Evolutionary History of True Crabs (Crustacea: Decapoda: Brachyura) and the Origin of Freshwater Crabs

Ling Ming Tsang,[1,2] Christoph D. Schubart,[3] Shane T. Ahyong,[4,5] Joelle C.Y. Lai,[6] Eugene Y.C. Au,[2] Tin-Yam Chan,[1] Peter K.L. Ng,[6] and Ka Hou Chu*[,2]

[1]Institute of Marine Biology, National Taiwan Ocean University, Keelung, Taiwan

[2]Simon F. S. Li Marine Science Laboratory, School of Life Sciences, The Chinese University of Hong Kong, Hong Kong, China

[3]Biology 1, University of Regensburg, Regensburg, Germany

[4]Australian Museum, Sydney, New South Wales, Australia

[5]School of Biological, Earth and Environmental Sciences, University of New South Wales, Kensington, New South Wales, Australia

[6]Department of Biological Sciences, National University of Singapore, Singapore

***Corresponding author:** E-mail: kahouchu@cuhk.edu.hk, khchu@netvigator.com.

Associate editor: Todd Oakley

Abstract

Crabs of the infra-order Brachyura are one of the most diverse groups of crustaceans with approximately 7,000 described species in 98 families, occurring in marine, freshwater, and terrestrial habitats. The relationships among the brachyuran families are poorly understood due to the high morphological complexity of the group. Here, we reconstruct the most comprehensive phylogeny of Brachyura to date using sequence data of six nuclear protein-coding genes and two mitochondrial rRNA genes from more than 140 species belonging to 58 families. The gene tree confirms that the "Podotremata," are paraphyletic. Within the monophyletic Eubrachyura, the reciprocal monophyly of the two subsections, Heterotremata and Thoracotremata, is supported. Monophyly of many superfamilies, however, is not recovered, indicating the prevalence of morphological convergence and the need for further taxonomic studies. Freshwater crabs were derived early in the evolution of Eubrachyura and are shown to have at least two independent origins. Bayesian relaxed molecular methods estimate that freshwater crabs separated from their closest marine sister taxa ~135 Ma, that is, after the break up of Pangaea (~200 Ma) and that a Gondwanan origin of these freshwater representatives is untenable. Most extant families and superfamilies arose during the late Cretaceous and early Tertiary.

Key words: **molecular phylogeny, freshwater crabs, Podotremata, Heterotremata, Thoracotremata, Gondwana.**

Introduction

Crabs of the infra-order Brachyura are among the most diverse groups of crustaceans with over 7,000 described species in 98 families, occurring in marine, freshwater, and terrestrial habitats (Ng et al. 2008; De Grave et al. 2009; Ahyong et al. 2011). The relationships among the brachyuran families are poorly understood, however, due to the high morphological diversity of the group. Guinot (1977, 1978, 1979) divided Brachyura into three sections according to the gonopore positions: Podotremata, Heterotremata, and Thoracotremata. Podotremata is considered to be primitive in retaining various presumably ancestral characteristics, whereas the sections Heterotremata and Thoracotremata together form the Eubrachyura with the latter section being the most derived.

The monophyly of Podotremata is, however, contentious, being justified on the basis of possibly pleisomorphic characters (Scholtz and McLay 2009). Ahyong et al. (2007) falsified the monophyly of Podotremata based on a molecular phylogenetic analysis of nuclear *18S* gene sequences and proposed a split of the former Podotremata into three sections: Dromiacea, Raninoida, and Cyclodorippoida. This view corroborates results from recent morphological analysis (Scholtz and McLay 2009) and was adopted in recent classifications of brachyuran crabs (De Grave et al. 2009; Ahyong et al. 2011; Karasawa et al. 2011; Števčić 2011).

On the other hand, the Eubrachyura are accepted as monophyletic (von Sternberg and Cumberlidge 2001a, 2001b; Ng et al. 2008; but see Brösing et al. 2007). Within Eubrachyura, however, the relationship between Heterotremata and Thoracotremata is unclear, chiefly whether the two sections are reciprocally monophyletic or whether Thoracotremata is derived from within Heterotremata, which effectively synonymises them under the Eubrachyura (Scholtz and Richter 1995, von Sternberg and Cumberlidge 2001a, 2001b; Dixon et al. 2003; Brösing et al. 2007). Within Heterotremata, the origins of the various families of exclusively freshwater crabs are one of the most contentious issues.

Freshwater crabs live exclusively in freshwater or terrestrial habitats and never enter brackish or marine waters for reproduction (Cumberlidge and Ng 2009). They all undergo direct development and complete their life cycle in freshwater (Ng et al. 2008; Yeo et al. 2008; Cumberlidge and Ng 2009). More than 1,300 species are known with additional species being regularly discovered (reviewed in Yeo et al. 2008; Cumberlidge and Ng 2009). Five families

(Pseudothelphusidae, Potamonautidae, Potamidae, Gecarcinucidae, and Trichodactylidae) are exclusively composed of freshwater species, the primary freshwater crabs (Ng et al. 2008; Yeo et al. 2008; Cumberlidge and Ng 2009; Klaus et al. 2009). The systematics of the primary freshwater crabs have received more attention recently due to their high diversity and conservation value (e.g., Daniels et al. 2006; Cumberlidge et al. 2008; Yeo et al. 2008; Cumberlidge and Ng 2009; Cumberlidge et al. 2009; Klaus et al. 2009, 2010). Although alpha level interrelationships are comparatively well resolved, the higher systematics of freshwater crabs is still unstable. The five families are generally considered to comprise two distinct lineages: the South American Trichodactylidae and a possibly monophyletic assemblage consisting of the other four families distributed almost worldwide. Some morphological characters point to a close affinity between Trichodactylidae and Portunoidea (Rodriguez 1992; von Sternberg et al. 1999; von Sternberg and Cumberlidge 2003), but this relationship is not supported by recent molecular analyses (Schubart and Reuschel 2009). The position of the other lineage is even more disputed. They are usually considered to be heterotremes (Martin and Davis 2001; Ng et al. 2008; De Grave et al. 2009; Ahyong et al. 2011), but some authors argue that they share a number of synapomorphies with thoracotremes (von Sternberg et al. 1999; von Sternberg and Cumberlidge 2001a, 2001b). A morphological cladistic analysis further suggested that Thoracotremata may constitute the marine sister group of the nontrichodactylid freshwater crabs and that the two groups possibly originated from xanthoid-like progenitors (von Sternberg et al. 1999). In addition, given the circumtropical distribution of the nontrichodactylid freshwater crabs, a single evolutionary origin would imply that the diversification and radiation of the group predated the break up of Gondwana (~184 Ma) or even Pangaea (~200 Ma) (Ng and Rodriguez 1995; Ng et al. 1995). Under this scenario, subsequent cladogenesis would have tracked tectonic events as a result of the split of Gondwana. This phylogenetic hypothesis, however, requires an ancient origin of freshwater crabs and the remaining Eubrachyura, which the fossil record does not support (earliest fossil of freshwater crabs dated <30 Ma; Feldmann et al. 2007, see Klaus et al. 2011 for a review). In sum, the origin of the primary freshwater crabs, status of eubrachyuran sections and interrelationships of the heterotremes, remains obscure—resolution requires a comprehensive phylogenetic framework.

Comprehensive morphological phylogenetic analysis of Brachyura is hampered by the large number of seemingly highly derived characters and extreme diversity of the group, whereas molecular phylogenetic studies of Brachyura have been restricted to particular subsets of taxa and small internal clades (Schubart, Cuesta, et al. 2000; Kitaura et al. 2002; Daniels et al. 2006; Schubart et al. 2006; Hultgren and Stachowicz 2008; Palacios-Theil et al. 2009; Schubart and Reuschel 2009; Sin et al. 2009; Wetzer et al. 2009; Lai et al. 2011). A comprehensive study of the overall phylogeny of Brachyura and the relationships among superfamilies and/or subsections is still lacking. Here, we attempt to construct a comprehensive phylogenetic framework of the Brachyura using sequences from six nuclear protein-coding genes and two mitochondrial rRNA genes. In particular, we aim to determine the following: 1) whether Podotremata, Heterotremata, and Thoracotremata are natural groups; 2) the origin and sister group of the freshwater crabs; and 3) the phylogenetic relationships and the divergence times of the major superfamilies and families.

Results

Phylogenetic Analyses

The combined data set consisted of 3,912 bp from eight gene fragments. Analyses under maximum likelihood (ML) and Bayesian inference (BI) of the combined data set resulted in topologies without conflicting nodes of strong support (here defined as ML bootstrap [BP] >70 or BI posterior probability [PP] >0.95). Hence, we present the nodal supports obtained from the two analyses together on the BI topology (fig. 1). Brachyura is strongly supported as monophyletic (fig. 1*A*) in relation to the included Anomura, corroborating the results of previous studies (Scholtz and Richter 1995; Dixon et al. 2003; Ahyong and O'Meally 2004; Porter et al. 2005; Ahyong et al. 2007; Tsang et al. 2008; Bracken et al. 2009, 2010). The former Podotremata (currently Dromiacea, Raninoida, and Cyclodorippoida) is paraphyletic, corroborating Ahyong et al. (2007). All the four sections proposed by Ahyong et al. (2007) and used in De Grave et al. (2009), namely Dromiacea, Raninoida, Cyclodorippoida, and Eubrachyura, are monophyletic. The Dromiacea represents the earliest diverging brachyuran lineage, while Cyclodorippoida forms a clade with Raninoida and the two together appear to be sister to Eubrachyura, but with low nodal support for these arrangements. This is consistent with the recent molecular (Ahyong et al. 2007) and morphological (Scholtz and McLay 2009) evidence. Moreover, the reciprocal monophyly of the two subsections of the Eubrachyura, Heterotremata, and Thoracotremata, is recovered with strong nodal support (fig. 1*B* and *C*).

Most families with multiple exemplars included are monophyletic except for Homolidae, Xanthidae, Menippidae, Epialtidae, and Majidae (fig. 1*B* and *C*). Homolidae and Xanthidae are paraphyletic, because of the inclusion of Latreilliidae and Panopeidae, respectively. Epialtidae and Majidae are polyphyletic in their present composition, with genera from the two families intermingling. The alternative a priori hypotheses for the monophyly of these families was not supported by the approximately unbiased (AU) test ($P < 0.05$), with the exception of Homolidae ($P = 0.094$) and Menippidae ($P = 0.104$). In contrast to the families, the status of most superfamilies was problematic. Calappoidea, Eriphioidea, Goneplacoidea, Ocypodoidea, and Grapsoidea are all found to be polyphyletic whereas Potamoidea is paraphyletic with respect to Gecarcinucoidea. The AU test strongly rejected the monophyly of Eriphioidea, Goneplacoidea, Ocypodoidea, and Grapsoidea ($P < 0.01$), but not Calappoidea ($P = 0.444$) and Potamoidea ($P = 0.205$). Dromioidea, Homoloidea, Dorippoidea,

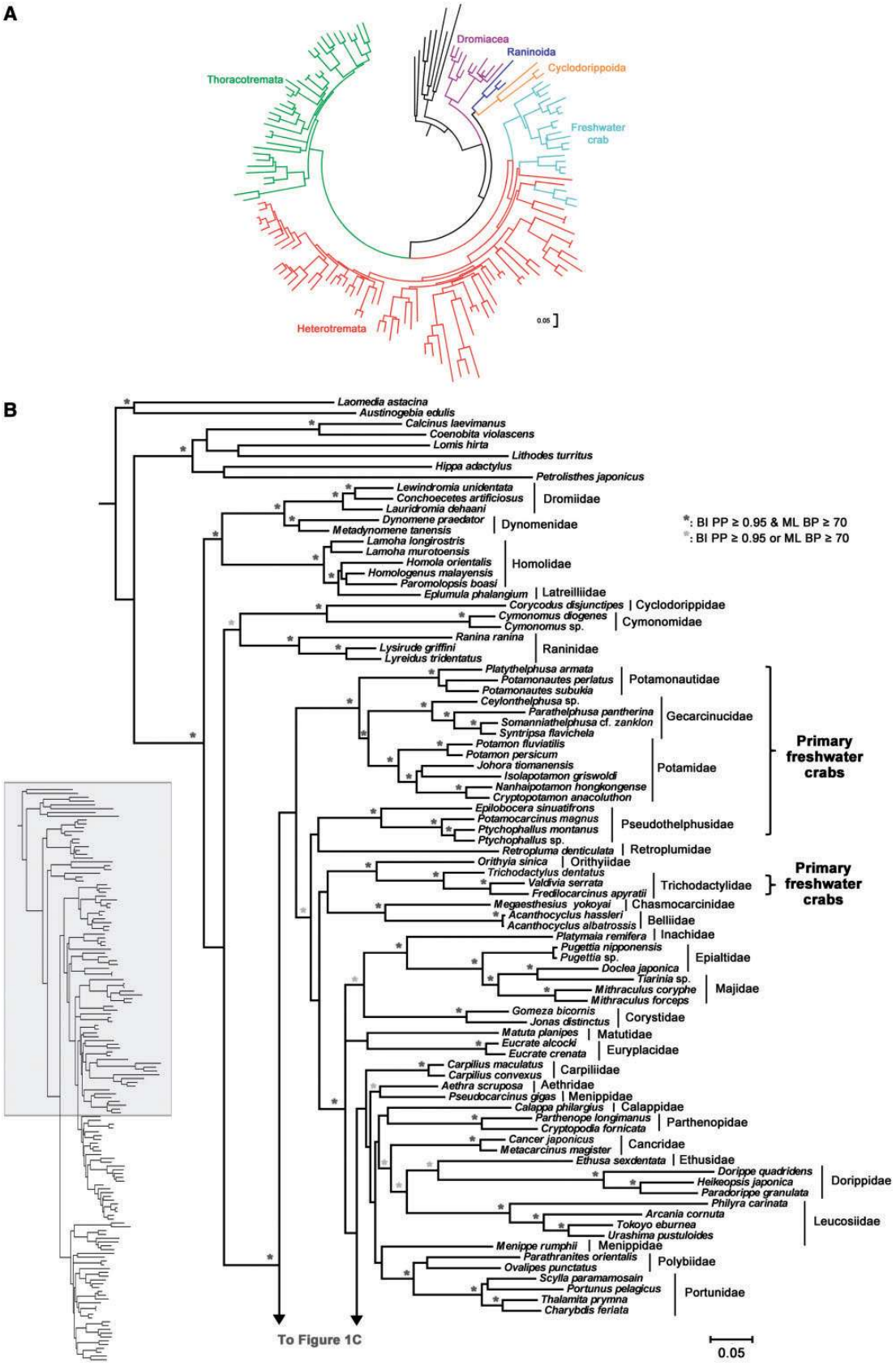

Fig. 1. Bayesian topology from a combined data set (3,912 bp from eight genes) analysis for the phylogenetic relationships among major brachyuran sections/subsections (*A*) and species (*B* and *C*). The color of the branches is encoded for the sections/subsections classification, with the exception of the true freshwater crabs belonging to Heterotremata denoted by light blue. The branches strongly supported by both BI (PP $\geq$ 0.95) and ML (BP $\geq$ 70) are indicated by black asterisks above, while those receiving strong support from one of the analyses are indicated by gray asterisks in (*B*) and (*C*). The family classification of the species based on Ng et al. (2008) and De Grave et al. (2009) are indicated on the right.

(continued)

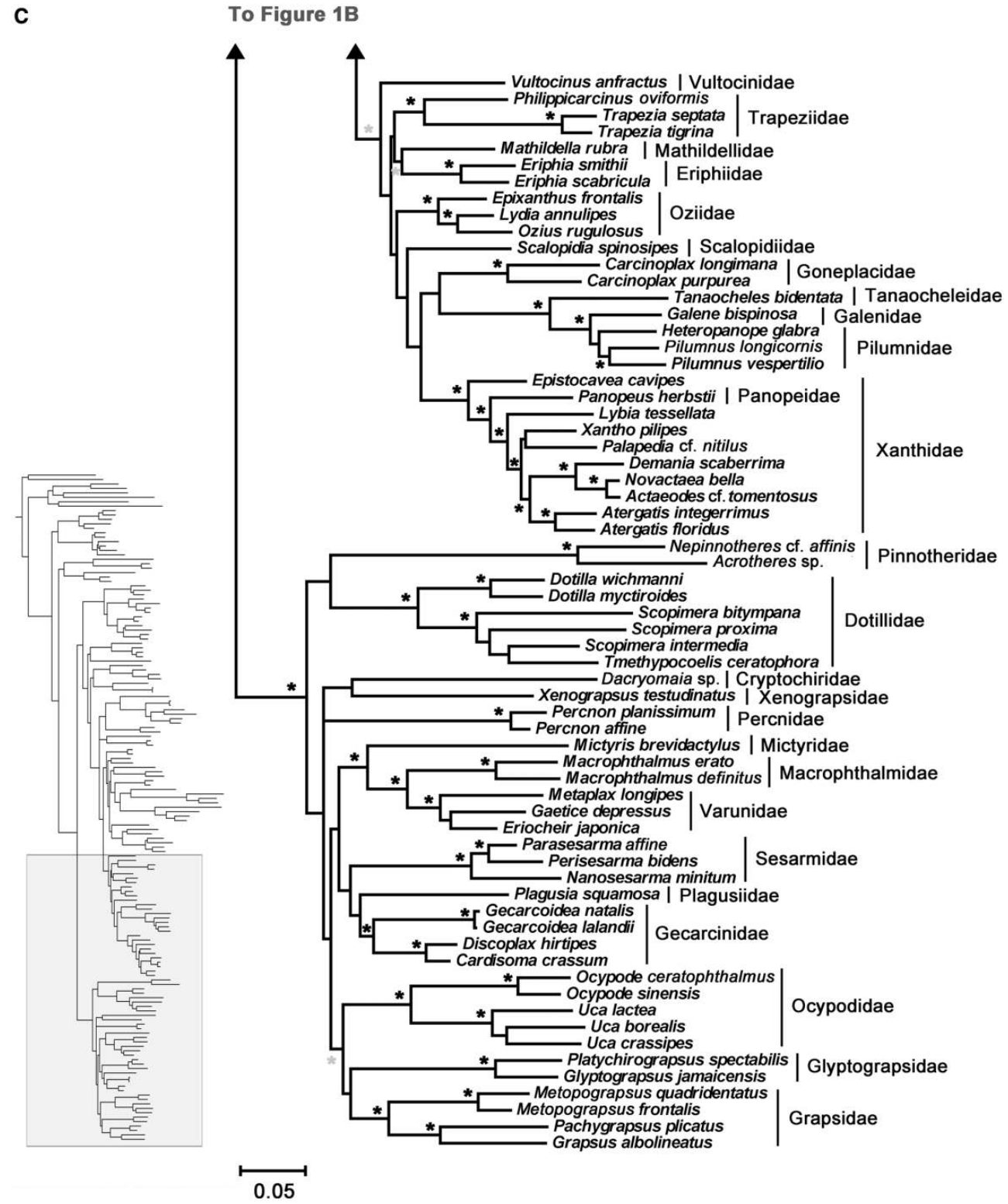

FIG. 1. (continued).

Majoidea, Pilumnoidea, Portunoidea, and Xanthoidea are recovered as monophyletic.

The three Old World freshwater crab families (Gecarcinucidae, Potamidae, and Potamonautidae) form a monophyletic assemblage, but not as sister of the New World Pseudothelphusidae. The two clades represent "early offshoots" of the Heterotremata and together form a paraphyletic grade with respect to the remaining heterotremes (fig. 1A). However, the AU test could not reject an alternative hypothesis of monophyly of the

Gecarcinucidae + Potamidae + Potamonautidae + Pseudothelphusidae clade ($P = 0.217$) and the four families form a clade in the maximum clade credibility tree generated from BEAST (fig. 2A). Trichodactylidae is distantly related to the other freshwater crabs and surprisingly aligned with the wholly marine Orithyiidae with strong nodal support (fig. 1B) and hence the monophyletic origin of all primary freshwater crabs was rejected by the AU test ($P < 0.001$).

Divergence Time Estimation and Lineage-Through-Time Plot

The BEAST analysis implies that the age of Brachyura is over 180 Ma, dating back to at least the early Jurassic (fig. 2A). The divergence of the sections/subsections (Dromiacea, Raninoida, Cyclodorippoida, Heterotremata, and Thoracotremata) would have occurred in the late Jurassic to early Cretaceous (~135–170 Ma). The origins of the major freshwater crab families, Gecarcinucidae, Potamidae, Potamonautidae, and Pseudothelphusidae are ancient, deriving from the early Cretaceous (~125 Ma; 95% credibility interval = 113–140 Ma). Most of the extant families and superfamilies arose during the late Cretaceous to early Tertiary. The lineage-through-time (LTT) plot revealed that Brachyura exhibited a fairly constant rate of diversification in its history (fig. 2B). There was deceleration in diversification as indicated by the negative γ value (-4.17; $P < 0.0001$). However, this value was not significantly higher than the critical value simulated (-11.9; $P = 1$), suggesting the apparent decline in diversification rate might be an artifact of incomplete taxon sampling.

Discussion

In this study, we have attempted to construct a phylogeny of Brachyura based on an extensive data set, both in terms of taxon sampling and number of molecular markers employed. Despite poorly resolved internal relationships within Eubrachyura, the topology provides important new insights into the evolution and systematics of Brachyura.

Higher Systematics and Tempo of Diversification in Brachyura

Our combined gene tree provides the first molecular evidence to provide strong support for the reciprocal monophyly of the extant Heterotremata and Thoracotremata (early indications in Schubart, Neigel, et al. 2000). The various freshwater crabs, despite sharing many characters with the thoracotremes, are shown to clearly align with Heterotremata rather than Thoracotremata. The monophyly of Heterotremata had been challenged by morphological cladistic analyses (Scholtz and Richter 1995, von Sternberg and Cumberlidge 2001a, 2001b; Dixon et al. 2003; Brösing et al. 2007). From their perspective, the two subsections represent two extremes with a series of transitional forms (Magalhães and Türkay 1996; von Sternberg and Cumberlidge 2001b). The two subsections are, indeed, characterized by two distinct morphological types, coxal male sexual apertures and sternal male sexual apertures, with no intermediate form known amongst extant taxa (von Sternberg and Cumberlidge 2001b). Coxal apertures are plesiomorphic, whereas the sternal apertures characterizing the thoracotremes are apomorphic. Therefore, the reciprocal monophyly of the two subsections here recovered suggests that the common ancestor of thoracotremes and heterotremes had coxal male apertures, with sternal condition evolving along the stemline to the Thoracotremata (see fig. 2). The exact relationships remain unknown, because Heterotremata is the most speciose of the two sections, and many heterotreme groups still need to be incorporated, among which could be the sister group to the Thoracotremata. The earliest known fossils of Brachyura, represented predominantly by Dromiacea, dated from the late Jurassic (reviewed in Brösing 2008; Karasawa et al. 2011), which is highly concordant with the hypothesis of late Jurassic to early Cretaceous origins of the brachyuran sections/subsections revealed by our molecular phylogeny and Bayesian relaxed molecular clock divergence time estimation. The earliest fossil identified as an anomuran dates from the Triassic (Chablais et al. 2011), indicating that Brachyura, as the sister group to Anomura, would also have been present by that time. Furthermore, our gene tree shows that the majority of extant brachyuran lineages originated during the late Cretaceous and throughout the Tertiary. This is highly consistent with the postulation that most heterotreme groups had undergone a significant post-Cretaceous radiation (Schram 1986; Schweitzer and Feldmann 2005; Brösing 2008).

Origin and Phylogenetic Position of Freshwater Crabs

The freshwater crabs have long been regarded as an excellent model for biogeographic studies owing to their circumtropical distribution, extraordinary species diversity, and high level of endemism (Ortmann 1902; Ng and Rodríguez 1995; Daniels et al. 2006; Klaus et al. 2009, 2010, 2011; Shih et al. 2009). Accordingly, the monophyly of the group is highly relevant to our understanding of the evolution of other freshwater groups and related biogeographic inferences of regional divergence events (e.g., Shih et al. 2009; Klaus et al. 2010). Although Trichodactylidae is generally regarded as an independent lineage, the phylogenetic connections between the other three Old World families, Gecarcinucidae and Potamidae in Eurasia, and Potamonautidae in the Afrotropical region, and New World Pseudothelphusidae, are more contentious. The major challenge in testing their monophyly is the unequivocal identification of their respective marine sister group (if the Old World families and Pseudothelphusidae form a monophylum) or sister groups if Pseudothelphusidae had an independent origin from the Old World families. Moreover, the tempo of their divergence is no less controversial as their phylogenetic placement. Some authors have postulated the origin of freshwater crabs may exceed 120 Ma (Ng and Rodriguez 1995; Ng et al. 1995). Yet, this hypothesis would imply that the diversification of these freshwater crabs probably predates the radiation of Heterotremata (based on known fossils), or that Brachyura as a whole is much more ancient than previously thought (see Klaus et al. 2011). The oldest fossil freshwater crabs known so far are relatively

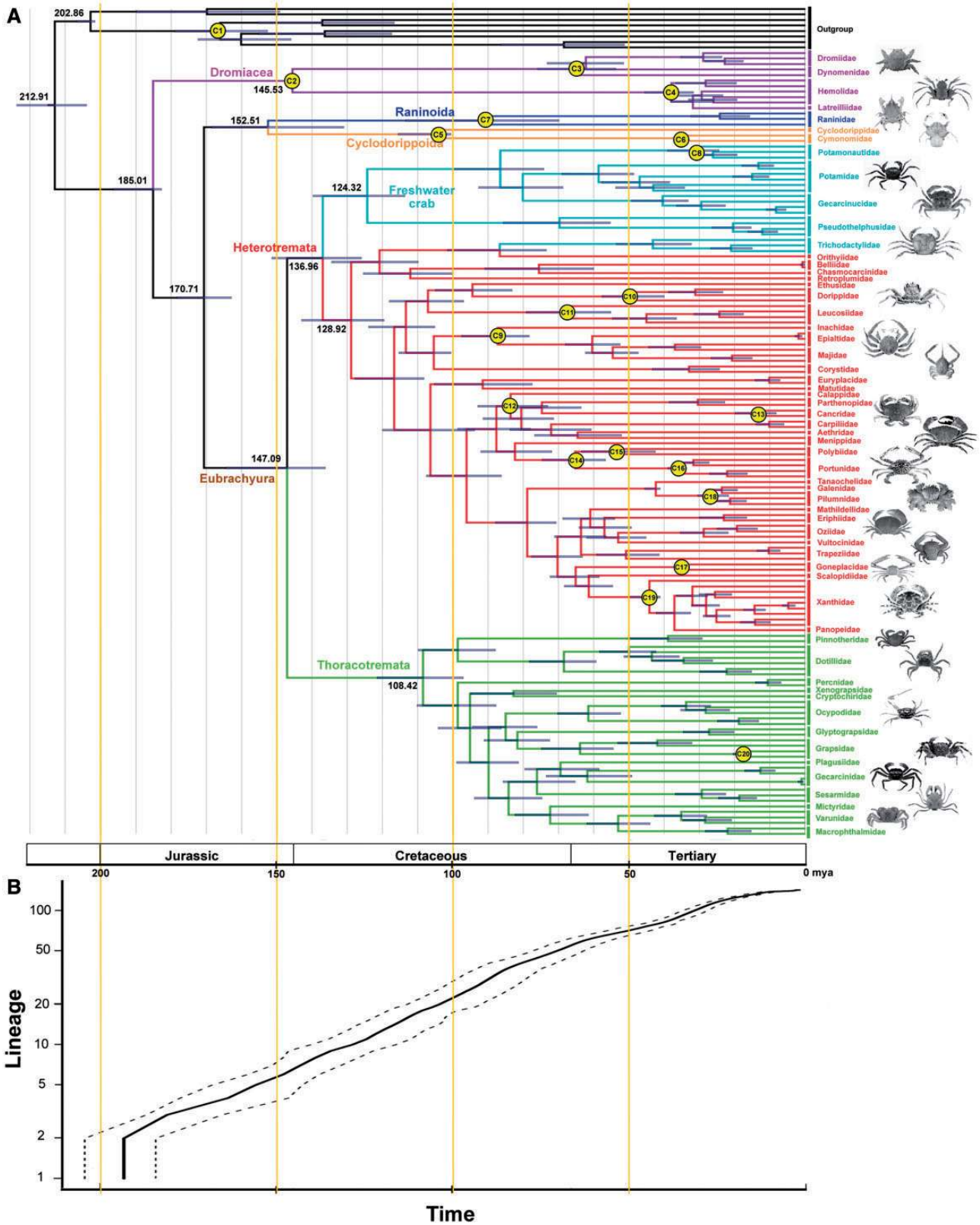

FIG. 2. (*A*) Maximum clade credibility tree based on 3,912 base pairs from eight genes showing divergence time estimates using BEAST. Fossil calibration points are indicated by "C," respectively. Only the family names are shown for ease of reference and the colour of the family name denotes the section/subsection grouping as in figure 1*A*. Please refer to supplementary figure S1 (Supplementary Material online) for a complete list of taxa and estimated nodal ages. Each interval between two yellow vertical lines represents 50 My with geological periods shown at the bottom. Blue nodal bars correspond to the 95% highest posterior density regions. The illustrations at the right show representatives of some common crab families analyzed. (*B*) Semilogarithmic LTT plots of 1,000 chronograms sampled from BEAST analyses. The solid line represents the mean value and the broken lines denote 5% and 95% CIs.

recent, dating back to the Late Oligocene (*Tanzanonautes tuerkai* from Potamonautidae; Feldmann et al. 2007). Heterotremata has probably undergone a post-Cretaceous radiation (Schram 1986) and hence it was suspected that the freshwater crabs diversified at approximately 30–65 Ma (Klaus et al. 2011). In a review of the present phylogenetic and paleontological evidence for the origin of freshwater crabs, Klaus et al. (2011) noted the huge discrepancies between the times estimated based on fossils of freshwater and marine brachyurans and rejected the hypothesis of Gondwanan origin of the group based on the existing evidence.

In our present molecular phylogenetic hypothesis, the Old World Potamoidea sensu lato (i.e., Potamidae, Gecarcinucidae, and Potamonautidae, see Klaus et al. 2009) and the New World Pseudothelphusidae were the earliest diverged lineages within Heterotremata, forming a paraphyletic grade with respect to the remaining heterotremes. Trichodactylidae appears to be the sister of the marine Orithyiidae, and may be distantly related to other freshwater crabs (albeit with low nodal support for its position). This suggests that there are at least two independent origins of the extant primary freshwater crabs, and the invasion of freshwater habitats by the marine ancestors mostly occurred early in eubrachyuran evolution. This excludes some Sesarmidae, which invaded fresh waters and achieved independence from the sea much more recently, see Schubart et al. (1998); and some Hymenosomatidae and Varunidae that normally live in freshwater but tolerate brackish water and exhibit short distance dispersal in the sea. Furthermore, some of the difficulties in previous studies of primary freshwater crab phylogeny are probably related to the fact that the nontrichodactylid freshwater crabs were derived early in the history of the heterotremes, during the initial radiation of Eubrachyura. This makes it unlikely that their sister group could be narrowed to any particular extant family clade, but rather is composed of the clade containing almost all nonfreshwater heterotremes. Hence, the phylogenetic position of freshwater crabs could not be determined without extensive sampling from the heterotremes as in this study. However, given that the freshwater crabs consist of 1,300 + extant species, the number of species analyzed in this study remains relatively small. Whether the nontrichodactylid freshwater crabs are monophyletic or polyphyletic requires further testing with broader taxon coverage. The current level of topological resolution and robustness among the heterotremes is generally low, and focused future analyses restricted to Heterotremata or perhaps Eubrachyura are required. An important corollary of the identification of the freshwater clades as basal or near basal heterotremes is a formal identification of these clades as the appropriate outgroups for further phylogenetic analyses of Heterotremata.

Divergence time estimates have greatly pushed back the origins of the primary freshwater crabs to the early Cretaceous (~130 Ma; 95% credibility interval = 127–151 Ma). It is widely acknowledged that the freshwater habitats of these crabs do not provide optimal conditions for fossil preservation, so the minimal fossil record is not surprising. If our estimated divergence time is close to reality, the divergence time of nontrichodactylid freshwater crabs would clearly postdate the break up of Pangaea (~200 Ma). A divergence time of over 200 Ma is one of the presumed prerequisites for the monophyletic origin of all the nontrichodactylid freshwater crabs considering their distribution on all continents except Antarctica and assuming that they diverged after becoming independent of the marine habitat. However, recent phylogenetic evidence strongly supports transoceanic dispersal in freshwater crabs. The monophyletic *Seychellum* originated from a single colonization event of the Seychelles from the Africa (Daniels 2011). Klaus et al. (2013) recently reported a single out-of-Borneo event, crossing the Wallace Line by the Sundaic freshwater crabs. Therefore, transoceanic dispersals across marine barriers are probably not impossible and may account for aspects of the modern distribution of primary freshwater crabs. If our divergence time estimation of a split between New and Old World nontrichodactylid freshwater crabs is approximately correct at ~125 Ma, then it might suggest that either the extant New or Old World freshwater crab families were derived from a single transoceanic dispersal across the relatively narrow marine barrier between continents during the early stage of breakup of Gondwana. This hypothesis requires further testing with broader taxon coverage from the freshwater crabs and divergence time estimation based on additional fossil evidence. As noted by other authors, the choice of marine and/or freshwater crab fossils as calibration points has a major effect on the divergence time estimates. To reduce bias and uncertainties, dating the divergence of brachyurans within a complete decapod phylogeny by incorporating molecular data from the same set of markers (Tsang et al. 2008, 2011; Chu et al. 2009; Ma et al. 2009; Tsang, Chan, Cheung, et al. 2009) and multiple calibration points from other fossil-rich decapod infra-orders (e.g., lobsters) would be a potential solution. For instance, Porter et al. (2005) placed the common ancestor of the heterotremes at approximately 240 Ma, based on a molecular phylogeny of all decapod infra-orders and multiple fossil calibrations using various decapods. In such a scenario, the divergence of freshwater crabs, as an early offshoot from heterotremes, would predate 200 Ma, that is, the Pangaea breakup. In any case, the divergence of freshwater crabs apparently occurred in much more ancient times than expected and a more objective divergence time estimation based on comprehensive dated decapod phylogeny in the future could provide us a more robust estimate of the origin of the group.

Most of the molecular phylogenetic studies of primary freshwater crabs revealed strong biogeographic correlations (Daniels et al. 2006; Klaus et al. 2009; Shih et al. 2009; but see Klaus et al. 2010). Our combined gene tree suggests that the Potamidae is more closely related to Gecarcinucidae than to Potamonautidae. The range of Potamidae and Gecarcinucidae overlaps to a large extent in the Asian region, whereas Potamonautidae is restricted to the Afrotropical area. This supports the proposal of Klaus et al. (2009) and other recent studies (e.g., von Sternberg and Cumberlidge 2001a; Klaus et al. 2006) to perhaps place all Old World freshwater crabs within a single superfamily,

Potamoidea. We herewith put forward this taxonomy once again.

Implications for the Superfamilial and Familial Classification

The taxonomy of Brachyura has been revised and refined continuously in recent years based on studies of adult and larval morphology, molecular evidence, and spermatozoal structure (reviewed in Ng et al. 2008). Unfortunately, the lack of a well-supported phylogenetic framework hampers the identification of synapomorphies and the inference of phylogenetic relationships among families and genera. Therefore, many controversies remain to be settled. From our inferred gene tree, we evaluate the validity of recent changes in brachyuran systematics.

Dromiacea

Our inferred topology is largely congruent with previous molecular work by Ahyong et al. (2007) in revealing "Podotremata" as paraphyletic, with Raninidae and Cyclodorippidae more closely related to Eubrachyura than the other podotremes. The monophyly of Podotremata has long been contentious and there is strong evidence from morphological studies arguing for the paraphyly of the group (Brösing et al. 2007; Scholtz and McLay 2009). In the most recent comprehensive classification of extant brachyuran species, Ng et al. (2008) provisionally retain the use of Podotremata, but in a subsequent updated classification of all Decapoda genera, De Grave et al. (2009) and subsequent authors (Ahyong et al. 2011) follow Ahyong et al. (2007) in not using the name Podotremata, and instead recognizing the three sections, Dromiacea, Raninoida, and Cyclodorippoida (see, however, discussion in van Bakel et al. 2012). In our previous study, using two of the nuclear protein-coding genes used herein, the podotreme exemplars formed a weakly supported clade (Tsang et al. 2008). In that study, however, the number of podotremes analyzed was small, and did not include Cyclodorippidae. Our phylogeny based on expanded taxon and genetic sampling supports the identity of the three sections, instead of a single Podotremata.

Within the Dromiacea, the familial relationships inferred in this study are highly concordant with the nuclear 18S topology of Ahyong et al. (2007). Dromiidae and Homolidae are shown to be paraphyletic with the incursion of Dynomenidae and Latreilliidae, respectively, in the 18S gene tree (Ahyong et al. 2007). We recover the monophyly of Dromiidae with strong statistical support. We could not, however, obtain sequences from *Hypoconcha*, the earliest diverged dromiid in the topology of Ahyong et al. (2007).

Xanthoidea Sensu Lato

The composition and taxonomy of Xanthoidea has been revised substantially over the years (Serène 1984; Števčić 2005; Karasawa and Schweitzer 2006; Ng et al. 2008). Many families, including Carpiliidae, Eriphiidae, Goneplacidae, Hexapodidae, Menippidae, Pilumnidae, and Trapeziidae, were placed in the Xanthoidea until recently (Martin and Davis 2001; Števčić 2005; Karasawa and Schweitzer 2006) and have since been elevated and/or removed to other superfamilies (Ng et al. 2008; De Grave et al. 2009). Our results find most of these families forming a large clade with moderate nodal support. This provides the first molecular indication for a possible affinity of the families formerly included in Xanthoidea sensu lato. Xanthoidea sensu stricto currently comprises Xanthidae, Panopeidae, and Pseudorhombilidae. We have included the first two families in this study and showed that they form a strongly supported clade. Panopeidae, however, is nested within the xanthids, corroborating previous molecular results (Lai et al. 2011).

Considering the validity of the newly raised superfamilies, a monophyletic Pilumnoidea is recovered. The monophyly of Eriphioidea and Goneplacoidea is not supported, with exemplars analyzed widely dispersed in the tree. The monophyly of Goneplacoidea is uncertain and the relationships among its constituent families remain poorly understood (Ng et al. 2008). Therefore, our gene tree generally supports the reappraisal of most of the superfamilies proposed by Ng et al. (2008), but it is clear that further refinement is needed.

Majoidea

Comprising more than 800 extant species, majoids are a diverse group of brachyurans (Ng et al. 2008; De Grave et al. 2009). Although the monophyly of the group as a whole is generally accepted (reviewed in Ng et al. 2008; but see Brösing et al. 2007; Guinot 2011), the phylogenetic patterns within the group remain to be examined in detail, and existing family and superfamily classifications are highly problematic. Guinot (2011) argues that Hymenosomatidae is not affiliated with Majoidea and recognized a separate superfamily for these small crabs. Our results corroborate the monophyly of Majoidea, supporting the terminal moult and highly abbreviated larval development as synapomorphies of the group. However, the monophyly of the majority of the majoid families is not recovered, with many being polyphyletic (see also Hultgren and Stachowicz 2008; Hultgren et al. 2009). This suggests that morphological synapomorphies supporting majoid clades are not congruent with traditionally used taxonomic characters. Significantly more effort is required to identify the natural groups within Majoidea and recognize their synapomorphies. On the other hand, the larval characters appear to be more congruent with the molecular phylogeny (Hultgren and Stachowicz 2008; Hultgren et al. 2009). Given the diversity of the majoids, there is no doubt that more extensive analyses, in particular of the Indo-Pacific genera, are needed to progress knowledge of the evolution of the spider crabs.

Grapsoidea and Ocypodoidea

Schubart, Cuesta, et al. (2000) presented the first molecular phylogeny of Grapsoidea based on North American species. The mitochondrial *16S* rRNA gene tree revealed that Gecarcinidae was closely related to the former grapsid subfamilies Grapsinae, Plagusiinae, Sesarminae, and Varuninae, so that they should be treated at the same taxonomic rank (i.e.,

Table 1. Primer Sequences Used for PCR Amplification, Annealing Temperature Used, and Their Sources.

Primer	Sequence (5′ to 3′)	Annealing Temperature Used (°C)	Source
AK		60	
AK for a-1	CTC CCC TST TTG AYC CCA TCA T		Tsang et al. (2011)
AK for a-2	ACC CCA TCA TTG AGG AYT AYC A		Tsang et al. (2011)
AK for b	ATA GAC GAC CAC TTC CTS TTC AA		Tsang et al. (2011)
AK rev 1	TGG AAC TCA GTC AGA CCC ATR CG		Tsang et al. (2011)
AK rev 2	CCG CCC TCA GCC TCR GTG TGY TC		Tsang et al. (2011)
AK rev 3	ATA CCG TCC TGC ATY TCY TT		This study
Enolase		52–54	
Enol EA1	CAG CAA TCA ATG TCA TCA AYG GWG G		Tsang et al. (2011)
Enol EA2	AGT TGG CTA TGC AGG ART TYA TGA T		Tsang et al. (2011)
Enol ES1	ACT TGG TCA AAT GGR TCY TCA AT		Tsang et al. (2011)
Enol ES2	ACC TGG TCG AAT GGR TCY TC		Tsang et al. (2011)
H3		55	
H3 AF	ATG GCT CGT ACC AAG CAG ACV GC		Colgan et al. (1998)
H3 AR	ATA TCC TTR GGC ATR ATR GTG AC		Colgan et al. (1998)
GAPDH		51–54	
GAPDH F2	ATG AAG CCA GAA AAC ATT CCA TGG		Tsang et al. (2011)
GAPDH GA	ATG GTG TAT ATG TTC AAG TAY GAY TC		Tsang et al. (2011)
GAPDH R	GAA TAG CCT AAC TCG TTG TCR TAC CA		Tsang et al. (2011)
GAPDH GR	TCG CTA GAT ACA ACA TCA TCY TCR GT		Tsang et al. (2011)
GAPDH GR2	GTG AAG TCA CAG GAG ACA ACA TCR TCY TC		This study
PEPCK		60	
PEPCK for	GTA GGT GAC GAC ATT GCY TGG ATG AA		Tsang et al. (2008)
PEPCK for2	GCA AGA CCA ACC TGG CCA TGA TGA C		Tsang et al. (2008)
PEPCK rev	GAA CCA GTT GAC GTG GAA GAT C		Tsang et al. (2008)
PEPCK rev3	CGG GYC TCC ATG CTS AGC CAR TG		Tsang et al. (2008)
NaK		57–60	
NaK for-a	GTG TTC CTC ATT GGT ATC ATT GT		Tsang et al. (2008)
NaK for-b	ATG ACA GTT GCT CAT ATG TGG TT		Tsang et al. (2008)
NaK rev	ACC TTG ATA CCA GCA GAT CGG CAC TTG GC		Tsang et al. (2008)
NaK rev2	ATA GGG TGA TCT CCA GTR ACC AT		Tsang et al. (2008)
NaK rev3	GGA GGR TCA ATC ATR GAC AT		This study
16S		48–52	
AR	CGC CTG TTT ATC AAA AAC AT		Simon et al. (1994)
SF	GAC CGT GCT AAG GTA GCA TAA TC		This study
SR	CCG GTC TGA ACT CAA ATC GTG		Tsang, Chan, Shih, et al. (2009)
BR	CCG GTC TGA ACT CAG ATC ACG T		Simon et al. (1994)
1472	AGA TAG AAA CCA ACC TGG		Crandall and Fitzpatrick (1996)
12S		48–52	
FB	GTG CCA GCA GCT GCG GTT A		Tsang, Chan, Shih, et al. (2009)
ai	AAA CTA GGA TTA GAT ACC CTA TTA T		Simon et al. (1994)
bi	AAG AGC GGG CGA TGT GT		Simon et al. (1994)
R2	CCT ACT TTG TTA CGA CTT ATC TC		Tsang, Chan, Shih, et al. (2009)

family level). Together with Gecarcinidae and the newly described Glyptograpsidae they were considered full families by Schubart et al. (2002) and tentatively kept within Grapsoidea. This was followed by Martin and Davis (2001) in their classification of Crustacea. Moreover, these authors included Mictyridae, which had been considered part of Grapsoidea (see Bowman and Abele 1982), in the superfamily Ocypodoidea. Martin and Davis (2001) still maintained a large and diverse family Ocypodidae with four subfamilies viz. Dotillinae, Heloeciinae, Macrophthalminae, and Ocypodinae. This classification was challenged by subsequent molecular phylogenetic studies (Kitaura et al. 2002; Schubart et al. 2006). Despite minor differences in the arrangements of some clades, their topologies consistently show that both Ocypodoidea and Grapsoidea are polyphyletic and some constituent families/subfamilies intermingle. Based on the evidence of all these gene trees, Schubart et al. (2006) argued against the traditional use of the Grapsoidea and Ocypodoidea as monophyletic superfamilies and treated the constituent families separately. Ng et al. (2008), however,

maintained the superfamilies in their classification, but gave full family ranking to the former subfamilies of Ocypodidae.

Our study confirms the results of previous mtDNA analyses that the two superfamilies are polyphyletic in their current composition. Some groupings revealed by Kitaura et al. (2002) and Schubart et al. (2006), for example, Varunidae + Macrophthalmidae, are recovered in the present tree with strong support, suggesting an overall congruence of the topology from molecular analyses using different types of molecular markers. Moreover, recent *16S* studies showed that Asthenognathinae is closely related to Varunidae rather than a pinnotherid as previously thought (Palacios-Theil et al. 2009), while Cryptochiridae may be a close ally of the Grapsidae (Wetzer et al. 2009). The varunid affinities of asthenognathids and some pinnotherids have also been supported by recent morphological studies (Naruse and Clark 2009; Komai 2011; Komai and Konishi 2012). These findings further challenge the validity of Ocypodoidea and Grapsoidea. At present, however, the interfamilial relationships are not sufficiently resolved to navigate changes in the overall classification. Therefore, further studies with more comprehensive taxon sampling are essential to obtain a well resolved, robust phylogeny for a consensus on the evolutionary history and taxonomy of Thoracotremata.

Materials and Methods

Taxon Sampling

The Brachyura currently comprise 98 extant families in 37 superfamilies (Ng et al. 2008; De Grave et al. 2009; Ahyong et al. 2011). We attempted to sample extensively from different families and genera to resolve the familial and superfamilial relationships with a total of 142 species from 58 families and 30 superfamilies, representing almost 60% of the extant brachyuran families and ~80% of the superfamilies, and including all four sections (supplementary table S1, Supplementary Material online). We therefore decided to analyze multiple genera from taxonomically diverse families (e.g., Majidae, Xanthidae). To evaluate the origin and phylogenetic position of freshwater crabs, we included multiple exemplars from all five families. Anomura is widely acknowledged as the sister group of Brachyura, together forming the Meiura (Scholtz and Richter 1995; Dixon et al. 2003; Ahyong and O'Meally 2004; Tsang et al. 2008). Six anomuran species were included to test for the monophyly of Brachyura and two members from the Gebiidea were used as outgroups.

Sequence Collection

Total genomic DNA was extracted from pereiopod tissue of the target species using the commercial QIAamp Tissue Kit (QIAGEN). Six nuclear protein-coding genes, *arginine kinase* (*AK*), *enolase*, *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*), *histone* 3 (*H3*), *sodium–potassium ATPase α-subunit* (NaK), and *phosphoenolpyruvate carboxykinase* (*PEPCK*), that were previously applied to higher level decapod phylogeny (Tsang et al. 2008,; Chu et al. 2009; Ma et al. 2009; Tsang, Chan, Cheung, et al. 2009; Tsang et al. 2011), were analyzed. The mitochondrial large (*16S*) and small subunit (*12S*) ribosomal RNA genes were also sequenced, resulting in a concatenated data set of segments from eight genes. The amplifications were conducted in a reaction mix containing 1−5 μl of template DNA, 1× polymerase chain reaction (PCR) reaction buffer, 3−6 mM $MgCl_2$, 200 nM of each primer, 200 μM deoxynucleotide triphosphates (dNTPs), 1.5 U *Taq* polymerase (TaKaRa), and ddH_2O to a total volume of 50 μl. The PCR profiles followed standard procedures that were described in previous literature (Colgan et al. 1998; Schubart et al. 2006; Tsang et al. 2008, 2011). The primers for individual gene regions and their sources and annealing temperatures utilized are listed in table 1. The successful PCR amplicons were then purified using the QIAquick gel purification kit (QIAGEN) according to manufacturer's instructions. Sequencing reactions were carried out using the same sets of primers and the ABI Big-dye Ready-Reaction mix kit, following the standard cycle sequencing protocol. The products were analyzed using an Applied Biosystems (ABI) 3700 automated sequencer.

Table 2. Best-Fit Nucleotide Substitution Model for Individual Partitions as Selected by jModelTest 0.1.

Partition	Model
*AK*12	SYM + I + G
*AK*3	SYM + I + G
*enolase*12	K80 + I + G
*enolase*3	SYM + I + G
*GAPDH*12	SYM + I + G
*GAPDH*3	SYM + I + G
*NaK*12	K80 + I + G
*NaK*3	SYM + I + G
*PEPCK*12	SYM + I + G
*PEPCK*3	K80 + I + G
H3	K80 + I + G
16S	GTR + I + G
12S	HKY + I + G

Phylogenetic Analyses

Sequences were aligned using MUSCLE (Edgar 2004) with default parameters and confirmed by translating into amino acid sequences for the protein-coding genes. The total data set was analyzed using ML and BI. The data were first partitioned by genes as the markers have different genomic locations and possibly mutation constraints. We further used PartitionFinder v1.0.1 (Lanfear et al. 2012) to determine the best partitioning strategy for the six protein-coding genes according to the Bayesian information criterion recommended by the authors. Splitting into two partitions, first + second codons and third codon position, were adopted for five genes (*AK*, *enolase*, *GADPH*, *NaK*, and *PEPCK*), whereas the three codon positions of *H3* gene were analyzed as a single partition. Hence, the final data set was divided into a total of 13 partitions and the best-fit models of nucleotide substitution for each partition (table 2), selected by jModeltest (Posada 2008), were used in ML and BI analyses. The ML analysis was implemented with RAxML 7.0.3 (Stamatakis

Table 3. Fossil Calibrations Used in Divergence Time Analyses and Prior Setting for the Log-Normal Distribution Used in BEAST.

Node Calibrated	Placement	Classification	Species	Age	Prior for Log-Normal Distribution
Infra-order Anomura	Stem	Platykottidae	*Platykotta akaina* Chablais et al. (2011)	Late Triassic (Norian/Rhaetian) 201.6–228	Offset = 201.6, SD = 2.5, 95% = 262.7
Dromiacea	Stem	Homolodromiidae	*Eoprosopon klugi* Förster (1986)	Pleisenbachian, early Jurassic 182.7–190.8	Offset = 182.7, SD = 2, 95% = 209.5
Dynomenidae	Stem	Dynomenidae	*Cyclothyreus* Remeš (1895)	Tithonian, Late Jurassic 145–152.1	Offset = 145, SD = 1.9, 95% = 167.8
Homolidae	Stem	Homolidae	*Doerflesia ornata* Feldmann and Schweitzer (2009)	Tithonian, Late Jurassic 145–152.1	Offset = 145, SD = 1.9, 95% = 167.8
Family Cyclodorippidae	Crown	Cyclodorippidae	*Hillius youngi* Bishop (1983)	Albian, early Cretaceous 100.5–113	Offset = 100, SD = 2, 95% = 127.3
Genus *Cymonomus*	Crown	Cymonomidae	*Cymonomus primitivus* Müller and Collins (1991)	Eocene, Priabonian 33.9–37.8	Offset = 33.9, SD = 1.2, 95% = 41.1
Subsection Raninoida	Stem	Palaeocorystidae	*Paranecrocarcinus hexagonalis* Van Straelen (1936)	Hauterivian, early Cretaceous 130.8–133.9	Offset = 130.8, SD = 1.8, 95% = 150.1
Family Potamonautidae	Stem	Potamonautidae	*Tanzanonautes tuerkai* Feldmann et al. (2007)	Late Oligocene 23–28.1	Offset = 23, SD = 1.3, 95% = 31.49
Superfamily Majoidea	Stem	Priscinachidae	*Cretamaja granulata* Klompmaker (2013)	late Albian, early Cretaceous 100.5–113	Offset = 100.5, SD = 2, 95% = 127.3
Family Dorippidae	Crown	Dorippidae	*Bartethusa hepatica* Quayle and Collins (1981)	Ypresian, early Eocene 47.8–56	Offset = 47.8, SD = 1.7, 95% = 64.18
Family Leucosiidae	Crown	Leucosiidae	*Typilobus modregoi* Via (1959)	Middle Eocene 41.2–47.8	Offset = 41.2, SD = 1.5, 95% = 52.99
Family Calappidae	Crown	Calappidae	*Calappa zinsmeisteri* Feldmann and Wilson (1988)	Late Eocene 33.9–41.2	Offset = 33.9, SD = 1.5, 95% = 45.69
Family Cancridae	Stem	Cancridae	*Notocarcinus sulcatus* Schweitzer and Feldmann (2000)	Middle Eocene 41.2–47.8	Offset = 41.2, SD = 1.5, 95% = 52.99
Superfamily Portunoidea	Crown	Macropipidae	*Ophthalmoplax stephensoni* Rathbun (1935)	Late Cretaceous 66–72.1	Offset = 66, SD = 1.6, 95% = 79.9
Family Polybiidae	Crown	Polybiidae	*Liocarcinus heintzi* Schweitzer and Feldmann (2010)	Oligocene, Rupelian 28.1–33.9	Offset = 28.1, SD = 1.4, 95% = 38.1
Family Portunidae	Crown	Portunidae	*Portunus* sp	Eocene 33.9–56	Offset = 33.9, SD = 2, 95% = 60.74
Genus *Carcinoplax*	Crown	Goneplacidae	*Carcinoplax temikoensis* Feldmann and Maxwell (1990)	Kaiatan or Runangan, Late Eocene 34.3–37.2	Offset = 34.3, SD = 1.2, 95% = 41.5
Family Pilumnidae	Stem	Pilumnidae	*Galenopsis* sp Milne-Edwards (1865)	Middle Eocene 41.2–47.8	Offset = 41.2, SD = 1.5, 95% = 52.99
Family Xanthidae	crown	Xanthidae	*Phlyctenodes tuberculosus* Milne-Edwards (1862)	Middle Eocene 41.2–47.8	Offset = 41.2, SD = 1.5, 95% = 52.99
Genus *Metopograpsus*	Crown	Grapsidae	*Metopograpsus badenis* Müller (2006)	Early Miocene 15.97–23	Offset = 15.97, SD = 1.4, 95% = 25.97

NOTE.—The geological ages of the fossil recovered from are provided in millions of years before present.

2006). The model GTRGAMMAI was used for the individual partitions, with individual-shape parameters, GTR-rates and base frequencies estimated and optimized for each partition. We conducted 1,000 bootstrap runs and searched for the best-scoring ML tree. Bayesian analysis was conducted using MrBayes v.3.21 (Ronquist et al. 2012). Two independent runs were carried out with four differentially heated Metropolis coupled Monte Carlo Markov Chains for 20,000,000 generations started from a random tree. Model parameters were estimated during the analysis. Chains were sampled every 2,000 generations. Convergence of the analyses was validated by the standard deviation of split frequencies (<0.01) and monitoring the likelihood values over time, graphically using Tracer v1.4 (Rambaut and Drummond 2007). All trees generated prior to the achievement of stationarity of the log likelihood values (5,000 trees) were discarded as burn-in. A 50% majority-rule consensus tree was constructed from the remaining trees to estimate PPs.

Alternative phylogenetic hypotheses from previous morphological and molecular studies were statistically tested using the likelihood-based AU test (Shimodaira 2002). The null hypothesis for all topology testing was that there was no difference between trees. The alternative topologies were inferred and optimized using RAxML with a prior phylogenetic hypothesis set as constraint. Subsequently, the per-site log likelihood values of individual sites for the trees were estimated with the same software and the confidence values of the tree topology were calculated by CONSEL (Shimodaira and Hasegawa 2001) with 1,000 BP replicates to obtain *P* values of the testing topology.

Divergence Time Estimation

We used BEAST v1.7.5 (Drummond et al. 2012) to estimate the divergence time of lineages using the uncorrelated relaxed clock proposed by Drummond et al. (2006) which allows the evolutionary rate to vary among branches. There are a number of brachyuran crab fossils with a reasonably broad taxonomic coverage (Schram 1986; Schweitzer and Feldman 2005; Brösing 2008; De Grave et al. 2009; Schweitzer et al. 2010). We have incorporated 20 fossils that represent the oldest known occurrences of clades that could be assigned with high confidence (table 3). We calibrated with the most recent common ancestor of the corresponding clades and followed the recommendations by Parham et al. (2012) in justifying fossil placement (either stem or crown nodes) according to the apomorphies shared among fossil and extant taxa or results of phylogenetic analyses whenever available (see supplementary material S1, Supplementary Material online, for discussion of individual fossils). We set constraints so that the divergence time of the clade is under a log-normal prior distribution of the age of the fossil, as the actual divergence event is most likely to have occurred sometime prior to the earliest appearance of the respective fossil (Drummond et al. 2006; Ho 2007). We used a Yule prior with a log-normal distribution for the rate of speciation. The data set was partitioned as in the BI and ML analyses to allow independent substitution rates and base frequencies. The BEAST analyses were first run with eight randomly selected fossil calibrations using UPGMA starting tree, with 50 million generations and a sample frequency of 5,000 generations. The first 5,000 trees were discarded as burn-in and a maximum clade credibility tree was produced by TreeAnnotator v1.7.5. Subsequently, we ran the BEAST analyses with full set of fossil calibrations (20 in total) using the chronogram produced in the first BEAST run as a starting tree to fulfill the topological and temporal constraints of the fossil calibrations. Two independent runs were performed with 200 million generations and a sample frequency of 10,000 generations. The first 10,000 trees were discarded as burn-in and the results of the two runs were combined using the LogCombiner v1.7.5. Convergence was assessed by trace plot in Tracer and the effective sampling size for all parameters was more than 200. The maximum clade credibility tree showing the mean nodal height was generated by TreeAnnotator v1.7.5. The final analyses were also run without data to ensure the prior settings will not bias the results.

Rate of Diversification

The tempo and rate of diversification was evaluated using an LTT plot. The LTT plot was generated based on the last 1,000 trees sampled in the BEAST analysis using the R package phytools (Revell 2012). The outgroups were pruned and the mean LTT curve was computed as well as the 95% confidence intervals (CIs). To test whether the rate of diversification has declined through time, we used the gamma (γ) statistic of Pybus and Harvey (2000) and accounted for the effect of incomplete taxon sampling using their Monte Carlo constant rate test as implemented in the LASER package version 2.2 (Rabosky 2006). In brief, a γ statistic from the empirical chronogram generated from BEAST with outgroups removed was calculated. Then 1,000 trees were simulated using the default pure-birth parameters of the birth-death model and containing the actual number of brachyuran species known (6,599 species; De Grave et al. 2009). The resulting trees were randomly pruned down to 143 terminals (the number of ingroup taxa analyzed) and hence a distribution of γ under pure birth process with 143 out of 6,599 extant species sampled were simulated to obtain an appropriate critical value of γ statistic (Pybus and Harvey 2000).

Supplementary Material

Supplementary material S1, figure S1, and table S1 are available at *Molecular Biology and Evolution* online (http://www.mbe.oxfordjournals.org/).

Acknowledgments

The authors thank B.K.K. Chan (Academia Sinica), K.J.H. Wong (National Taiwan University), Theodor Poettinger, and Alireza Keikhosravi (University of Regensburg) for their assistance in identifying and/or collecting some specimens used in the study. They also thank two anonymous reviewers who provided invaluable comments that substantially improved the manuscript. This work was supported by the Research Grants Council (RGC) of the Hong Kong Special Administrative Region, China (project no. CUHK463810) to

K.H.C.; the Germany/Hong Kong Joint Research Scheme of DAAD and RGC to C.D.S. and K.H.C. (project no. G_HK008/08), respectively; and partially supported by the Research Fellowship Scheme of The Chinese University of Hong Kong grant to L.M.T.

References

Ahyong ST, Lai JCY, Sharkey D, Colgan DJ, Ng PKL. 2007. Phylogenetics of the brachyuran crabs (Crustacea: Decapoda): the status of Podotremata based on small subunit nuclear ribosomal RNA. *Mol Phylogenet Evol.* 45:576–586.

Ahyong ST, Lowry JK, Alonso M, Bamber RN, Boxshall GA, Castro P, Gerken S, Karaman GS, Goy JW, Jones DS, et al. 2011. Subphylum Crustacea Brünnich, 1772. In: Zhang Z-Q, editor. Animal biodiversity: an outline of higher-level classification and survey of taxonomic richness. *Zootaxa* 3148:165–191.

Ahyong ST, O'Meally D. 2004. Phylogeny of the Decapoda Reptantia: resolution using three molecular loci and morphology. *Raffles Bull Zool.* 52:673–693.

Bishop GA. 1983. Fossil decapod crustaceans from the Lower Cretaceous, Glen Rose Limestone of central Texas. *Trans San Diego Soc Nat Hist.* 20:27–55.

Bowman TE, Abele LG. 1982. Classification of the recent Crustacea. In: Abele LG, editor. The biology of Crustacea. Vol. 1: Systematics, the fossil record and biogeography. New York: Academic Press. p. 1–27.

Bracken HD, De Grave S, Toon A, Felder DL, Crandall KA. 2010. Phylogenetic position, systematic status, and divergence time of the Procaridididea (Crustacea: Decapoda). *Zool Scr.* 39:198–212.

Bracken HD, Toon A, Felder DL, Martin JW, Finley M, Rasmussen J, Palero F, Crandall KA. 2009. The decapod tree of life: compiling the data and moving toward a consensus of decapod evolution. *Arthropod Syst Phylogeny.* 67:99–116.

Brösing A. 2008. A reconstruction of an evolutionary scenario for the Brachyura (Decapoda) in the context of the Cretaceous-Tertiary Boundary. *Crustaceana* 81:271–287.

Brösing A, Richter S, Scholtz G. 2007. Phylogenetic analysis of the Brachyura (Crustacea, Decapoda) based on characters of the foregut with establishment of a new taxon. *J Zool Syst Evol Res.* 45: 20–32.

Chablais J, Feldmann RM, Schweitzer CE. 2011. A new Triassic decapod, *Platykotta akaina*, from the Arabian shelf of the northern United Arab Emirates: earliest occurrence of the Anomura. *Paläontol Zeitschr.* 85:93–102.

Chu KH, Tsang LM, Ma KY, Chan TY, Ng PKL. 2009. Decapod phylogeny: what can protein-coding genes tell us? In: Martin JW, Crandall KA, Felder DL, editors. Crustacean Issues 18: Decapod crustacean phylogenetics. Boca Raton (FL): CRC Press. p. 89–100.

Colgan DJ, McLauchlan A, Wilson GDF, Livingston SP, Edgecombe GD, Macaranas J, Cassis G, Gray MR. 1998. Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Aust J Zool.* 46: 419–437.

Crandall KA, Fitzpatrick JF. 1996. Crayfish molecular systematics: using a combination of procedures to estimate phylogeny. *Syst Biol.* 45: 1–26.

Cumberlidge N, Ng PKL. 2009. Systematics, evolution and biogeography of freshwater crabs. In: Martin JW, Crandall KA, Felder DL, editors. Crustacean Issues 18: Decapod crustacean phylogenetics. Boca Raton (FL): CRC Press. p. 491–508.

Cumberlidge N, Ng PKL, Yeo DCJ, Magalhães C, Campos MR, Alvarez F, Naruse T, Daniels SR, Esser LJ, Attipoe FYK, et al. 2009. Freshwater crabs and the biodiversity crisis: importance, threats, status, and conservation challenges. *Biol Conserv.* 142:1665–1673.

Cumberlidge N, von Sternberg RM, Daniels SR. 2008. A revision of the higher taxonomy of the Afrotropical freshwater crabs (Decapoda: Brachyura) with a discussion of their biogeography. *Biol J Linn Soc.* 93:399–413.

Daniels SR. 2011. Reconstructing the colonisation and diversification history of the endemic freshwater crab (*Seychellum alluaudi*) in the granitic and volcanic Seychelles Archipelago. *Mol Phylogenet Evol.* 61:534–542.

Daniels SR, Cumberlidge N, Pérez-Losada M, Marijnissen SAE, Crandall KA. 2006. Evolution of Afrotropical freshwater crab lineages obscured by morphological convergence. *Mol Phylogenet Evol.* 40: 227–235.

De Grave S, Pentcheff ND, Ahyong ST, Chan T-Y, Crandall KA, Dworschak PC, Felder DL, Feldmann RM, Fransen CHJM, Goulding LYD, et al. 2009. A classification of living and fossil genera of decapod crustaceans. *Raffles Bull Zool Suppl.* 21:1–109.

Dixon CJ, Ahyong ST, Schram FR. 2003. A new hypothesis of decapod phylogeny. *Crustaceana* 76: 935–975.

Drummond AJ, Ho SYW, Phillips MJ, Rambaut A. 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biol.* 4:e88.

Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol Biol Evol.* 29: 1969–1973.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 32:1792–1797.

Feldmann RM, Maxwell PA. 1990. Late Eocene decapod Crustacea from North Westland, South Island, New Zealand. *J Paleont.* 64:779–797.

Feldmann RM, O'Connor PM, Stevens NJ, Gottfried MD, Roberts EM, Ngasala S, Rasmusson EL, Kapilima S. 2007. A new freshwater crab (Decapoda: Brachyura: Potamonautidae) from the Paleogene of Tanzania, Africa. *N Jb Geol Paläont Abh.* 244:71–78.

Feldmann RM, Schweitzer CE. 2009. Revision of Jurassic Homoloidea De Haan, 1839, from the Ernstbrunn and Štramberk limestones, Austria and the Crezh Republic. *Ann Naturhist Mus Wien.* 111A: 183–206.

Feldmann RM, Wilson MT. 1988. Eocene decapod crustaceans from Antarctica. In: Feldmann RM, Woodburne MO, editors. Geology and Paleontology of Seymour Island, Antarctic Peninsula. Geological Society of America, Memoir 169. p. 465–488.

Förster R. 1986. Der erste Nachweis eines brachyuren Krebses aus dem Lias (Oberes Pliensbach) Mitteleuropas. *Mitteilungen der Bayerischen Staatssammlung für Paläontologie und Historische Geologie* 26:25–31.

Guinot D. 1977. Propositions pour une nouvelle classification des Crustacés Décapodes Brachyoures. *Comp Rend Acad Sci Paris D.* 285:1049–1052.

Guinot D. 1978. Principes d'une classification évolutive des crustacés décapodes brachyoures. *Bull Biol France Belgique.* 112:211–292.

Guinot D. 1979. Donneés nouvelles sur la morphologie, la phylogenèse et la taxonomie des Crustacés Décapodes Brachyoures. *Mém Mus Nat Hist nat Paris (A) Zool.* 112:1–354.

Guinot D. 2011. The position of the Hymenosomatidae MacLeay, 1838, within the Brachyura. *Zootaxa* 2890:40–52.

Ho SYW. 2007. Calibrating molecular estimates of substitution rates and divergence times in birds. *J Avian Biol.* 38:409–414.

Hultgren KM, Guerao G, Marques FPL, Palero FP. 2009. Assessing the contribution of molecular and larval morphological characters in a combined phylogenetic analysis of the superfamily Majoidea. In: Martin JW, Crandall KA, Felder DL, editors. Crustacean Issues 18: Decapod Crustacean Phylogenetics. Boca Raton (FL): CRC Press. p. 437–455.

Hultgren KM, Stachowicz JJ. 2008. Molecular phylogeny of the brachyuran crab superfamily Majoidea indicates close congruence with trees based on larval morphology. *Mol Phylogenet Evol.* 48: 986–996.

Karasawa H, Schweitzer CE. 2006. A new classification of the Xanthoidea sensu lato (Crustacea: Decapoda: Brachyura) based on phylogenetic analysis and traditional systematic and evaluation of all fossil Xanthoidea sensu lato. *Contrib Zool.* 75:23–73.

Karasawa H, Schweitzer CE, Feldmann RM. 2011. Phylogenetic analysis and revised classification of podotrematous Brachyura (Decapoda) including extinct and extant families. *J Crustac Biol.* 31:523–565.

Kitaura J, Wada K, Nishida M. 2002. Molecular phylogeny of grapsoid and ocypodoid crabs with special reference to the genera *Metaplax* and *Macrophthalmus*. *J Crustac Biol.* 22:682–693.

Klaus S, Brandis D, Ng PKL, Yeo DCJ, Schubart CD. 2009. Phylogeny and biogeography of Asian freshwater crabs of the family Gecarcinucidae (Brachyura: Potamoidea). In: Martin JW, Crandall KA, Felder DL, editors. Crustacean Issues 18: Decapod crustacean phylogenetics. Boca Raton (FL): CRC Press. p. 509–531.
Klaus S, Schubart CD, Brandis D. 2006. Phylogeny, biogeography and a new taxonomy for the Gecarcinucoidea Rathbun, 1904 (Decapoda: Brachyura). *Org Divers Evol.* 6:99–217.
Klaus S, Schubart CD, Streit B, Pfenninger M. 2010. When Indian crabs were not yet Asian—biogeographic evidence for Eocene proximity of India and Southeast Asia. *BMC Evol Biol.* 10:287.
Klaus S, Selvandran S, Goh JW, Wowor D, Brandis D, Koller P, Schubart CD, Streit B, Meier R, Ng PKL, et al. 2013. Out of Borneo: Neogene diversification of Sundaic freshwater crabs (Crustacea: Brachyura: Gecacinucidae: Parathelphusa). *J Biogeogr.* 40:63–74.
Klaus S, Yeo DCJ, Ahyong ST. 2011. Freshwater crab origins—laying Gondwana to rest. *Zool Anz.* 250:449–456.
Klompmaker AA. 2013. Extreme diversity of decapod crustaceans from the mid-Cretaceous (late Albian) of Spain: Implications for Cretaceous decapod paleoecology. *Cret Res.* 41:150–185.
Komai T. 2011. A new species of the varunid crab genus *Gopkittisak* (Crustacea: Decapoda: Bracyhura: Grapsoidea) from the Ryukyu Islands. *Species Diversity* 16:103–111.
Komai T, Konishi K. 2012. Reappraisal of the systematic position of the supposed pinnotherid crab *Pseudopinnixa carinata* (Crustacea: Decapoda: Brachyura). *Species Diversity* 17:29–37.
Lai JCY, Mendoza JCE, Guinot D, Clark PF, Ng PKL. 2011. Xanthidae MacLeay, 1838 (Decapoda: Brachyura: Xanthoidea) systematics: a multi-gene approach with support from adult and zoeal morphology. *Zool Anz.* 250:407–448.
Lanfear R, Calcott B, Ho SYW, Guindon S. 2012. Partition Finder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Mol Biol Evol.* 29:1695–1701.
Ma KY, Chan T-Y, Chu KH. 2009. Phylogeny of penaeoid shrimps (Decapoda: Penaeoidea) inferred from nuclear protein-coding genes. *Mol Phylogenet Evol.* 53:45–55.
Magalhães C, Türkay M. 1996. Taxonomy of the neotropical freshwater crab family Trichodactylidae II. The genera *Forsteria*, *Melocarcinus*, *Sylviocarcionus*, and *Zilchiopsis* (Crustacea: Decapoda: Brachyura). *Senckenbergiana Biol.* 75:97–130.
Martin JW, Davis GE. 2001. An updated classification of the recent Crustacea. *Nat Hist Mus Los Angeles County Sci Ser.* 39:1–124.
Milne-Edwards A. 1862. Monographie des Crustacés fossils de la famille Cancériens. *Ann Sci Nat Zool.* 18:31–85.
Milne-Edwards A. 1865. Monographie des Crustacés fossils de la famille Cancériens. *Ann Sci Nat Zool.* 3:297–351.
Müller P. 2006. New decapods from the Miocene of Hungary with remarks about their environment. *Földtani Közlöny* 136:37–49.
Müller P, Collins JSH. 1991. Late Eocene coral-associated decapods (Crustacea) from Hungary. *Contr Tert Quatern Geol.* 28:47–92.
Naruse T, Clark PF. 2009. Establishment of a new genus for *Asthenognathus gallardoi* Serène & Soh, 1976 within Gaeticinae Davie and N.K. Ng, 2007 (Crustacea: Decapoda: Brachyura: Varunidae). *Zootaxa* 1987:61–68.
Ng PKL, Guinot D, Davie PJF. 2008. Systema Brachyuorum: Part I. An annotated checklist of extant Brachyuran crabs of the world. *Raffles Bull Zool Suppl.* 17:1–286.
Ng PKL, Rodriguez G. 1995. Freshwater crabs as poor indicators: a critique of Bănărescu (1990). *Crustaceana* 68:636–645.
Ng PKL, Števcic Z, Pretzmann G. 1995. A revision of the family Deckeniidae, Ortmann 1897 (Crustacea, Decapoda, Brachyura, Potamoidea) from the Seychelles, Indian Ocean. *J Nat Hist.* 29: 581–600.
Ortmann AE. 1902. The geographical distribution of freshwater decapods and its bearing upon ancient geography. *Proc Am Phil Soc.* 41: 267–400.
Palacios-Theil E, Cuesta JA, Campos E, Felder DL. 2009. Molecular genetic re-examination of subfamilies and polyphyly in the family Pinnotheridae. In: Martin JW, Crandall KA, Felder DL, editors. Crustacean Issues 18: Decapod crustacean phylogenetics. Boca Raton (FL): CRC Press. p. 457–474.
Parham J, Donoghue P, Bell C, Calway T, Head J, Holroyd P, Inou J, Irmis R, Joyce WG, Ksepka DT, et al. 2012. Best practices for justifying fossil calibrations. *Syst Biol.* 61:346–359.
Porter ML, Pérez-Losada M, Crandall KA. 2005. Model-based multi-locus estimation of decapod phylogeny and divergence times. *Mol Phylogenet Evol.* 37:355–369.
Posada D. 2008. jModelTest: phylogenetic model averaging. *Mol Biol Evol.* 25:1253–1256.
Pybus OG, Harvey PH. 2000. Testing macro-evolutionary models using incomplete molecular phylogenies. *Proc R Soc Lond B.* 267: 2267–2272.
Quayle WJ, Collins JSH. 1981. New Eocene crabs from the Hampshire Basin. *Palaeontology* 24:733–758.
Rabosky DL. 2006. Likelihood methods for detecting temporal shifts in diversification rates. *Evolution* 60:1152–1164.
Rambaut A, Drummond AJ. 2007. Tracer v1.4. [cited 2013 Oct 15]. Available from: http://beast.bio.ed.ac.uk/Tracer.
Rathbun MJ. 1935. Fossil Crustacea of the Atlantic and Gulf coastal plain. *Geo Soc Am Special Paper* 2:1–160.
Remeš M. 1895. Beiträge zur Kenntnis der Crustaceen der Stramberger Schichten. *Bull Int Acad Sci Boheme.* 2:200–204.
Revell LJ. 2012. Phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol Evol.* 3:217–223.
Rodriguez G. 1992. The freshwater crabs of America. Family Trichodactylidae. Paris (France): Office de la Recherche Scientifique d'Outre Mer.
Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Hōhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol.* 61:539–542.
Scholtz G, McLay CL. 2009. Is the Brachyura Podotremata a monophyletic group? In: Martin JW, Crandall KA, Felder DL, editors. Crustacean Issues 18: Decapod crustacean phylogenetics. Boca Raton (FL): CRC Press. p. 417–435.
Scholtz G, Richter S. 1995. Phylogenetic systematics of the reptantian Decapoda (Crustacea, Malacostraca). *Zool J Linn Soc.* 113:289–328.
Schram FR. 1986. Crustacea. New York: Oxford University Press.
Schubart CD, Cannicci S, Vannini M, Fratini S. 2006. Molecular phylogeny of grapsoid crabs (Decapoda, Brachyura) and allies based on two mitochondrial genes and a proposal for refraining from current superfamily classification. *J Zool Syst Evol Res.* 44:193–199.
Schubart CD, Cuesta JA, Diesel R, Felder DL. 2000. Molecular phylogeny, taxonomy, and evolution of nonmarine lineages within the American grapsoid crabs (Crustacea: Brachyura). *Mol Phylogenet Evol.* 15:179–190.
Schubart CD, Cuesta JA, Felder DL. 2002. Glyptograpsidae, a new brachyuran family from Central America: larval and adult morphology, and a molecular phylogeny of the Grapsoidea. *J Crustac Biol.* 22: 28–44.
Schubart CD, Diesel R, Hedges SB. 1998. Rapid evolution to terrestrial life in Jamaican crabs. *Nature* 393:363–365.
Schubart CD, Neigel JE, Felder DL. 2000. The use of the mitochondrial 16S rRNA gene for phylogenetic and biogeographic studies of Crustacea. The biodiversity crisis and Crustacea. Proceedings of the Fourth International Crustacean Congress, Amsterdam, Netherlands, 20–24 July 1998. Vol. 2. Crustacean Issues 12. Boca Raton (FL): CRC Press. p. 817–830.
Schubart CD, Reuschel S. 2009. A proposal for a new classification of Portunoidea and Cancroidea (Brachyura: Heterotremata) based on two independent molecular phylogenies. In: Martin JW, Crandall KA, Felder DL, editors. Crustacean Issues 18: Decapod crustacean phylogenetics. Boca Raton (FL): CRC Press. p. 533–549.
Schweitzer CE, Feldmann RM. 2000. Re-evaluation of the Cancridae Latreille, 1802 (Decapoda: Brachyura) including three new genera and the new species. *Contri Zool.* 69:223–250.
Schweitzer CE, Feldmann RM. 2005. Decapod crustaceans, K/P event, and Palaeocene recovery. In: Koenemann S, Jenner R, editors.

Crustacean Issues 16: Crustacea and arthropod relationships. Boca Raton (FL): CRC Press. p. 17–53.
Schweitzer CE, Feldmann RM. 2010. New fossil decapod crustaceans from the Remy Collection, Muséum national d'Histoire naturelle, Paris. *Geodiversitas* 32:399–415.
Schweitzer CE, Feldmann RM, Garassino A, Karasawa H, Schweigert G. 2010. Systematic list of fossil decapod crustacean species. *Crustaceana Monogr.* 10:1–222.
Serène R. 1984. Crustaces Decapodes Brachyoures de l'Ocean Indien occidental et de la mer Rouge, Xanthoidea: Xanthidae et Trapeziidae. Avec un addendum par CROSNIER (A.): Carpiliidae et Menippidae. *Faune Tropicale* no. XXIV: 1–349.
Shih H-T, Yeo DCJ, Ng PKL. 2009. The collision of the Indian plate with Asia: molecular evidence for its impact on the phylogeny of freshwater crabs (Brachyura: Potamidae). *J Biogeogr.* 36:703–719.
Shimodaira H. 2002. An approximately unbiased test of phylogenetic tree selection. *Syst Biol.* 51:492–508.
Shimodaira H, Hasegawa M. 2001. CONSEL: for assessing the confidence of phylogenetic tree selection. *Bioinformatics* 17:1246–1247.
Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann Entomol Soc Am.* 87:652–701.
Sin YW, Lai JCY, Ng PKL, Chu KH. 2009. Phylogeny of Dorippoidea (Crustacea: Decapoda: Brachyura) inferred from three mitochondrial genes. *Invertebr Syst.* 23:223–230.
Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22:2688–2690.
Stevčić Z. 2005. The reclassification of brachyuran crabs (Crustacea: Decapoda: Brachyura). *Fauna Croatica* 14:1–150.
Števčić Z. 2011. Addition to the reclassification of the brachyuran crabs (Crustacea: Decapoda: Brachyura) part I. New taxa. *Natura Croatica* 20:125–139.
Tsang LM, Chan BKK, Shih F-L, Chu KH, Chen AC-L. 2009. Host associated speciation in the coral barnacle *Wanella milleporae* (Cirripedia: Pygomatidae) inhabiting the *Millepora* coral. *Mol Ecol.* 18:1463–1475.
Tsang LM, Chan T-Y, Cheung MK, Chu KH. 2009. Molecular evidences for the Southern Hemisphere origin and deep sea invasions of spiny lobster (Crustacea: Decapoda: Palinuridae). *Mol Phylogenet Evol.* 51: 304–311.
Tsang LM, Chan T-Y, Ahyong ST, Chu KH. 2011. Hermit to king, or hermit to all: multiple transitions to crab-like forms from hermit crab ancestors. *Syst Biol.* 60:616–629.
Tsang LM, Ma KY, Ahyong ST, Chan T-Y, Chu KH. 2008. Phylogeny of Decapoda using two nuclear protein-coding genes: origin and evolution of the Reptantia. *Mol Phylogenet Evol.* 48: 359–368.
van Bakel BWM, Guinot D, Artal P, Fraaije RHB, Jagt JWM. 2012. A revision of the Palaeocorystoidea and the phylogeny of raninoidian crabs (Crustacea, Decapoda, Brachyura, Podotremata). *Zootaxa* 3215:1–216.
Van Straelen V. 1936. Crustacés décapodes nouveaux ou peu connus de l'époque crétacique. *Bulletin du Musée royal d'Histoirenaturelle de Belgique* 12:1–50.
Via L. 1959. Decápodos fósiles del Eoceno espaȕol. (Resumeavance de la tesis doctoral). *Bol I.G.M.E.* 70:331–402.
von Sternberg R, Cumberlidge N. 2001a. Notes on the position of the true freshwater crabs within the brachyrhynchan Eubrachyura (Crustacea: Decapoda: Brachyura). *Hydrobiologia* 449: 21–39.
von Sternberg R, Cumberlidge N. 2001b. On the heterotreme-thoracotreme distinction in the Eubrachyura de Saint Laurent, 1980 (Decapoda, Brachyura). *Crustaceana* 74: 321–338.
von Sternberg R, Cumberlidge N. 2003. Autapomorphies of the endophragmal system in trichodactylid freshwater crabs (Crustacea: Decapoda: Eubrachyura). *J Morph.* 256:23–28.
von Sternberg R, Cumberlidge N, Rodriguez G. 1999. On the marine sister groups of the freshwater crabs (Crustacea: Decapoda). *J Zool Syst Evol Res.* 37:19–38.
Wetzer R, Martin JW, Boyce SL. 2009. Evolutionary origin of the gall crabs (family Cryptochiridae) based on 16S rDNA sequence data. In: Martin JW, Crandall KA, Felder DL, editors. Crustacean Issues 18: Decapod crustacean phylogenetics. Boca Raton (FL): CRC Press. p. 475–490.
Yeo DCJ, Ng PKL, Cumberlidge N, Magalhães C, Daniels SR, Campos MR. 2008. Global diversity of crabs (Crustacea: Decapoda: Brachyura) in freshwater. *Hydrobiologia* 595:275–286.