopod, (2) the third, fourth, and fifth pereopods with slender and simple dactyli, (3) the chelipeds with usually elongate subsymmetrical to somewhat asymmetrical chelae, and (4) the broadly rounded orbital hoods (Hart, 1964; Marin, 2010). None of these characters, however, are exclusive to Clade II. The position of *B. emarginatus* is more uncertain: both morphological and molecular analyses failed to provide a robust position

for this taxon. Morphologically, *B. emarginatus* appears to be somewhat “intermediate” between some species of Clade II (e.g. *B. longidactylus*) and *B. truncatus* from Clade III (see below).

The Pacific–Atlantic Clade III (Fig. 5) includes three species with a more disparate distribution: two species in the contiguous SEP–SWA area (*B. truncatus*, *B. liliana*), and one species in the NWP (*B. granulimanus*). The biogeographically puzzling sister position of *B. liliana* (SWA) and *B. granulimanus* (NWP) (see below) is supported by one strong morphological synapomorphy: the presence of a large sharp tooth on the mesial face of the first antennular article. This tooth is unique not only in the genus *Betaeus*, but also in the family Alpheidae. In addition, large males of both *B. liliana* and *B. granulimanus* have strongly unequal and asymmetrical chelae (Fig. 2G and H), more asymmetrical than in the other species of the genus. All three species of Clade III (*B. truncatus*, *B. liliana*, *B. granulimanus*) are characterized by the presence of a stout lateral tooth on the transverse suture of the uropodal exopod. In addition, the transverse suture is not finely toothed, as in most members of Clades I and II (except *B. emarginatus*). All three species of Clade III share a pale band on the pollex, at the base of the dactylus (Fig. 2E, G and H).

Clade IV (Fig. 5) includes three species of *Betaeopsis* from the tropical IWP and cool-temperate SWP, sharing a number of synapomorphies. The most important of them are (1) the presence of hook-like spines on the uropodal endopods, (2) the absence of an articulated plate on the sixth abdominal somite, and (3) the somewhat reduced number of mastigobranchs (Anker and Jeng, 2002). The monophyly of Clade IV is therefore indisputable.

The only species of *Betaeus* present in the temperate SWP is *B. australis*, which in the morphological analysis comes out as sister to *B. harrimani*, however, with a weak support (Fig. 3A). In the 16S + H3 analysis, *B. australis* is sister to Clades I + II (Fig. 3B), but in the combined analyses, *B. australis* is recovered as sister to Clades I + II + III (Fig. 4). Thus, in the combined analyses, *B. australis* represents a sister lineage to all the remaining species of *Betaeus s. str.* Importantly, *B. australis* and *B. harrimani* uniquely share the presence of a dorsal pilosity on the palm of the chelae, which from the morphological point of view represents a synapomorphy. These two species also have very similar chelipeds, orbital hoods, uropodal exopods (with a finely toothed transverse suture), and color patterns (for *B. harrimani* see Fig. 2A). Therefore, the position of *B. australis* as sister to Clades I + II, as recovered by the molecular analyses (Fig. 3B), seems to be less contradictory with morphology (Fig. 3A) than its position as sister to Clades I + II + III, in the combined analyses (Fig. 4).

More problematic and possibly leading to taxonomic consequences is the position of the South African *B. jucundus* (SEA) as a sister lineage to *Betaeus s. str.* + *Betaeopsis*. The above tree topology obviously results in the paraphyly of *Betaeus* (Figs. 3A, 4, and 5). Importantly, the same position for *B. jucundus* was recovered in the morphological analysis of alpheid genera by Anker et al. (2006), where *Betaeus* was represented by *B. jucundus*, *B. australis*, *B. harfordi*, and *Betaeopsis* by *Bp. aequimanus*. The position of *B. jucundus* as sister to the remaining species of *Betaeus* is expected given several morphological differences between *B. jucundus* and the remaining species of *Betaeus*. At least some of these differences may represent plesiomorphies within the *Betaeus* – *Betaeopsis* clade. For instance, in *B. jucundus*, the eyestalks are unarmed, the chelipeds are small and weakly differentiated (Fig. 11), the anal tubercles are poorly developed, and the uropodal exopod has a straight transverse suture, unlike in other species of the genus (Barnard, 1950). In most other characters, *B. jucundus* is similar to the other species of *Betaeus*. However, *B. jucundus* also shares one character with *Betaeopsis*, namely the presence of small setiferous tubercles on the chelae (Barnard, 1950; Anker and Jeng, 2002). The obvious morphological differences between *Betaeopsis* and *Betaeus s. str.*, and the well-supported sister position of

*Betaeopsis* and *Betaeus s. str.* in our combined morphology + DNA analyses, favor the retention of *Betaeopsis* as a valid genus and suggest the establishment of a new genus for *B. jucundus*. However, any taxonomic rearrangements within *Betaeus* would be premature in the absence of molecular data for *B. jucundus*.

#### 4.2. Biogeography of *Betaeus* and *Betaeopsis*

With seven species (*B. gracilis*, *B. harfordi*, *B. macginitieae*, *B. setosus*, *B. longidactylus*, *B. harrimani*, *B. ensenadensis*), the NEP (west coast of North America from Alaska to Baja California) is the most species rich region for *Betaeus* (Hart, 1964). Other geographical regions harbor many fewer taxa. For instance, the NWP (Russia’s Far East and Japan south to Taiwan) and the adjacent SEP and SWA regions (from Peru to Argentina) harbor three species each (NWP: *B. granulimanus*, *B. levifrons*, *B. gelasinifer*; SWA: *B. emarginatus*, *B. truncatus*, *B. liliana*). Only one species of *Betaeus* (*B. australis*) is present in southern Australia (SWP) and another one (*B. jucundus*) on the Atlantic side of southern Africa (SEA). In contrast, *Betaeopsis* (Clade IV) has representatives in both tropical (IWP, *Bp. indicus*, *Bp. acanthops*) and cool-temperate waters (SWP, *Bp. aequimanus*) (refer to Fig. 5 and Table 1).

Herein, we tested the hypothesis that the cool-temperate waters of the NEP represent the center of origin of *Betaeus* + *Betaeopsis*, with a more intense speciation compared to other geographical regions. Importantly, Clades I and II, composed in large part of NEP species (Fig. 5), constitute the most derived ingroup clades, e.g., compared to the geographically more distant *B. australis* (SWP), *Betaeopsis* (Clade IV, IWP + SWP), and especially *B. jucundus* (SEA). Furthermore, Clade I (NEP + NWP) is more derived than Clade III (NWP + SEP + SWA). At first glance, these observations agree reasonably well with one of the predictions of the CoFo model. An historical scenario can be drawn, in which species of *Betaeus* arise in the NEP to later expand their geographic ranges, spreading out toward other geographical zones (NWP, SEP). However, in partial disagreement with this scenario, no correlation between species age and geographic range was found.

In addition, our results suggest that speciation in *Betaeus* also took place in regions other than the NEP. For instance, our morphological and molecular (+total evidence) datasets strongly support the sister relationship between *B. granulimanus* (NWP) and *B. liliana* (SWA), suggesting that their most recent common ancestor probably did not live in the NEP. The same is true for the three species of *Betaeopsis*, which seems to have undergone a small radiation in the Indo-West and South-West Pacific (IWP–SWP). Thus, to explain the current distribution pattern of hooded shrimps, both centripetal speciation (at the center of origin) and peripheral speciation (out of the center of origin) processes need to be invoked. Various groups of marine invertebrates and vertebrates have their center of diversity in the Indo-Australian Archipelago. Several studies revealed that speciation in these groups occurs in regions of both high and low species richness (e.g., Barber and Bellwood, 2005 and references therein). Thus, regions with high biodiversity may represent not only a “cradle” (center of origin) but also a “museum” (center of accumulation) of species.

The CoFo model assumes that dispersal is the most relevant component of diversification: new species arise and spread out from a geographic center of distribution toward the periphery, where they eventually become extinct (Briggs, 1974, 2000, 2003). This model does not consider vicariance to be of major importance in the speciation process. However, vicariance has been recognized as the most important process favoring diversification in many marine organisms, including snapping shrimps (*Alpheus* spp. – Knowlton and Weigt, 1998). If both dispersal and vicariance (and not only dispersal) are taken into account, and if the geographic range of each species is considered stable through time,

our phylogenetic results can be interpreted very differently. For instance, in our combined phylogenetic analyses, *B. jucundus* (SEA) was found to be sister to *Betaeopsis* (IWP, SWP) + *Betaeus* s. str. (NEP, NWP, SWP, SEP, SWA), whereas *B. australis* (SWP) was recovered in a sister position to Clades I, II, III (NEP, NWP, SEP, SWA) (Fig. 4). These findings suggest that the entire *Betaeus* – *Betaeopsis* clade may have originated in subtropical or temperate waters of the southern Indo-Pacific (and not in the NEP, as per CofO model), subsequently spreading into the northern Pacific (NEP, NWP), where its main radiation took place (Fig. 5). This hypothesis would be also supported by the mainly tropical–subtropical affinities of other alpheid genera, including genera phylogenetically close to *Betaeus* and *Betaeopsis* (Anker et al., 2006).

The high species diversity in the NEP combined with two independent NWP–NEP connections, more precisely between *B. gelasinifer* and *B. setosus* + *B. cf. gracilis* in Clade I, and between *B. levifrons* and *B. ensenadensis* in Clade II (Fig. 5), suggest that climate change might have been responsible for compressing the distribution range of originally more widely distributed ancestral species during periods of global cooling. Such cooling periods may have facilitated local differentiation and/or allopatric speciation within these ancestral species on the opposite sides of the Pacific Ocean. This hypothesis based on vicariance was proposed for several groups with amphipacific distribution, including marine snails from the genus *Nucella* (Amano et al., 1993) and the smelt family Osmeridae (McAllister, 1963). An alternative explanation would be a radiation of these clades in the NEP followed by two more recent and independent dispersal events from the NEP to the NWP, resulting in the formation of *B. gelasinifer* and *B. levifrons* in the NWP.

Two geographically isolated taxa, *B. liliana* (SWA) and *B. granulimanus* (NWP), are supported as geminate taxa by both morphology and DNA separately, as well as by the total evidence analyses (Figs. 3–5). A possible explanation for this geographically incongruent sister relationship is a much wider distribution of the ancestors of Clade III in the Pacific Ocean, with lineages eventually colonizing the NWP (*B. granulimanus*) and the SEP (*B. truncatus*), from there penetrating into the SWA (*B. truncatus*, *B. liliana*), and possibly going extinct elsewhere. In this case, a putative extinction over large areas of ancestral range offers a more “parsimonious” explanation than short-scale temporal but large-scale spatial dispersal events, followed by allopatric speciation. Nonetheless, short temporal dispersal (e.g., via rafting) has been suggested to account for cases of extremely disjoint distributions (several thousands km apart) in various marine organisms (see review in Thiel and Haye, 2006).

The biogeographic scenarios discussed above are considerably different from that predicted by the simple CofO model. At least some of these scenarios appear to be more likely than those predicted by the CofO model in the general context of the family Alpheidae. We argue in favor of additional studies examining the phylogeography of different members of *Betaeus* and *Betaeopsis* to have a more detailed picture of their genetic structure and diversification.

#### 4.3. Evolution of symbiosis in *Betaeus* and *Betaeopsis*

*Betaeus* includes both free-living species and species living in either facultative or obligate symbioses with other invertebrates. Eight species of *Betaeus* (*B. truncatus*, *B. emarginatus*, *B. liliana*, *B. australis*, *B. granulimanus*, *B. pingi*, *B. gelasinifer*, *B. gracilis*) and all three species of *Betaeopsis* (*Bp. aequimanus*, *Bp. indicus*, *Bp. acanthops*) are primarily free-living shrimps, dwelling in crevices under rocks, among kelp holdfasts, or in small coastal lava caves (Hart, 1964; Boschi, 1966; Miya, 1972; Bruce and Iliffe, 1991; Jensen, 1995; Nomura and Komai, 2000). Symbiotic relationships are known in at least nine species of *Betaeus* (see Table 1). An infaunal

(e.g., burrow-associated) lifestyle is known in five species: *B. jucundus*, *B. levifrons*, *B. ensenadensis*, *B. harrimani*, and *B. longidactylus*. Three of them (*B. levifrons*, *B. jucundus*, *B. ensenadensis*) may be obligate symbionts in burrows of mud and ghost shrimps (*Upogebia*, *Neotrypaea*, *Callichirus*) (Hart, 1964; Branch et al., 1994; Marin, 2010). The other two species (*B. harrimani*, *B. longidactylus*) are known as facultative symbionts in burrows of mud and ghost shrimps (*Upogebia*, *Neotrypaea*) and innkeeper worms (*Urechis*), but are also occasionally found free-living under rocks (Hart, 1964; Butler, 1980; Chace and Abbott, 1980; Jensen, 1995). Two species, *B. macginitieae* and *B. harfordi*, are obligate symbionts of sea urchins (*Strongylocentrotus* spp.) and abalones (*Haliotis* spp.), respectively (Hart, 1964; Jensen, 1995). Finally, two species, *B. setosus* and *B. liliana*, often share shelter with porcelain crabs and xanthid crabs, respectively, apparently gaining some protection from their larger and stronger hosts (Butler, 1980; Jensen, 1986, 1995; Baeza et al., 2010). Thus *Betaeus* is no exception to the general propensity of alpheid shrimps to engage in symbioses with other marine organisms (e.g., Bruce, 1976; Knowlton and Keller, 1985; Anker, 2001; Anker et al., 2005; Anker and Jeng, 2007).

The mapping of the symbiosis – free-living continuum on the combined (morphology + DNA) phylogeny of *Betaeus* and *Betaeopsis* suggests that the commensal-infaunal habits evolved more than once in *Betaeus* (Fig. 5). This evolutionary plasticity emulates the microhabitat flexibility of some species, such as *B. harrimani* and *B. longidactylus*, which may be free-living or associated with burrows of upogebiids, callianassids or echinurans. Similarly, shelter sharing with anomuran and brachyuran crabs appears to have evolved independently in two non-related and geographically distant lineages, viz. in *B. setosus* (Clade I, NEP) and in *B. liliana* (Clade III, SWA). Finally, one small lineage of *Betaeus* within Clade I evolved from free-living forms to obligate symbionts of slowly moving invertebrates: *B. macginitieae* is always found dwelling under or among the spines of sea urchins of the genus *Strongylocentrotus* (Fig. 2B), whereas its sister species, *B. harfordi*, lives commensally in the mantle cavity of abalones, *Haliotis* spp. (Fig. 2C). Both *B. macginitieae* and *B. harfordi* appear to be obligate symbionts, leaving their hosts for foraging only during dark periods and returning back to them on the onset of light. Hosts are recognized mainly via chemical (*B. harfordi*) or both chemical and visual stimuli (*B. macginitieae*) (Ache and Davenport, 1972).

#### 4.4. Evolution of sociobiology in *Betaeus* and *Betaeopsis*

The sociobiology of species of *Betaeus* and *Betaeopsis* is quite diverse (at least compared to other genera within the Alpheidae, see below), although for several species it is known incompletely. Five species of *Betaeus* (*B. truncatus*, *B. liliana*, *B. emarginatus*, and probably *B. australis* and *B. granulimanus*) and one species of *Betaeopsis* (*Bp. aequimanus*) typically occur in small groups of up to several dozen individuals that include adults of both sexes (sometimes a larger dominant male) and a number of immature individuals; these groups are called aggregations (Baeza et al., 2010; A. Anker, pers. obs.; J.A. Baeza, pers. obs.). Some ecological aspects of this gregariousness were recently studied in *B. liliana* in Mar del Plata, Argentina (Baeza et al., 2010), where this species lives in aggregations of up to 17 individuals in large intertidal rock pools. These aggregations feature female-biased sex ratios more frequently than expected by chance, but have no complex social structure that could suggest a harem-like grouping (Baeza et al., 2010). In contrast, several species of *Betaeus* appear to live in pairs (e.g., *B. harfordi* and *B. macginitieae* – Hart, 1964), or solitarily (e.g., *B. setosus* – Jensen, 1986).

In contrast to *Betaeus*, the vast majority of alpheid shrimps, including species of *Alpheus* and *Synalpheus*, are socially monogamous, i.e. they live in male–female pairs (e.g., Mathews, 2003;

Rahman et al., 2003). However, at least some species of *Athanas* are often found in tide pools or under rocks in small, apparently “loose” groups, containing several males, females and younger individuals (A. Anker, pers. obs.). In addition, a few sponge-dwelling species of *Synalpheus* are eusocial, living in colonies ranging from a dozen to several hundred individuals, with only one or a few reproductive females called “queens” (Duffy, 1996; Morrison et al., 2004).

Mapping sociobiology as a trait with two states, i.e. (1) solitary + social monogamy vs. (2) gregariousness, on the phylogeny of *Betaeus* and *Betaeopsis* (Fig. 5) shows that all gregarious species belong to lineages closer to the root of the tree: Clade IV (*Bp. aequimanus*, ecology unknown in the other two species), *B. australis*, Clade III (*B. truncatus*, *B. liliana*, *B. granulimanus*), and *B. emarginatus* (sister to Clades I + II). Based on this repartition of sociobiology traits, two scenarios of their evolution are plausible. According to the first scenario, gregariousness represents an ancestral character state of *Betaeus s. str.* (excluding *B. jucundus*) + *Betaeopsis*, which means that living in pairs or solitarily evolved more than once independently within *Betaeus s. str.* Importantly, a shift from gregarious to a solitary/pair living life is observed in lineages with symbiotic tendencies, i.e. in Clade I (*B. harfordi*, *B. macginitieae*) and in Clade II (*B. levifrons*, *B. ensenadensis*, *B. harrimani*), but also in *B. jucundus* (Fig. 5). The alternative scenario is that solitary/pair living evolved once in the ancestor of Clades I and II and then switched back to gregariousness in *B. emarginatus*; this second scenario requires the same number of steps as does the first scenario. It must be noted here that the sociobiology of most infaunal species of *Betaeus* (*B. ensenadensis*, *B. harrimani*, *B. levifrons*, *B. jucundus*) is poorly known, but based on field collection data and general observations (Hart, 1964; Branch et al., 1994; Jensen, 1995; A. Anker, pers. obs.) they do not seem to live in burrows in aggregations.

#### 4.5. Outlook

In summary, *Betaeus* appears to be a very interesting model group for ecological, behavioral, and evolutionary studies. More detailed ecological and behavioral studies in several species of *Betaeus* (similar to that of Baeza et al., 2010 on *B. liliana*) are needed to have a better knowledge of their life habits and (for symbiotic species) interactions with their hosts. Additional molecular analyses are needed, especially for two species for which H3 and 16S sequences are still unavailable (*B. jucundus* and *B. gelasinifer*); these sequences would be extremely important to conclude about the phylogenetic position and taxonomic status of *B. jucundus*. Use of different molecular markers (e.g., MyHC, COI used in other alpheid studies, e.g., Mathews and Anker, 2009) could add to the robustness of the phylogenetic hypothesis for the *Betaeus*–*Betaeopsis* clade. A more robust and comprehensive phylogeny may be used to propose and test hypotheses on the evolution of social systems and symbiotic interactions in hooded shrimps, as well as their possible interdependence (Baeza and Thiel, 2003, 2007). Comparative studies on phylogeography and historical demography of several species of *Betaeus* and *Betaeopsis* are needed to better understand the diversification processes in this interesting clade of caridean shrimps in particular, and in temperate marine invertebrates in general.

#### Acknowledgments

J.A.B. was supported by a postdoctoral fellowship from SMSFP. A.A. was supported by a postdoctoral fellowship from CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) of the Brazilian Government. This study was partially funded by a National Geographic research grant to J.A.B. Some specimens used in this study were provided by our colleagues: Constance Gramlich

(San Diego State University), Gregory Jensen (University of Washington), Richard Taylor (Leigh Marine Laboratory, New Zealand), Charles Griffiths (University of Cape Town, Republic of South Africa), Ernesto Campos (Universidad Autónoma de Baja California, Mexico), Gustav Paulay (FLMNH, USA), Sammy De Grave (OUMNH, United Kingdom), Luis Ignacio Vilchis and William A. Newman (Scripps Institution of Oceanography, University of California at San Diego, USA), Jimmy O'Donnell (University of California at Santa Cruz, USA), Ivan N. Marin (A.N. Severtzov Institute of Ecology and Evolution, RAS, Moscow, Russia), Ivan Hinojosa (UCN, Chile), and Tomas Luppi, Nahuel Farias, Eduardo Spivak, and Marcelo Scelzo (UNdMP, Argentina). Special thanks to Martin Thiel (UCN, Chile) for inviting both of us to co-instruct (together with Raymond T. Bauer) a short course on biology of caridean shrimps in Coquimbo in 2007. Jeff Hunt and Lee Weigt (Laboratory of Analytical Biology, USNM) provided logistical support for the molecular analysis. Additional color photographs used in Fig. 2 were provided by Richard Taylor, Gustav Paulay, Kevin Lee ([www.diverkevin.com](http://www.diverkevin.com), USA), Larry Jon Friesen ([www.sbnature.net](http://www.sbnature.net), USA), and Madoka Itoh (Japan). The comments by three anonymous referees improved considerably the present manuscript. This is Smithsonian Marine Station at Fort Pierce contribution number 882.

#### Appendix A. List of morphological characters used in the phylogenetic analysis of the genus *Betaeus*

Abbreviations used in the text: Mxp3 – maxilliped; P – pereopod.

- (1) Frontal margin of carapace: with rostrum (0), without rostrum, broadly rounded or truncate (1), without rostrum, with deep median emargination (2).
- (2) Antennular stylocerite: not exceeding distal margin of second antennular article (1), exceeding distal margin of second antennular article (0).
- (3) Second antennular article: conspicuously elongate (1), not or only slightly elongate (0).
- (4) First antennular segment: with large acute distolateral tooth (1), without such a tooth (0).
- (5) Uropodal endopod: with two curved, hook-like spines distally (1), without hook-like spines (0).
- (6) Uropodal diaeresis: sinuous and usually with large lateral tooth (0), finely toothed (1), straight (2).
- (7) P3 dactylus: without dents or ungui (0), with ventral margin bearing large unguis subdistally (1), minute dent at about mid-length (2).
- (8) P3 dactylus: without indentations (0), with dorsal margin bearing minute dent (1).
- (9) P3 merus: without spines or with at least two smaller spines on ventrolateral surface (0), with one large stout spine on ventrolateral surface (1).
- (10) P3 ischium: with spine on ventrolateral surface (0), without spine on ventrolateral surface (1).
- (11) Uropodal protopod: simple, not bidentate (0), lateral lobe with two teeth situated close to each other, forming half-moon (1), with two teeth situated at some distance from each other (2).
- (12) Eystalks: without tooth (0), with projecting, lanceolate tooth (1), with small triangular tooth (2).
- (13) Mastigobranchs: present on P1–3 or P1–4 (0), present on P1–2 only (1).
- (14) P3 carpus: without distal spine(s), at most with a long seta, weaker than propodus spines (0), with one or two stout distal spine(s), comparable in thickness to the spines of propodus (1).

- (15) Antennal scaphocerite: without distinct cleft (0), with cleft between distolateral tooth and blade reaching 0.6 length of blade (1), with cleft reaching at most distal 0.8 length of blade (2).
- (16) P2, first carpal article: as long or longer than the sum of following three articles (0), shorter than the sum of following three articles (1).
- (17) Antennal carapocerite: at most reaching to the tip of distolateral tooth of scaphocerite (0), exceeding scaphocerite (1).
- (18) P5 propodal brush: well developed, with more than four rows (0), reduced to at most three seral rows (1).
- (19) Mxp3, tip of ultimate article: with at least one stout seta or spinule (0), unarmed (1).
- (20) Color pattern: with brown–red bands dorsally on abdomen, flanks colorless (1), uniform dark purple (2), semitransparent yellowish (3), otherwise (0).
- (21) Color pattern, P1 palm: without pale transverse band (0), with whitish or pale transverse band (1).
- (22) P1: smooth (0), covered with fine granules (1), with large bumpy tubercles (2).
- (23) P1: more or less equal, symmetrical or subsymmetrical (0), somewhat unequal, asymmetrical (1).
- (24) P1 palm: without pilosity on dorsal face (0), with pilosity on dorsal face (1).
- (25) Sixth abdominal somite: without articulated plate (0), with articulated plate (1).
- (26) P1 merus: without tubercles on mesial margin (0), with tubercles on mesial margin (1).
- (27) P1 in adult males, dactylus: unarmed or with continuous teeth (0), with one proximal tooth and one stout distal tooth (1).
- (28) P1 chela: neither rounded nor elongate (0), broadly rounded (1), conspicuously elongate (2).
- (29) P1 palm: without setiferous tubercles on mesial face (0), with setiferous tubercles on mesial face (1).
- (30) P1 fingers when extended: dactylus in dorsolateral or dorsal position (0), in ventral position (1).
- (31) Uropodal exopod, distolateral tooth: adjacent to distolateral spine (0), at some distance from distolateral spine (1).
- (32) Telson, spines on posterior margin: long, mesial much longer than lateral (0), short, subequal (1), short, mesial curved upwards (2).
- (33) Telson, dorsal spines: large (0), small (1) absent (2).
- (34) Articulated plate: present, more subtriangular (0), present, somewhat quadrangular (1), absent (2).
- (35) P1 in adult males, gap between fingers: small or absent (0), very large (1).
- (36) Lateral antennular flagellum, fused portion (proximal to accessory branch): short, with less than five articles (0), long, with usually more than seven articles (1).
- (37) Anal tubercles: poorly developed or absent (0), well-developed (1).
- (38) Uropods: exceeding telson only slightly (0), by far exceeding telson (1).
- (39) Ocellar beak: not projecting, invisible laterally (0), projecting, visible laterally (1).

## Appendix B

Matrix of morphological character states from Appendix A. Character states are ordered for characters from 1 to 39. 0–3 – Character states as defined in Appendix A; ? – Character state unknown; P – Character state polymorphic (0 or 1).

<i>B. australis</i>	100001011012002000010001111001000111101
<i>B. emarginatus</i>	110000211112012010100110111001000011101
<i>B. ensenadensis</i>	111001001022010010110110110001100111101
<i>B. gelasinifer</i>	210001101112011110130000111101000011101
<i>B. cf. gracilis</i>	21000110111201111013000011P0010000P1101
<i>B. granulimanus</i>	210100001112001110100210111201000011101
<i>B. harfordi</i>	21100110111201110130000111101011011101
<i>B. harrimani</i>	10000100111201200001011111001000111101
<i>B. jucundus</i>	11000200100001000000000011001100000000
<i>B. levifrons</i>	1110010011220100101001001102011001?110?
<i>B. lilianae</i>	110100001112002110101110111201000011101
<i>B. longidactylus</i>	100001011112002010000010111201000011101
<i>B. macginitieae</i>	111001101112011010120000111101011011101
<i>B. setosus</i>	21100110111201110130000111101000011101
<i>B. truncatus</i>	110000001012002010101110111001000111101
<i>Bp. acanthops</i>	1000101011011000110?000000011022?01011
<i>Bp. aequimanus</i>	20001010110110001100000000011020?01011
<i>Bp. indicus</i>	1000101011011000110?000000011020?01011
<i>At. nitescens</i>	00000000000000000000000000000000000000
<i>Al. yaldwyni</i>	00000000000001000000000010000000000000
<i>M. minutus</i>	010000000000000100000000000000?00000

## References

- Ache, B.W., Davenport, D., 1972. The sensory basis of host recognition by symbiotic shrimps, genus *Betaeus*. *Biol. Bull.* 143, 94–111.
- Amano, K., Vermeij, G.J., Narita, K., 1993. Early evolution and distribution of the gastropod genus *Nucella*, with special reference to Miocene species from Japan. *Trans. Proc. Paleontol. Soc. Jpn. N. Ser.* 171, 237–248.
- Anker, A., 2001. Taxonomie et evolution des Alpheidae (Crustacea, Decapoda). Thèse de Doctorat, Muséum national d'Histoire naturelle, Paris. 2 vols, 547 + 331 pp.
- Anker, A., Ah Yong, S.T., Noel, P.Y., Palmer, A.R., 2006. Morphological phylogeny of alpheid shrimps: parallel preadaptation and the origin of a key morphological innovation, the snapping claw. *Evolution* 60, 2507–2528.
- Anker, A., Jeng, M.-S., 2002. Redefinition of *Betaeopsis* Yaldwyn, 1971, and invalidation of *Hamalpheus* Bruce & Iliffe, 1991 (Crustacea: Decapoda: Alpheidae). *Proc. Biol. Soc. Wash.* 115, 570–584.
- Anker, A., Jeng, M.-S., 2007. Establishment of a new genus for *Arete borradalei* Coutière, 1903 and *Athanas verrucosus* Banner and Banner, 1960, with redefinitions of *Arete* Stimpson, 1860 and *Athanas* Leach, 1814 (Crustacea: Decapoda: Alpheidae). *Zool. Stud.* 46, 454–472.
- Anker, A., Murina, G.V., Lira, C., Vera Caripe, J.A., Palmer, A.R., Jeng, M.S., 2005. Macrofauna associated with echiuran burrows: a review with new observations of the innkeeper worm, *Ochetostoma erythrogrammon* Leuckart and Rüppel in Venezuela. *Zool. Stud.* 44, 157–190.
- Antezana, T., Fagetti, E., Lopez, M.T., 1965. Observaciones bioecológicas en decápodos comunes de Valparaíso. *Rev. Biol. Mar., Valparaíso* 12, 1–60.
- Baeza, J.A., 2010. The symbiotic lifestyle and its evolutionary consequences: social monogamy and sex allocation in the hermaphroditic shrimp *Lysmata pederseni*. *Naturwissenschaften* 97, 729–741.
- Baeza, J.A., Fariás, N.E., Luppi, T.A., Spivak, E.D., 2010. Habitat size, social grouping and symbiosis: testing the “resource economic monopolization” hypothesis with the shrimp *Betaeus lilianae* and description of its partnership with the crab *Platyxanthus crenulatus*. *J. Exp. Mar. Biol. Ecol.* 389, 85–92.
- Baeza, J.A., Schubart, C.D., Zillner, P., Fuentes, S., Bauer, R.T., 2009. Molecular phylogeny of shrimps from the genus *Lysmata* (Caridea: Hippolytidae): the evolutionary origins of pair-living, protandric simultaneous hermaphroditism and species status. *Biol. J. Linn. Soc.* 96, 415–424.
- Baeza, J.A., Thiel, M., 2003. Predicting territorial behavior in symbiotic crabs using host characteristics: a comparative study and proposal of a model. *Mar. Biol.* 142, 93–100.
- Baeza, J.A., Thiel, M., 2007. The mating system of symbiotic crustaceans: a conceptual model based on optimality and ecological constraints. In: Duffy, J.E., Thiel, M. (Eds.), *Reproductive and Social Behavior: Crustaceans as Model Systems*. Oxford University Press, Oxford, pp. 245–255.
- Banner, A.H., Banner, D.M., 1960. Contributions to the knowledge of the alpheid shrimp of the Pacific Ocean. Part V. The Indo-Pacific members of the genus *Athanas*. *Pac. Sci.* 14, 129–155.
- Banner, D.M., Banner, A.H., 1973. The alpheid shrimp of Australia. Part I: The lower genera. *Rec. Aust. Mus.* 28, 291–382.
- Barber, P.H., Bellwood, D.R., 2005. Biodiversity hotspots: evolutionary origins of biodiversity in wrasses (Halichoeres: Labridae) in the Indo-Pacific and new world tropics. *Mol. Phyl. Evol.* 35, 235–253.

- Barnard, K.H., 1950. Descriptive catalogue of South African decapod Crustacea (crabs and shrimps). *Ann. South Afr. Mus.* 38, 1–837.
- Bauer, R.T., 2004. Remarkable Shrimps: Adaptations and Natural History of the Carideans. Oklahoma University Press, Norman.
- Bernardi, G., Bucciarelli, G., Costagliola, D., Robertson, D.R., Heiser, J.B., 2004. Evolution of coral reef fish *Thalassoma* spp. (Labridae). 1. Molecular phylogeny and biogeography. *Mar. Biol.* 144, 369–375.
- Boschi, E.E., 1966. Una nueva especie de crustáceo decápodo Caridea para las aguas costeras de la Provincia de Buenos Aires, Argentina. *Physis* 26, 83–88.
- Branch, G., Griffiths, C.L., Branch, M.L., Beckley, L.E., 1994. Two Oceans: A Guide to the Marine Life of Southern Africa. D. Philip, Cape Town.
- Briggs, J.C., 1974. Marine Zoogeography. McGraw-Hill, Toronto.
- Briggs, J.C., 2000. Centrifugal speciation and centres of origin. *J. Biogeogr.* 27, 1183–1188.
- Briggs, J.C., 2003. Marine centers of origin as evolutionary engines. *J. Biogeogr.* 30, 1–18.
- Bruce, A.J., 1976. Shrimps and prawns of coral reefs, with special reference to commensalism. In: Jones, O.A., Dean, R.M. (Eds.), *Biology and Geology of Coral Reefs*, vol. 3. Academic Press, New York, pp. 37–94.
- Bruce, A.J., Iliffe, T.M., 1991. *Hamalpheus acanthops*, new genus, new species, a stygiophilic alpheid shrimp from a Samoan lava tube. *J. Crustac. Biol.* 11, 583–593.
- Butler, T.H., 1980. Shrimps of the Pacific coast of Canada. *Can. Bull. Fish. Aquat. Sci.* 202, 1–280.
- Castresana, J., 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol. Biol. Evol.* 17, 540–552.
- Chace Jr., F.A., Abbott, D.P., 1980. Chapter 23 Caridea: the shrimps. In: Morris, R.H., Morris, R.H., Abbott, P.D., Haderlie, E.C. (Eds.), *Intertidal Invertebrates of California*. Stanford University Press, Stanford, p. 690.
- Colgan, D., McLauchlan, A., Wilson, G.D.F., Livingston, S., Edgecombe, G.D., Macaranas, J., Cassis, G., Gray, M.R., 1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Aust. J. Zool.* 46, 419–437.
- Coutière, H., 1899. Les “Alpheidae”, morphologie externe et interne, formes larvaires, bionomie. *Ann. Sci. Nat., Zool., série 8–9*, 1–560, pls. 1–6. Masson et Cie Ed, Paris.
- De Grave, S., Pentcheff, N.D., Ah Yong, S.T., Chan, T.-Y., Crandall, K.A., Dworschak, P.C., Felder, D.L., Feldmann, R.M., Fransen, C.H.J.M., Goulding, L.Y.D., Lemaitre, R., Low, M.E.Y., Martin, J.W., Ng, P.K.L., Schweitzer, C.E., Tan, S.H., Wetzer, R., 2009. A classification of living and fossil genera of decapod crustaceans. *Raffles Bull. Zool. (Suppl. 21)*, 1–109.
- Duffy, J.E., 1996. Eusociality in a coral-reef shrimp. *Nature* 381, 512–514.
- Duffy, J.E., Morrison, C.L., Ríos, R., 2000. Multiple origins of eusociality among sponge-dwelling shrimps (*Synalpheus*). *Evolution* 54, 503–516.
- Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucl. Acids Res.* 32, 1792–1797.
- Glassell, S.A., 1938. New and obscure decapod Crustacea from the west American coasts. *Trans. San Diego Soc. Nat. Hist.* 8, 411–454, pls. 27–36.
- Grant, T., Kluge, A.G., 2003. Data exploration in phylogenetic inference. Scientific, heuristic, or neither. *Cladistics* 19, 379–418.
- Hart, J.L.F., 1964. Shrimps of the genus *Betaeus* on the Pacific coast of North America with descriptions of three new species. *Proc. US Natl. Mus.* 115, 431–466.
- Holthuis, L.B., 1952. Reports of the Lund University Chile Expedition 1948–1949 5. The Crustacea Decapoda Macrura of Chile. *Lunds Universitets Årsskrift N.F. Avd. 2*, 47, 1–110.
- Huelsenbeck, J.P., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17, 754–755.
- Hultgren, K.M., Guerao, G., Marques, F.P.L., Palero, F.P., 2009. Assessing the contribution of molecular and larval morphological characters in a combined phylogenetic analysis of the Superfamily Majoidea. In: Martin, J.W., Crandall, K.A., Felder D.L. (Eds.), *Decapod Crustacean Phylogenetics*. Crustacean Issues. Koenemann, S. (series ed.) vol. 18. CRC Press, Taylor & Francis Group, Boca Raton, London, New York, pp. 437–455.
- Jensen, G.C., 1986. Some observations of the alpheid shrimp *Betaeus setosus* Hart with its host *Pachycheles rudis* Stimpson. *Bull. South Cal. Acad. Sci.* 85, 180–181.
- Jensen, G.C., 1995. Pacific Coast Crabs and Shrimps. Sea Challengers, Monterey.
- Jobb, G., von Haeseler, A., Strimmer, K., 2004. TREEFINDER: a powerful graphical analysis environment for molecular phylogenetics. *BMC Evol. Biol.* 4, 18.
- Jordan, D.S., 1908. The law of geminate species. *Am. Nat.* 42, 73–80.
- Kass, R.E., Raftery, A.E., 1995. Bayes factors. *J. Am. Stat. Assoc.* 90, 773–795.
- Kim, H.S., 1977. Macrura. Illustrated Flora and Fauna of Korea, 19: 1–414, pls. 1–56. Samwha Publishing Co., Seoul (in Korean).
- Knowlton, N., Keller, B.D., 1985. Two more sibling species of alpheid shrimps associated with the Caribbean sea anemones *Bartholomea annulata* and *Heteractis lucida*. *Bull. Mar. Sci.* 37, 893–904.
- Knowlton, N., Weigt, L.A., 1998. New dates and new rates for divergence across the Isthmus of Panama. *Proc. R. Soc. Biol. Sci.* 265, 2257–2263.
- Maddison, W.P., Maddison, D.R., 2010. Mesquite: A Modular System for Evolutionary Analysis. Version 2.73 <<http://mesquiteproject.org>>.
- Marin, I., 2010. Redescription of the alpheid shrimp *Betaeus levifrons* Vinogradov, 1950 (Crustacea, Decapoda, Alpheidae) from Peter the Great Bay, Russian coast of the Sea of Japan. *Zootaxa* 2613, 51–60.
- Mathews, L.M., 2003. Tests of the mate-guarding hypothesis for social monogamy: male snapping shrimp prefer to associate with high-value females. *Behav. Ecol.* 14, 63–67.
- Mathews, L.M., Anker, A., 2009. Molecular phylogeny reveals extensive ancient and ongoing radiations in a snapping shrimp species complex (Crustacea, Alpheidae, *Alpheus armillatus*). *Mol. Phylog. Evol.* 50, 268–281.
- McAllister, D.E., 1963. A revision of the smelt family, Osmeridae. *Bull. Natl. Mus. Canada* 191, 1–53.
- Miya, Y., 1972. The Alpheidae (Crustacea, Decapoda) of Japan and its adjacent waters. Part I. *Pub. Amakusa Mar. Biol. Lab.* 3, 23–101.
- Morrison, C.L., Ríos, R., Duffy, J.E., 2004. Phylogenetic evidence for an ancient rapid radiation of Caribbean sponge-dwelling snapping shrimps (*Synalpheus*). *Mol. Phylogenet. Evol.* 30, 563–581.
- Nomura, K., Komai, T., 2000. A new alpheid shrimp of the genus *Betaeus* from the Pacific coast of central Japan (Crustacea: Decapoda: Alpheidae). *Crustac. Res.* 29, 45–57.
- Nylander, J.A.A., Ronquist, F., Huelsenbeck, J.P., Nieves-Aldrey, J.L., 2004. Bayesian phylogenetic analysis of combined data. *Syst. Biol.* 53, 47–67.
- Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14, 817–818.
- de Queiroz, A., Donohue, M.J., Kim, J., 1995. Separate versus combined analysis of phylogenetic evidence. *Annu. Rev. Ecol. Syst.* 26, 657–681.
- Rahman, N., Dunham, D.W., Govind, C.K., 2003. Social monogamy in the big-clawed snapping shrimp, *Alpheus heterochelis*. *Ethology* 109, 457–473.
- Rocha, L.A., Rocha, C.R., Robertson, D.R., Bowen, B.W., 2008. Comparative phylogeography of Atlantic reef fishes indicates both origin and accumulation of diversity in the Caribbean. *BMC Evol. Biol.* 8, 157. <http://dx.doi.org/10.1186/1471-2148-8-157>.
- Schubart, C.D., Neigel, J.E., Felder, D.L., 2000. Use of the mitochondrial 16S rRNA gene for phylogenetic and population studies of Crustacea. *Crustac. Issues* 12, 817–830.
- Swofford, D.L., 2002. PAUP\*: Phylogenetic Analysis Using Parsimony (\* and other methods), version 4.0b10. Sinauer, Sunderland, Massachusetts.
- Thiel, M., Haye, P., 2006. The ecology of rafting in the marine environment. III. Biogeographical and evolutionary Consequences. *Oceanogr. Mar. Biol. Annu. Rev.* 44, 323–429.
- Vinogradov, L.G., 1950. Classification of shrimps, prawns and crabs from the Far East. *Izvestiya Tikhookeanskogo Nauchno-Issledovatel'skogo Instituta Rybnogo Khozyaistva i Okeanografii* 33, 179–358, in Russian.
- Wahlberg, N., Braby, M.F., Brower, A.V.Z., de Jong, R., Lee, M.M., Nylin, S., Pierce, N.E., Sperling, F.A.H., Vila, R., Warren, A.D., Zakharov, E., 2005. Synergistic effects of combining morphological and molecular data in resolving the phylogeny of butterflies and skippers. *Proc. R. Soc. Lond. B Biol. Sci.* 272, 1577–1586.
- Yaldwyn, J.C., 1971. Preliminary descriptions of a new genus and twelve new species of natant decapod Crustacea from New Zealand. *Rec. Dominion Mus.* 7, 85–94.
- Yang, H.J., Koo, H., Kim, W., 2007. A new record of *Betaeus gelasinifer* Nomura and Komai (Decapoda: Caridea: Alpheidae) from Korea. *Korean J. Syst. Zool.* 23, 99–102.
- Yu, S.C., 1930. Deux nouvelles crevettes de Chine. *Bull. Soc. Zool. France* 55, 454–462.