Molecular phylogeography reveals multiple Pleistocene divergence events in estuarine crabs from the tropical West Pacific

Adnan Shahdadi¹, Katharina von Wyschetzki², Hung-Chang Liu³, Ka Hou Chu⁴,⁵, Christoph D. Schubart⁶*

¹ Department of Marine Biology, Faculty of Marine Sciences and Technology, University of Hormozgan, Bandar Abbas, Iran, ² Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, Cambridge, United Kingdom, ³ Land Crab Ecology Research Laboratory, Chenggong, Hsinchu City, Hsinchu County, Taiwan, ⁴ Simon F. S. Marine Science Laboratory, School of Life Sciences, The Chinese University of Hong Kong, Shatin, Hong Kong, China, ⁵ Hong Kong Branch of Southern Marine Science and Technology Guangdong Laboratory (Guangzhou), The Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong, China, ⁶ Zoology & Evolution, University of Regensburg, Regensburg, Germany

* Christoph.Schubart@biologie.uni-regensburg.de

Abstract

Due to the lack of visible barriers to gene flow, it was a long-standing assumption that marine coastal species are widely distributed, until molecular studies revealed geographically structured intraspecific genetic differentiation in many taxa. Historical events of sea level changes during glacial periods are known to have triggered sequential disjunctions and genetic divergences among populations, especially of coastal organisms. The Parasesarma bidens species complex so far includes three named plus potentially cryptic species of estuarine brachyuran crabs, distributed along East to Southeast Asia. The aim of the present study is to address phylogeography and uncover real and hidden biological diversity within this complex, by revealing the underlying genetic structure of populations and species throughout their distribution ranges from Japan to West Papua, with a comparison of mitochondrial COX1 and 16S rRNA gene sequences. Our results reveal that the P. bidens species complex consists of at least five distinct clades, resulting from four main cladogenesis events during the mid to late Pleistocene. Among those clades, P. cricotum and P. sangui-manus are recovered as monophyletic taxa. Geographically restricted endemic clades are encountered in southeastern Indonesia, Japan and China respectively, whereas the Philippines and Taiwan share two clades. As individuals of the Japanese clade can also be found in Taiwan, we provide evidence of a third lineage and the occurrence of a potential cryptic species on this island. Ocean level retreats during Pleistocene ice ages and present oceanic currents appear to be the main triggers for the divergences of the five clades that are here addressed as the P. bidens complex. Secondary range expansions converted Taiwan into the point of maximal overlap, sharing populations with Japan and the Philippines, but not with mainland China.
sequences have been submitted to the GenBank (NCBI) and are available in S1 Table. The materials examined are deposited in the zoological collections and the vouchers are available in S1 Table.

Funding: Two travel grants under the Germany/Hong Kong Joint Research Scheme of the German Academic Exchange Service (DAAD) and Research Grants Council (RGC), Hong Kong in 2009-2010 (ID 50022239/G_HK008/08) and in 2012-2013 (ID S4385238/G-H022/11). The travel grants [German Academic Exchange Service (DAAD) and Research Grants Council (RGC)] were solely used to support travel expenses between universities and collection sites. Laboratory expenses were supported by departmental funds of: - University of Regensburg, Dept. Zoology & Evolution, chair: Prof. Jürgen Heinze - Hong Kong Research Institute of Textiles and Apparel (HK), HK008/08, chair: Prof. Ka Hou Chu. From those funding organizations, only Prof. KH Chu had a role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Introduction

The biogeography of marine species is generally determined by different abiotic and biotic factors. Historical events of sea level changes during glacial periods in different geological epochs [1, 2] have triggered sequential disjunction/junctions of coastal populations and consequently resulted in successive genetic divergence [3–6]. Former and current oceanic currents are also important agents for the "transport" of alleles between distant populations [7–9] or can act as barriers, preventing reciprocal gene flow among nearby populations [10–12]. The mode of reproduction, early developmental motility, and dispersal ability of different ontogenetic stages of marine organisms are additional important players in structuring their biogeography [12–14]. Some species have even been able to perform trans-Pacific dispersal, resulting in certain degree of genetic differentiation [15].

The field of molecular phylogeography has contributed significantly to the development of our present understanding of marine biogeographic patterns [14]. For decades, the lack of visible geographic barriers was responsible for the general belief that most marine species are widely, or some of them even globally, distributed and dispersed via oceanic currents [16]. Molecular comparisons, however, have revealed genetic disjunctions and phylogeographic structures among and within many marine animals [17], including decapod crustaceans [18]. Such genetic disconnections and locally structured patterns are also common among partly pelagic [19] and meroplanktonic (e.g. coastal crabs with planktonic larvae) species [20].

Brachyuran crabs of the family Sesarmidae Dana, 1851 are among the most important faunal components of tropical estuarine habitats, including marshes and mangroves, with a high species diversity, especially throughout the Indo-West Pacific (IWP) [21–25]. This zoogeographic region is well known for its high biodiversity, especially among marine taxa [26–29]. Several studies have attempted to understand and describe phylogeographic patterns of different representatives in this marine realm [9, 30–32]. Ragionieri et al. [33] studied the phylogeography of sesarmid crabs referred to as Neosarmatium meinerti (De Man, 1887), with a presumably wide distribution throughout the IWP. This taxon showed a clear genetic structure composed of four distinct clades (i.e. East Africa, western Indian Ocean, Southeast Asia and northern Australia), with the north Australian clade being most clearly separated from others. As these clades turned out to be similarly diverged from one another as from the West Pacific species N. fourmanoiri Serène, 1973, all four of them were recognized and described as valid species [33, 34].

With currently 54 extant species, Parasesarma De Man, 1895 is one of the two largest sesarmid genera [35]. Its representatives are distributed throughout the IWP, mostly in East and Southeast Asia [36]. In a recent phylogenetic analysis, we recovered several stable clades among species of Parasesarma [36]. One of these clades showed similar patterns as those reported for the N. meinerti species group, with representatives in East Africa (P. guttatum (A. Milne-Edwards, 1869) and P. capensis (Fratini, Cannicci and Innocenti, 2019), India (P. bengalense (Davie, 2003)), Southeast Asia (P. coticum (Rahayu and Davie, 2002)), Australia (P. brevicristatum (Campbell, 1967), P. darwinense (Campbell, 1967) and P. holthuisi (Davie, 2010)), and East Asia (P. bidens (De Haan, 1835) and P. sanguimans Li, Shih & Ng, 2019) [36].

Within the IWP, Southeast Asia sticks out as a general biodiversity hotspot, harbouring the highest species richness among all other marine phylogeographic provinces [37, 38]. Numerous studies have thus been conducted to uncover the mechanisms and history of the diversification in this area and to address the local barriers and reasons for lineages’ divergences [16, 39, 40]. On the other hand, the East Asian coastline was affected by many changes during its geological history, and therefore has been given increased attention in several phylogeographic studies [20, 41–43].
According to the presence of several marginal seas in the area and their role in genetic divergences during ice ages [41, 44], a thorough phylogeographic study can potentially uncover undetected biodiversity in this group and date potential divergence events. Moreover, such study could also map the distribution ranges and boundaries of the corresponding phylogenetic groups. With this intention, the present study focuses on the coastal crab species occurring in this area, i.e. *Parasesarma cricotum* from Southeast Asia vs. *P. sanguimanus* and *P. bidens* from East Asia, in order to reveal underlying causes for species divergence and biogeographic distribution patterns in these two Asian subregions. *Parasesarma bidens* was originally described from Japan [45], with a supposed wide distribution throughout East to Southeast Asia [46]. Within mainland China, *P. bidens* has been characterized by its genetic homogeneity [47], whereas morphological and genetic distinction of West Papuan [48] and Taiwanese [49] populations led to the relatively recent descriptions of the two regionally confined species *P. cricotum* [48] and *P. sanguimanus* [49].

The present study aimed to gain deeper insights into the phylogeography and genetic structure of these species and other populations still belonging to *Parasesarma bidens*, which will here be referred to as the *P. bidens* species complex. For this purpose, and to document overall diversity within this complex, we molecularly screened 142 individuals originating from a wide distribution range (from Japan to West Papua) by comparing DNA sequences corresponding to the mitochondrial COX1 and 16S rRNA genes. Furthermore, a molecular clock approach was applied to estimate divergence times for the genetic groups.

**Materials and methods**

**Materials examined**

Specimens of the *P. bidens* group (including *P. bidens*, *P. cricotum* and *P. sanguimanus*) were gathered from Japan (Hiroshima, Nagasaki, Iriomote), China (Hong Kong, Hainan), Taiwan (Pingtung, Taichung), Philippines (Bohol, Cebu, Luzon) and Indonesia (West Papua, Sulawesi) (Fig 1). Materials from Gaomei (Taiwan, Taichung) were loaned from the Zoological Collections of the Department of Life Science, National Chung Hsing University (Taichung, Taiwan). Some of the specimens from the Philippines and Japan were loaned from the Florida Museum of Natural History (Florida, USA) and the Ryukyus University Museum (Fujukun, Okinawa, Japan), respectively (see S1 Table). All other materials, which were collected during different official field expeditions between 1995 to 2011 from non-protected mangrove forests and estuaries were legally integrated in biological collections in Germany, Japan, Hong Kong and Singapore (see S1 Table for localities, museum voucher numbers, and DNA extraction code of the CD Schubart lab).

**Laboratory methods and sequence preparations**

Genomic DNA was isolated using a modified Puregene method (Gentra Systems, Minneapolis, MN) from muscular leg tissue. A segment of nearly 800 base pairs (bp) from the 3’ end of the mitochondrial protein-coding gene cytochrome oxidase subunit 1 (COX1) was selected as the main molecular marker for our genetic analyses. After preliminary analyses of these sequences revealed the underlying phylogenetic relationships, for a subset of specimens an approximately 650 bp segment of the 5’ end of the COX1 gene, corresponding to the barcode region, as well as a segment of a more conserved mitochondrial gene, encoding the RNA of the large ribosomal subunit (16S rRNA), was also sequenced. Polymerase chain reactions (PCRs) were performed with the following profile: initial denaturation step for 4 min at 94˚C; 40 cycles with 45 s at 95˚C for denaturing, 60 s at 48˚C for annealing, 60 s at 72˚C for extension; and 5 min at 72˚C as final extension step. To amplify the 3’ end of COX1, the primers COL8 (forward) and
COH1b (reverse) were used. In case this combination did not work, the alternative forward primers COL1b or COL11 were used to amplify a segment of about 680 or 420 bp, respectively. To amplify the barcode region of COX1, the primer set COL6-COH6, and for the 16S rRNA gene the primer combination 16L29-16H10, was used (see S2 Table for primer information). PCR products were outsourced for sequencing to Macrogen Europe (for accession numbers see S1 Table). Sequences were proofread using Chromas Lite (v. 2.1.1) (Technelysium Pty Ltd, Queensland, Australia). Primer regions were removed and the remaining sequences were aligned with ClustalW [50] implemented in BioEdit 7.0.5 [51].
Phylogenetic and phylogeographic analyses

To uncover phylogeographic structure and genetic groups among the obtained sequences, two phylogenetic algorithms were applied for the sequences of our large COX1 dataset, corresponding to its 3’ end. A maximum likelihood (ML) tree was constructed using the software raxmlGUI v. 1.5b2 [52] with 1000 bootstrap replicates. To estimate the clades’ divergence times, we conducted a Bayesian inference (BI) analysis with the software BEAST 2.6.2 [53], using a strict clock model (Yule Model) with a rate of evolution for the COX1 of 2.33% per million years (my), following [54]. Markov chains were run for 10 million generations, sampling every 1000th iterations and discarding the first 25% as burnin. The remaining 7500 trees were used to calculate the maximum clade credibility tree in TreeAnnotator v.1.6.1 (part of the BEAST package). Phylogenetic analyses were based on the General Time Reverse plus Gamma (GTR + G, [55]) evolutionary model, as suggested by the AIC algorithm in jModelTest 0.1.1 [56]. A sequence of *P. bengalense* (Davie, 2003) was also included to the analyses as a representative of the sister clade, consisting of *P. bengalense*, *P. capensis* and *P. guttatum*, to the *P. bidens* group [36, 49, 57], in order to re-examine their phylogenetic relationship. Based on previous phylogenetic analyses [36, 57], sequences of three other species of the genus, *P. eumolpe* (De Man, 1895), *P. indiarum* (Tweedie, 1940) and *P. peninsulare* Shahdadi, Ng & Schubart, 2018, were included in the analyses as outgroups.

To obtain a better resolution of the genetic relationships among and within groups, a maximum parsimony haplotype network [58] was built via the software PopART [59]. Mean genetic distances (Kimura 2 Parameter = K2P) between phylogenetic groups (clades) were calculated with the software MEGA version X [60]. To find the phylogenetic positions of some sequences that were recovered from GenBank (https://www.ncbi.nlm.nih.gov/), a haplotype network was also constructed for the sequences of the barcode region of the COX1 gene. To check the genetic relationships in a second and more conserved marker, a maximum parsimony haplotype network [58] was also built for the 16S rRNA gene, using the software PopART [59].

Analyses of population genetics and demography

To calculate the genetic diversity indices (i.e. the values of haplotype diversity (Hd), nucleotide diversity (π), number of segregating sites (S), and average number of nucleotide differences (k)) of the large COX1 dataset, DnaSP 5.10 [61] was used. Demographic changes at levels of mitochondrial DNA lineages were analyzed by the neutrality test using estimation of Fu’s Fs [62] in ARLEQUIN 3.5 [63] with 1,000 permutations. To trace population size changes, we analyzed the distribution of pairwise differences (mismatch distribution) [64] in DnaSP 5.10 [61] with the model of constant population size for expected values, and the graphs were created in Microsoft Excel 2013 [65]. In order to measure the smoothness of the mismatch distribution, we also analyzed the distribution of pairwise differences in ARLEQUIN 3.5 [63] under sudden expansion models, with calculation of Harpending’s raggedness index (r) [66] under the null hypothesis of neutral evolution using 1,000 bootstrap iterations. The neutrality estimations and mismatch distribution were calculated for each of the five evolutionary clades (recovered from the phylogenetic analyses). The diversity indices were calculated for each of these clades, as well as for the populations comprised in each clade separately. To identify genetic differentiation among populations of each clade, the fixation index, $F_{ST}$ (in mitochondrial DNA $\Phi_{ST}$), and the analyses of molecular variance (AMOVA) were also performed with ARLEQUIN [63] and 1,000 permutations.

Sequences of two localities from the main islands of Japan had to be lumped, because of a low number of available sequences: Hiroshima (4 sequences) and Nagasaki (5 sequences).
Results

In total, COX1 sequences were obtained from 142 specimens of *P. bidens* species complex (Fig 2A, S1 Table), with variable lengths from different localities as the main ingroup of the present analyses. Most sequences had a length of 742 bp, after removal of the primer sequences and adjacent regions. Two phylogenetic trees, one BI with BEAST (Fig 2B, S1 Fig) and one ML (S2 Fig) were constructed with all available sequences of the 3’ end. 139 sequences had a length of at least 618 bp and were batched in a second alignment for building a haplotype network, calculating K2P distances, diversity indices and other demographic tests. For the barcode region (5’ end of COX1) 13 sequences (eight sequences from GenBank and five from the present study) with a length of 610 bp, and for the 16S gene 15 sequences (present study) with a length of 527 bp were available (See S1 Table) to build the haplotype networks.

For the COX1 sequences, no sequence contained stop codons or resulted in alignment indels, which may have indicated the presence of pseudogenes.
Phylogeny and phylogeography

The topologies of BI and ML phylogenetic trees were largely congruent (Fig 2B, S1 and S2 Figs) and allowed to recover five well supported clades (hereafter referred to as clades I–V) for the *P. bidens* group in the Western Pacific (Fig 2B, S1 and S2 Figs). These five clades form a monophyletic group, sister to *P. bengalense*. The five clades of the *P. bidens* group form two main clusters, one composed of clades I, II and III, and the other including clades IV and V. The sequences from China (Hainan and Hong Kong) (*P. bidens* Clade I) form a stable monophyletic group together with the closely related Clade II (*P. bidens*), which is composed of all Japanese specimens (main islands and Iriomote) together with few Taiwanese individuals. The monophylum consisting of clades I and II holds a sister position with the Indonesian (West Papua and Sulawesi) Clade III (= *P. cricotum*). Most Taiwanese sequences, together with some from the Philippines, cluster in a solid clade, separated from all others by a relatively long branch (Clade IV). This clade shows a sister relationship to *P. sanguimanus* (Clade V). Most specimens of Clade V were from the Philippines, with a single one (out of 17 sequences) from Taiwan.

The result from the divergence time estimation in the Bayesian analyses (Fig 2B) reveal that the five clades of the *P. bidens* species complex diverged from their sister group (*P. bengalense* and allies) approximately 2.27 million years ago (1.64–2.96 mya). The results further show that these five clades shared a last common ancestor about 1.56 million years ago (1.15–2.02 mya). Following the first divergence event at about 1.56 mya, which resulted in the two main clusters, there were two divergence events at seemingly different times. At about 1.14 (0.78–1.50) mya one of these events separated *P. cricotum* (Clade III) from *P. bidens* (clades I and II), and the second at about 1.08 (0.71–1.46) mya separated Clade IV from Clade V (*P. sanguimanus*). The divergence of Clade I from China and Clade II from Japan dates back to about 0.69 (0.45–0.94) mya. The earliest divergence events within each of these five clades are recorded at about 0.42 mya (Fig 2B).

The haplotype networks based on the COX1 gene (both segments) (Fig 3A and S2 and S3 Figs) recovered exactly the same five groupings as described above. In the main network (the 3’ end) (Fig 3A), all clades except for Clade III (*P. cricotum*) include a common haplotype and to some extent show a star-shape structure. In Clade III, specimens of the two localities (West Papua and Sulawesi) revealed a very close association, but without sharing haplotypes. In the haplotype network the 5’ end (the barcode region), the sequences from South Korea grouped with the Japanese specimens and the one from Palau Island clustered with those of Sulawesi and West Papua (S3 Fig).

To confirm these patterns of genetic differentiation with a more conserved mitochondrial marker 15 sequences with 527 bp of the 16S rRNA gene (after removing primer sequences and adjacent regions) were compared. Genetic relationships based on the 16S rRNA haplotype network (Fig 3B) were generally similar to those of COX1 (Fig 3A) and equivalent genetic groups were recovered, but with fewer mutation steps between groups (Fig 3B).

Among the geographic areas covered in this study, Taiwan holds a special position by hosting members of three clades, II, IV and V (Fig 1). The Philippines harbour specimens of two clades (IV and V), while other regions only include members of single clades (Fig 1). Clade IV is the most common clade in Taiwan, while in the Philippines Clade V is the most common one (Fig 1). With regard to the K2P distances (S3 Table), clades I and II show the least distance, 1.4%, while other clades are separated by K2P values of more than 2.4%. The largest distance is 4.0%, between clades III and IV. The mean K2P distance between two superimposed clusters is 3.6%. The mean distance between Clade III and clades I and II is 2.6%.
Population genetic and historical demography

Clades II and III show the highest total genetic diversity among all clades (Hd = 0.755, π = 0.00356, k = 2.194 in Clade II; Hd = 0.857, π = 0.00354, k = 2.186 in Clade III) (see Table 1), whereas Clade IV has the least genetic diversity (Hd = 0.672, π = 0.00160, k = 0.987), especially within Taiwan with even lower diversity indices (Hd = 0.350, π = 0.00061, k = 0.375). Among the studied localities, the Philippines revealed a high genetic diversity in Clade IV (Hd = 0.910, π = 0.00631, k = 3.897).

Comparisons of $F_{ST}$ values among populations (of each clade) revealed the lowest values in the two Chinese populations, Hainan and Hong Kong ($F_{ST} = 0.07094$), compared to all other population pairs (Table 1). In contrast, highest values were found between the Indonesian populations from Sulawesi and West Papua (both Clade III, $F_{ST} = 0.56503$) (Table 1). The result of the AMOVA analysis revealed low levels of molecular variance among populations within clades I and II. A higher value of variation was found between the two Indonesian populations (Clade III) compared to the other examined population pairs (Table 2).

https://doi.org/10.1371/journal.pone.0262122.g003

Fig 3. Haplotype networks. Maximum parsimony haplotype network, constructed with PopART. A. for the 3’ end of COX1 (618 bp). B. for 16S rRNA (529 bp). Hatch marks represent mutation steps. Ph, Philippines; Ch, China; Jp, Japan; In, Indonesia.
Generally, the significant negative values of the neutrality test (Fu’s $F_s$) (Table 1) confirmed demographic expansions in all the five clades, with clades I and IV showing stronger signs of expansion with lower values (Table 1). In the analyses of mismatch distribution, all the five clades showed a unimodal pattern with one main peak (Fig 4). Clades II and V, however, showed signs of a small second peak (Fig 4). The values of Harpending’s $r$ were statistically non-significant in the case of all clades, except for Clade III, implying that population expansion is evident in all clades, except for Clade III.

### Discussion

During the Pleistocene (since 2.58 mya), more than 11 major and many minor glacial events could be identified [67]. As a consequence, global sea levels experienced sequential falling and rising throughout these successions of glacial and interglacials [68]. During the cold periods, sea basins were shallow and, depending on their depth, the global ocean shape or local sea

### Table 1. Diversity indices

| Clade       | Locality         | n    | h    | $Hd$±SD | $\pi$     | S   | k   | Fu’s $F_s$ | $\Phi_{ST}$ | Harpending’s $r$ |
|-------------|------------------|------|------|---------|-----------|-----|-----|------------|-------------|-----------------|
| I           | Hainan           | 20   | 5    | 0.505±0.126 | 0.00103   | 4   | 0.637 |            |             |                 |
|             | Hong Kong        | 14   | 8    | 0.868±0.068   | 0.00245   | 7   | 1.516 |            |             |                 |
|             | Total            | 34   | 11   | 0.690±0.077   | 0.00167   | 9   | 1.032 | -7.91287*  | 0.07094     | 0.0877* (p = 0.1) |
| II          | Jp. main islands | 9    | 5    | 0.861±0.087   | 0.00252   | 5   | 1.556 |            |             |                 |
|             | Iriomote         | 21   | 7    | 0.614±0.116   | 0.00371   | 12  | 2.286 |            |             |                 |
|             | Total            | 30   | 11   | 0.755±0.077   | 0.00356   | 15  | 2.194 | -3.66265*  | 0.08285     | 0.0484* (p = 0.8) |
| III         | West Papua       | 16   | 5    | 0.650±0.108   | 0.00195   | 4   | 1.483 |            |             |                 |
|             | Sulawesi         | 14   | 6    | 0.780±0.081   | 0.00659   | 5   | 1.110 |            |             |                 |
|             | Total            | 30   | 11   | 0.857±0.040   | 0.00354   | 9   | 2.186 | -3.79208*  | 0.56503*    | 0.0668 (p = 0.05) |
| IV          | Taiwan           | 16   | 4    | 0.350±0.148   | 0.00061   | 3   | 0.375 |            |             |                 |
|             | Philippines      | 13   | 8    | 0.910±0.056   | 0.00631   | 22  | 3.897 |            |             |                 |
|             | Total            | 29   | 10   | 0.672±0.097   | 0.00160   | 9   | 0.987 | -7.25174*  | 0.22907*    | 0.0645* (p = 0.2) |
| V           | Total            | 16   | 6    | 0.683±0.120   | 0.00191   | 6   | 1.183 | -2.16352*  | 0.1425*     | 0.1425* (p = 0.9) |

n = Number of sequences; h = Number of haplotypes; $Hd$ = Haplotype diversity; SD = Standard deviation; $\pi$ = Nucleotide diversity; S = Number of segregating sites; k = Average number of nucleotide differences

* = P < 0.05 in Fu’s $F_s$ and $\Phi_{ST}$, with support values for population expansion.

https://doi.org/10.1371/journal.pone.0262122.t001

### Table 2. AMOVA

| Clades and populations | source of variation | d.f. | Sum of squares | Components of competence | Percentage of variation |
|------------------------|---------------------|------|----------------|--------------------------|------------------------|
| I (Hainan & Hong Kong) | Among populations   | 1    | 1.122         | 0.03796 (0.05767)        | 7.09                   |
|                        | Within populations  | 32   | 15.907        | 0.49710                  | 92.91                  |
| II (Iriomote & main islands of Japan) | Among populations | 1    | 2.221         | 0.09382 (0.06647)        | 8.28                   |
|                        | Within populations  | 28   | 29.079        | 1.03855                  | 91.72                  |
| III (West Papua & Sulawesi) | Among populations | 1    | 13.361        | 0.85083 (0.00000)        | 56.50                  |
|                        | Within populations  | 28   | 18.339        | 0.65497                  | 43.50                  |
| IV (Philippines & Taiwan) | Among populations | 1    | 2.176         | 0.12738 (0.00000)        | 22.91                  |
|                        | Within populations  | 26   | 11.146        | 0.42869                  | 77.09                  |

The numbers in parentheses under the column of ‘Components of competence’ are $P$ values after 1000 permutations.

https://doi.org/10.1371/journal.pone.0262122.t002
ranges successively changed [69]. Furthermore, many organisms migrated to warmer areas in the vicinity of the equator. In consequence of these refugial retreats followed by geographic expansions during warmer periods, genetic divergences among isolated populations occurred repeatedly [70]. Present phylogeographic structures of the majority of tropical and subtropical marine species, including those in East and Southeast Asia, are therefore known to have been shaped during these Pleistocene climate fluctuations [2, 4, 71, 72]. Several studies attempted to time-calibrate genetic divergences among related groups from different phyla, using available fossil data and other geological evidence [73–75]. In the present study, we use the substitution rate of 1.66–2.6% (mean = 2.33) per my for COX1, calibrated by Schubart et al. [54] for sesarmid crabs, based on the closure of the Isthmus of Panama.

East and Southeast Asian marine waters consist of several deep basins and trenches surrounded by shallow waters (e.g. Sea of Japan, South China Sea, Philippine Sea, Sulu Sea, Celebes Sea and Banda Sea). These deeper water basins served as refugia for many marine species during periods of low sea level [5, 76], and several studies addressed phylogeographic patterns and the history of this area [16, 20, 39, 41, 77]. However, since animals have a wide variety of life cycles and dispersal capabilities [78], the present-day structures of marine taxa are a mosaic and do not follow a universal pattern [76]. Horne et al. [79] found no phylogeographic structure in two surgeonfish species across their Indo-Central Pacific ranges. A lack of divergence and genetic structure was also found among populations of the rock snail Thais clavigera (Küster, 1860) throughout the northwestern Pacific [44]. On the other hand, Chang et al. [80] recovered four lineages among the soft shore barnacle Fistulobalanus albicostatus (Pilsbry, 1916) in East Asia and estimated their divergence to have occurred during the Pleistocene, as a result of past and present oceanographic regimes. Congruently, Shin et al. [20] also detected a shallow genetic gap between Taiwanese and Korean-Japanese populations of two littoral crab species of the genus Hemigrapsus (Brachyura: Varunidae).

Previous phylogenetic analyses on species of Parasesarma [36, 49, 57, 81] revealed that members of the *P. bidens* species complex from the Western Pacific (*P. bidens, P. cricotum* and *P. sanguimanus*) hold a sister relationship with a clade from within the Indian Ocean consisting of *P. bengalense, P. capensis* and *P. guttatum*. Under a larger clade, these six species form two reciprocally monophyletic groups, a western clade versus an eastern one [36, 49, 57, 81]. This pattern highlights the importance of the Indo-Pacific barrier [31] in structuring phylogeny of these marine invertebrates. Present results also recovered representatives of the Western Pacific (the five clades of *P. bidens* group) as a monophyletic group under a supported clade (Fig 2B, S1 and S2 Figs). Based on the divergence time estimation, it seems that the eastern cluster (the five clades of *P. bidens* group from the West Pacific) has been separated from their western sister clade at about 2.27 mya (Fig 2B).

**Phylogeny, phylogeography and evolutionary history of the *P. bidens* species complex**

In comparison to the previously mentioned studies, the present one covers a larger geographic range along East to Southeast Asia, reaching southward to New Guinea and Sulawesi (Indonesia), in the north to Japan (Hiroshima), and west to Hainan Island (China), furthermore including major islands in eastern Asia like Bohol, Cebu, Luzon from the Philippines and Taiwan (Fig 1, S1 Table, S1 and S2 Figs). Our results reveal that members of the *P. bidens* species complex, which were considered to be a single species until 20 years ago, and so far include...
three described species (i.e. *P. bidens*, *P. cricotum* and *P. sanguimanus*), consist of at least five distinct lineages, with different genetic divergence (Figs 2B, 3A, 3B, S1 Table, S1–S3 Figs). The corresponding clades resulted from four main cladogenesis events at different time periods (Fig 2B). According to the estimated divergence times, the most recent common ancestor of the five clades dates back to about 1.56 (2.02–1.15) mya. This means that all divergences and formations of the clades must have happened during the mid to late Pleistocene (Calabrian to Chibanian). The first divergence at about 1.56 mya resulted in two main clusters. The smaller cluster, with representatives in the Philippines and Taiwan experienced another divergence event at about 1.08 (0.71–1.46) mya, giving rise to two currently geographically overlapping clades IV and V, with the latter corresponding to *P. sanguimanus*. It is very likely that this later divergence happened during one of the major glacials, with sea level at about 100 m below its current level. This large water retreat could have separated the Sulu Sea from surrounding water bodies (i.e. South China Sea in the north, Philippine Sea in the east and Celebes Sea in the south) (see fig. 1 in Voris, 2000). The two lineages corresponding to clades IV and V may have evolved as a result of isolation, but rising sea levels probably led to geographical expansion and subsequent secondary contact.

The phylogenetic history of the larger of the two main clusters seems to be more complex, as it resulted in a wider, but detached distribution of the three comprised clades. This cluster has representatives in southeast Indonesia (*P. cricotum*, Clade III), Japan and Taiwan (*P. bidens*, Clade II), and Hong Kong and Hainan (*P. bidens*, Clade I), but it is absent in the Philippines. Therefore, the history of this cluster can only be explained with the aid of a different scenario. It is conceivable that the origin of this cluster was to be found in its northern current range, for example in the Sea of Japan, as a refugial population during one of the mid-Pleistocene ice ages. Alternatively, it could also have evolved in one of the southern marginal seas like Celebes or Banda seas. However, at about 1.14 mya, so about 420,000 years after its origin, this early lineage split into two groups. These two groups (northern = clades I & II; southern = Clade III) do not overlap in their distribution, and they are not even geographically closest neighbours in their current ranges. Seemingly, a long-distance founding event must be purported, explaining the establishment of a new lineage. This colonization could have taken place via the Pacific Ocean, using the Micronesian Islands (e.g. Palau, Guam, and Mariana) as stepping stones. This hypothesis would be supported by the fact that *P. cricotum* (here Clade III) was recently recorded from Palau [82] (see also S3 Fig). Another possible explanation for the present-day disjunct distribution of *P. bidens* (clades I and II) vs. *P. cricotum* (Clade III) could be that there used to be a continuous distribution, but competition with crabs occupying similar ecological niches, like representatives of Clade IV and *P. sanguimanus* (Clade V), may have forced the first group into regions with different climatic or other ecological conditions. The most recent divergence leading to the five described clades, occurred about 0.69 mya and separated the Japanese Clade II from the Chinese Clade I. This divergence was probably triggered by another historic biogeographic event.

Among the studied areas, Taiwan with representatives from three clades (*P. bidens* Clade II, *P. sanguimanus* & *Parasesarma* sp. Clade IV) hosts crab populations from more clades than any other sampled regions (Fig 1). The majority of the examined materials from this island belongs to Clade IV (in Clade IV, 16 sequences out of 28, are from Taiwan), whereas one of the present sequences from Taiwan is placed in Clade II and one in clade of *P. sanguimanus* (Clade V) (Fig 1, S1 and S2 Figs). According to the distribution and frequency of the clades, it seems that Taiwan is not the region of origin for clades II and V, but was later reached by representatives from more northern (Japanese) and southern (Philippines) clades. This phenomenon of a “melting pot” with migrants from adjacent regions converts the island into a biogeographically interesting hotspot and explains the high biodiversity of Taiwanese waters.
as previously described [83, 84]. Members of Clade IV are distributed equally in Taiwan and the Philippines with low $\Phi_{ST}$ values (Table 1) and low genetic differentiation among the two island populations (Table 2), suggesting high connectivity and regular gene flow among the areas.

The Philippine Archipelago is home for crabs of clades IV and V, but at the same time acts as a barrier between relict populations of marginal seas (South China Sea, Sulu Sea, Philippine Sea, and Celebes Sea) during ice ages. This could have played a significant role in the formation of other evolutionary lineages. The corresponding area is already known to be the centre of Southeast Asian marine biodiversity [85]. West Papua and Sulawesi (both Indonesia) comprise the area of distribution of $P. cricotum$ (Clade III), and at the same time the southernmost distribution range of this complex. Apparently, members of this clade have not dispersed to northern areas (with the exception of Palau, see Shahdadi et al. [82]), while the Philippine individuals of clades IV and V have not distributed to southern Indonesian islands. Thus, it appears that there is no reciprocal gene flow between islands from the Philippines and those from eastern Indonesia, possibly because of the Northern Equatorial Current that originates in the Pacific Ocean, flows through the Celebes Sea and Makassar Strait [85, 86] and may restrict reciprocal larval exchange. Members of Clade II are distributed in Japan (Hiroshima to southern islands) (Fig 2B and S1 Fig), South Korea [87] (S3 Fig), and likely Chinese coasts of the Yellow Sea. A single haplotype belonging to Clade II was also found in Taiwan (Fig 2B and S1 Fig). According to the geographical distribution of this clade and low frequency in Taiwan, it seems that members of this clade originated in Japan and reached the Taiwanese coast through rare migration event(s). A similar pattern was revealed by Tsang et al. [84] for an acorn barnacle $H. pilsbryi$ (Hiro 1936). Shin et al. [20] also revealed that Japanese and Taiwanese populations of two species of $Hemigrapsus$ share no common haplotype and are genetically distinct, confirming gene flow restriction between Taiwan and Japanese islands. Congruently, crabs from Clade IV of the present study, which are abundant in Taiwan, were not found in Japanese islands. This gene flow restriction between Taiwan and Ryukyu in marine invertebrates were previously attributed to a combination of biological factors (e.g. spawning season) and oceanographic regime (e.g. the strong summer upwelling along the northeastern coastline of Taiwan and the Ryukyu Islands) (see Tsang et al. [88]). Clade I, distributed in mainland China westward to Hainan (present study) and eastward at least to Fujian [47] (see also S3 Fig), was apparently isolated from more northern areas of China, South Korea and main islands of Japan (range of clade II). Similar patterns of isolation were also discovered among other mangrove animals (e.g. in bubbler crabs [89], barnacles [80] and a mudskipper [90]). As previously hypothesized [80, 91, 92], the Yangtze River freshwater discharge could represent a possible barrier by interfering reciprocal gene flow between northern and southern areas (clade I vs. II). A similar case has been described for the role of the Orinoco freshwater discharge by restricting larval transport in western Atlantic fiddler crabs [93]. Amazon freshwater and sediment outflow are also known as a strong barrier responsible for most of the endemism found in Brazilian coastal reefs [12, 94]. While Taiwan hosts members of three clades of the $P. bidens$ group, none of them were found in mainland China (Fig 1 and S1 Fig). Reciprocally, members of Clade I occurring in mainland China, apparently, have not colonized Taiwanese coasts. This pattern of isolation was also recorded for other marine invertebrates (e.g. $Tetraclita squamosa$ [95] and $Chthamalus malayensis$ [72]). In contrast, populations of some species seem to be identical in the two areas (e.g. [96, 97]). Two parallel northward summer currents (South China Sea Current & Taiwan Current) and the southward winter coastal current (Minzhe Current) passing through the Taiwan Strait [42, 44, 98] could be possible candidates for biogeographic barriers separating the populations of Taiwan and mainland China in some species. This diversity of phylogeographic structure has been attributed to differences
in their reproductive strategies (e.g. spawning season), dispersal abilities (e.g. duration of larval stages) as well as their nesting ecology (e.g. utilization of a wide range of habitats) [44]. Members of the P. bidens group, however, are apparently not able to cross this hydrographic barrier, despite high offspring production during a prolonged spawning time (April to July [99] and January to October in Taiwan, H-C Liu, personal observation), probably because of their short planktonic larval phase (about 16 days for four zoeal stages [100]).

Population genetic and historical demography

Regarding the genetic diversity, the southern (III = Indonesian) and the northern (II = Japanese) clades show higher values of the different indices (Table 1). However, the differences among the five clades are not considerable, as for example the haplotype diversity (Hd) ranging from 0.85 in Clade III to 0.67 in Clade IV (Philippines & Taiwan). The Philippine specimens of Clade IV are characterized by the highest genetic diversity among all examined populations (Table 1). This might confirm that the Philippine islands are probably the centre of origin for this clade, and Taiwan was colonized after the population expansion, likely mediated by the Kuroshio Current [84]. However, for more conclusive inference a larger sample size from both areas is needed in order to run the corresponding statistical tests.

Concerning populations connectivity, it seems that regular gene flow is maintained between the two Chinese populations (Hainan and Hong Kong) (Clade I), with a low fixation index value ($\Phi_{ST} = 0.07094$) (Table 1). Although this value is not significant, the low value of percentage of variation among the two populations in AMOVA (7.09%) (Table 2) could confirm the relatedness of these populations. This is congruent with the previous analyses by Zhou et al. [47] showing a high connectivity among Chinese mainland population of this species. A similar status could be inferred for the Japanese populations of the present study, based on our results (Tables 1 and 2). According to the fixation index ($\Phi_{ST} = 0.22907$) and differentiation among populations in AMOVA (percentage of variation among population = 22.91%), the gene flow between the Philippines and Taiwan (in Clade IV) seems to be less common as in the cases of the Chinese and the Japanese populations. Sulawesi and West Papua (Clade III) also appear to be less connected, with a significantly high $\Phi_{ST}$ value and high differentiation between the two localities and with a high percentage of variation (Table 2). Like in many other animal lineages, according to present demographic analyses, clades of the here studied P. bidens species complex apparently have also been affected by recent historic events (e.g. the latest glacial maximum).

Conclusion

Our molecular data reveal that the here defined P. bidens species complex consists of at least five well separated phylogenetic groups, of which three have so far been described as nominal species, whereas two may be considered as cryptic. The discovery of this additional case of relatively recent sequential differentiation and speciation in East and Southeast Asia provides additional evidence for the amazingly high marine biodiversity in this region. Phylogenetic analyses indicate that these five lineages have originated not more than 1.6 mya, during the mid to late Pleistocene. Being relatively young lineages, and without any evidence of different habitat requirements, and thus external adaptations, they are not morphologically well differentiated. As occurring in many freshwater organisms that are isolated in different river systems [101, 102], here we can show that the real biodiversity is frequently underestimated and overlooked, in consequence of the fact that there are not enough taxonomic units to name all extant evolutionary significant lineages. Considering that all of these phylogenetic groups are
unrepeatable and irreplaceable witnesses of unique evolutionary heritage, each of them merits separate management efforts when it comes to biodiversity conservation [103, 104].

Supporting information

S1 Fig. COX1 (the 3' end; 742 bp) consensus Bayesian tree topology constructed with BEAST. Values on tree branches refer to posterior probabilities in BI for each corresponding node. *P. eumolpe*, *P. indiarum* and *P. peninsulare* were selected as outgroups. Abbreviations: Ph, Philippines; Tw, Taiwan; Ch, China; Jp, Japan; In, Indonesia.

S2 Fig. COX1 (the 3' end; 742 bp) Maximum Likelihood (ML) tree topology constructed with RaxmlGUI. Numbers are bootstrap values after 1000 pseudoreplicates. *P. eumolpe*, *P. indiarum* and *P. peninsulare* were selected as outgroups. Abbreviations: Ph, Philippines; Tw, Taiwan; Ch, China; Jp, Japan; In, Indonesia.

S3 Fig. Maximum parsimony haplotype network of COX1 (the 3' end; 610 bp) for a subset of specimens and sequences recovered from GenBank, constructed with PopART. Hatch marks represent mutation steps.

S1 Table. Material of *Parasesarma* examined for this study with locality, sex (M = male, F = female), size (maximum carapace width in millimeters), museum voucher number and year of collection, DNA extraction number and GenBank (NCBI) Accession number for COX1 (segments of the 5' end and the 3' end, respectively) and 16S rRNA.

S2 Table. Primers used in the present study with corresponding DNA sequences (5´-3´) and references.

S3 Table. Mean pairwise K2P distances (expressed in %) based on 618 bp of the COX1 gene (the 3’ end) between five phylogenetic clades recovered in the present study and calculated with MEGA version X.

Acknowledgments

We appreciate the contribution of Niko Ramisch, who obtained preliminary data during his Diplom thesis work at the University of Regensburg. We thank Richard Landstorfer and Ling Ming Tsang for participation in collecting activities in Hong Kong, Hainan, and the Philippines, which was carried out during an academic exchange project from 2009 to 2010 between the Chinese University of Hong Kong and the University of Regensburg (DAAD project D/09/ 00532). In 2000, CDS collected mangrove crabs in Sulawesi, with the help of Dr. Daisy Wowor and Tse-Ming Leong and funding support from the National University of Singapore during a postdoc employment with Prof. Peter K.L. Ng. We also thank Dwi Listyo Rahayu, Hsi-Te Shih, Daisuke Uyeno and Tohru Naruse for sending additional materials. The latter as well as Roland Melzer and Stefan Friedrich from the Staatsammlung in Munich, Jose C.E Mendoza and Muhammad Dzaki Bin Safaruan from the Lee Kong Chian Natural History Museum in Singapore kindly facilitated new museum accession numbers, while Gustav Paulay from Florida Museum of Natural History loaned important material. The manuscript benefitted greatly
from repeated comments by our esteemed colleague Ling Ming Tsang from the Chinese University of Hong Kong as well as by additional suggestions by two anonymous reviewers and the editor Dr. Benny Chan.

**Author Contributions**

**Conceptualization:** Ka Hou Chu.

**Data curation:** Adnan Shahdadi, Katharina von Wyschetzki, Christoph D. Schubart.

**Formal analysis:** Adnan Shahdadi.

**Funding acquisition:** Ka Hou Chu, Christoph D. Schubart.

**Investigation:** Adnan Shahdadi, Katharina von Wyschetzki, Ka Hou Chu, Christoph D. Schubart.

**Methodology:** Adnan Shahdadi, Katharina von Wyschetzki, Ka Hou Chu, Christoph D. Schubart.

**Project administration:** Christoph D. Schubart.

**Resources:** Hung-Chang Liu, Ka Hou Chu, Christoph D. Schubart.

**Supervision:** Ka Hou Chu, Christoph D. Schubart.

**Validation:** Adnan Shahdadi, Ka Hou Chu.

**Visualization:** Christoph D. Schubart.

**Writing – original draft:** Katharina von Wyschetzki.

**Writing – review & editing:** Adnan Shahdadi, Hung-Chang Liu, Ka Hou Chu, Christoph D. Schubart.

**References**

1. Porter SC. Some geological implications of average Quaternary glacial conditions. Quaternary Research. 1989; 32(3):245–61.

2. Woodruff DS. Biogeography and conservation in Southeast Asia: how 2.7 million years of repeated environmental fluctuations affect today’s patterns and the future of the remaining refugial-phase biodiversity. Biodiversity and Conservation. 2010; 19(4):919–41.

3. Springer VG, Williams JT. Widely distributed Pacific plate endemics and lowered sea-level. Bulletin of Marine Science. 1990; 47(3):631–40.

4. Hewitt G. The genetic legacy of the Quaternary ice ages. Nature. 2000; 405(6879):907–13. [https://doi.org/10.1038/35016000](https://doi.org/10.1038/35016000) PMID: 10879524

5. Barber PH, Erdmann MV, Palumbi SR. Comparative phylogeography of three codistributed stomatopods: origins and timing of regional lineage diversification in the coral triangle. Evolution. 2006; 60(9):1825–39. PMID: 17089967

6. Beck SV, Carvalho GR, Barlow A, Rüber L, Hui Tan H, Nugroho E, et al. Plio-Pleistocene phylogeography of the Southeast Asian blue panchax killifish, Aplocheilus panchax. PLoS One. 2017; 12(7): e0179557. [https://doi.org/10.1371/journal.pone.0179557](https://doi.org/10.1371/journal.pone.0179557) PMID: 28742862

7. Hellberg ME. Gene flow and isolation among populations of marine animals. Annual Review of Ecology, Evolution, and Systematics. 2008; 40:291–310.

8. Dawson MN, Barber PH, González-Guzmán LI, Toonen RJ, Dugan JE, Grosberg RK. Phylogeography of Emerita analoga (Crustacea, Decapoda, Hippidae), an eastern Pacific Ocean sand crab with long-lived pelagic larvae. Journal of Biogeography. 2011; 38(8):1600–12.

9. Borsa P, Durand J-D, Chen W-J, Hubert N, Muths D, Mou-Tham G, et al. Comparative phylogeography of the western Indian Ocean reef fauna. Acta Oecologica. 2016; 72:72–86.
10. Silva IC, Mesquita N, Paula J. Genetic and morphological differentiation of the mangrove crab *Perisesarma guttatum* (Brachyura: Sesarmidae) along an East African latitudinal gradient. *Biological Journal of the Linnean Society*. 2010; 99(1):28–46.

11. Shahdadi A, Davie PJ, Schubart CD. Systematics and phylogeography of the Australasian mangrove crabs *Parasesarma semperi* and *P. longicristatum* (Decapoda: Brachyura: Sesarmidae) based on morphological and molecular data. *Invertebrate Systematics*. 2018; 32(1):196–214.

12. Thurman C, Alber R, Hopkins M, Shih H-T. Morphological and genetic variation among populations of the fiddler crab *Minuca burgersi* (Holthuis, 1967) (Crustacea: Brachyura: Ocypodidae) from shores of the Caribbean Basin and western South Atlantic Ocean. *Zoological Studies*. 2021; 60(19).

13. Grosberg R, Cunningham C. Genetic structure in the sea: From populations to communities. *En: Bertness MD, Gaines SD, Hay ME(Eds.) Marine Community Ecology: 61–84*. *Sinauer Associates, Sunderland, MA, USA; 2001.*

14. Bowen BW, Rocha LA, Toonen RJ, Karl SA. The origins of tropical marine biodiversity. *Trends in Ecology & Evolution*. 2013; 28(6):359–66. [https://doi.org/10.1016/j.tree.2013.01.018 PMID: 23453048](https://doi.org/10.1016/j.tree.2013.01.018 PMID: 23453048)

15. Hongjasril W, Murase A, Miki R, Hastings PA. Journey to the west: trans-Pacific historical biogeography of fringehead blennies in the genus *Neoclinus* (Teleostei: Blenniformes). *Zoological Studies*. 2020; 59(09).

16. Benzie J, Ballment E, Forbes A, Demetriades N, Sugama K, Moria S. Mitochondrial DNA variation in Indo-Pacific populations of the giant tiger prawn, *Peneaus monodon*. *Molecular Ecology*. 2002; 11(12):2553–69. [https://doi.org/10.1046/j.1365-294x.2002.01638.x PMID: 12453239](https://doi.org/10.1046/j.1365-294x.2002.01638.x PMID: 12453239)

17. Briggs JC. Centrifugal speciation and centres of origin. *Journal of Biogeography*. 2000; 27(5):1183–8.

18. Briggs JC. The marine East Indies: diversity and speciation. *Journal of Biogeography*. 2005; 32(9):1517–22.

19. Costello MJ, Coll M, Danovaro R, Halpin P, Ojaveer H, Miloslavich P. A census of marine biodiversity knowledge, resources, and future challenges. *PLoS One*. 2010; 5(8):e12110. [https://doi.org/10.1371/journal.pone.0012110 PMID: 20899850](https://doi.org/10.1371/journal.pone.0012110 PMID: 20899850)

20. Wafar M, Venkataraman K, Ingole B, Khan SA, LokaBharathi P. State of knowledge of coastal and marine biodiversity of Indian Ocean countries. *PLoS One*. 2011; 6(1):e14613. [https://doi.org/10.1371/journal.pone.0014613 PMID: 21297949](https://doi.org/10.1371/journal.pone.0014613 PMID: 21297949)

21. Bowen BW, Gaither MR, DiBattista JD, Iacchei M, Andrews KR, Grant WS, et al. Comparative phylogeography of the ocean planet. *Proceedings of the National Academy of Sciences*. 2016; 113(29):7962–9. [https://doi.org/10.1073/pnas.1602404113 PMID: 27432983](https://doi.org/10.1073/pnas.1602404113 PMID: 27432983)
32. Ma KY, Chow LH, Wong KJ, Chen H-N, Ip BH, Schubart CD, et al. Speciation pattern of the horned ghost crab *Ocypode ceratophthalus* (Pallas, 1772): an evaluation of the drivers of Indo-Pacific marine biodiversity using a widely distributed species (vol 45, pg 2658, 2018). Journal of Biogeography. 2019; 46(4):830.

33. Ragionieri L, Fratini S, Vannini M, Schubart CD. Phylogenetic and morphometric differentiation reveal geographic radiation and pseudo-cryptic speciation in a mangrove crab from the Indo-West Pacific. Molecular Phylogenetics and Evolution. 2009; 52(3):825–34. https://doi.org/10.1016/j.ympev.2009.04.008 PMID: 19394431

34. Ragionieri L, Fratini S, Schubart CD. Revision of the *Neosarmatium meinerti* species complex (Decapoda: Brachyura: Sesarmidae), with descriptions of three pseudocryptic Indo-West Pacific species. Raffles Bulletin of Zoology. 2012; 60(1).

35. Shahdadi A, Davie PJ, Rahayu DL, Schubart CD. The synonymy of *Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research. 1994; 22(22):4673–80. https://doi.org/10.1093/nar/22.22.4673 PMID: 7984417

36. Shahdadi A, Fratini S, Schubart CD. Taxonomic reassessment of *Parasesarma* (Crustacea: Brachyura: Decapoda: Sesarmidae) based on genetic and morphological comparisons, with the description of a new genus. Zoological Journal of the Linnean Society. 2020; 190(4):123–58.

37. Benzie J. Genetic structure of marine organisms and SE Asian biogeography. Biogeography and Geological Evolution of SE Asia. 1998; 30:197–209.

38. Roberts CM, McClean CJ, Veron JE, Hawkins JP, Allen GR, McAllister DE, et al. Marine biodiversity hotspots and conservation priorities for tropical reefs. Science. 2002; 295(5558):1280–4. https://doi.org/10.1126/science.1067728 PMID: 11847338

39. Barber PH, Bellwood DR. Biodiversity hotspots: evolutionary origins of biodiversity in wrasses (Hali-....

40. Barber PH, Bellwood DR. Biodiversity hotspots: evolutionary origins of biodiversity in wrasses (Hali-....

41. Xu J, Chan T-Y, Tsang LM, Chu KH. Phylogeography of the mitten crab *Eriocheir* sensu stricto in East Asia: Pleistocene isolation, population expansion and secondary contact. Molecular Phylogenetics and Evolution. 2009; 52(1):45–56. https://doi.org/10.1016/j.ympev.2009.02.007 PMID: 19236929

42. Cheng J, Wang Z, Song N, Yanagimoto T, Gao T. Phylogeographic analysis of the genus *Platycepha-....

43. Haan HMD. Crustacea. In: von Siebold P. F., P.F., Fauna Japonica sive Descrip-....

44. Guo X, Zhao D, Jung D, Li Q, Kong L-F, Ni G, et al. Phylogeography of the rock shell *Thais clavigera* (Mollusca): evidence for long-distance dispersal in the northwestern Pacific. PloS One. 2015; 10(7): e0129715. https://doi.org/10.1371/journal.pone.0129715 PMID: 26171966

45. Haan HMD. Crustacea. In: von Siebold P. F., P.F., Fauna Japonica sive Descriptive Animalium, quae in Itinere per Japoniam, Jussu et Auspiciis Superiorum, qui Summum in India Batava Imperium Tenent, Suspecto, Annis 1823–1830 Collegit, Notis, Observationibus et Adumbrationibus Illustravit. Leiden, the Netherlands: Published by the author; 1833–1850. 243 p.

46. Dai AY, Yang SL, Song YZ, Chen GX. Crabs of the China Seas. Beijing: China Ocean Press (Chinese edition); Berlin, Heidelberg, New York, Tokyo: Springer (English edition).1991. 682 p.

47. Zhou H, Xu J, Yang M, Wu B, Yan B, Xiong Y. Population genetic diversity of sesarmid crab (*Perisesarma bidens*) in China based on mitochondrial DNA. Mitochondrial DNA Part A. 2016; 27(5):3255–62. https://doi.org/10.3109/19401736.2015.1015002 PMID: 25693695

48. Rahayu DL, Davie PJ. Two new species and a new record of *Perisesarma* (Decapoda, Brachyura, Grapsidae, Sesarmidae) from Indonesia. Crustaceana. 2002;579–607.

49. Li J-J, Shih H-T, Ng PKL. Three new species and two new records of *Parasesarma* De Man, 1895 (Crustacea: Brachyura: Sesarmidae) from Taiwan and the Philippines from morphological and molecular evidence. Zoological Studies. 2019;58.

50. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research. 1994; 22(22):4673–80. https://doi.org/10.1093/nar/22.22.4673 PMID: 7984417
51. Hall TA, editor. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In: Nucleic Acids Symposium Series; 1999. [London]: Information Retrieval Ltd.; c1979–c2000.

52. Silvestro D, Michalak I. raxmlGUI: a graphical front-end for RAxML. Organisms Diversity & Evolution. 2012; 12(4):335–7.

53. Bouckaert R, Vaughan TG, Barido-Sottani J, Duchêne S, Fourment M, Gavryushkina A, et al. BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. PLoS Computational Biology. 2019; 15(4):e1006650. https://doi.org/10.1371/journal.pcbi.1006650 PMID: 30958812

54. Schubart CD, Diesel R, Hedges SB. Rapid evolution to terrestrial life in Jamaican crabs. Nature. 1998; 393(6683):363–5.

55. Rodriguez F, Oliver JL, Marín A, Medina JR. The general stochastic model of nucleotide substitution. Journal of Theoretical Biology. 1990; 142(4):485–501. https://doi.org/10.1016/0022-5193(05)80104-3 PMID: 2338834

56. Posada D, Buckley TR. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. Systematic Biology. 2004; 53(5):793–808. https://doi.org/10.1080/10635150490522304 PMID: 15545256

57. Shahdhadi A, Schubart CD, Mendoza JCE. Conspicuous genetic similarity within a widely distributed and newly described species of Parasesarma De Man, 1895 from Western Pacific oceanic islands, with notes on the allied P. calypso group (Crustacea: Brachyura: Sesarmidae). Invertebrate Systematics. 2021; 35(5):542–69.

58. Templeton AR, Crandall KA, Sing CF. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics. 1992; 132(2):619–33. https://doi.org/10.1093/ genetics/132.2.619 PMID: 1385265

59. Leigh JW, Bryant D. popart: full-feature software for haplotype network construction. Methods in Ecology and Evolution. 2015; 6(9):1110–6.

60. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution. 2018; 35(6):1547. https://doi.org/10.1093/molbev/m sy096 PMID: 29722887

61. Librado P, Rozas J. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics. 2009; 25(11):1451–2. https://doi.org/10.1093/bioinformatics/btp187 PMID: 19346325

62. Fu Y-X. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics. 1997; 147(2):915–25. https://doi.org/10.1093/genetics/147.2.915 PMID: 9335623

63. Excoffier L, Lischer HE. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources. 2010; 10(3):564–7. https://doi.org/10.1111/j.1755-0998.2010.02847.x PMID: 21565059

64. Rogers AR, Harpending H. Population growth makes waves in the distribution of pairwise genetic differences. Molecular Biology and Evolution. 1992; 9(3):552–69. https://doi.org/10.1093/oxfordjournals.molbev.a040727 PMID: 1316531

65. Carlberg C. Statistical analysis: Microsoft excel 2013. Indianapolis: Que Publishing; 2014.

66. Harpending H. Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. Human Biology. 1994;591–600. PMID: 8088750

67. Richmond GM, Fullerton DS. Summation of Quaternary glaciations in the United States of America. Quaternary Science Reviews. 1986; 5:183–96.

68. Murray-Wallace CV, Woodroffe CD. Quaternary sea-level changes: a global perspective. Cambridge University Press; 2014.

69. Voris HK. Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. Journal of Biogeography. 2000; 27(5):1153–67.

70. Marko PB, Hoffman JM, Emme SA, McGovern TM, Keever CC, Nicole Cox L. The 'Expansion–Contraction'model of Pleistocene biogeography: rocky shores suffer a sea change? Molecular Ecology. 2010; 19(1):146–69. https://doi.org/10.1111/j.1365-294x.2009.04417.x PMID: 20092033

71. Avise J. Phylogeography: the history and formation of species. Cambridge University press; 2000.

72. Tsang LM, Chan BKK, Wu TH, Ng WC, Chatterjee T, Williams GA, et al. Population differentiation in the barnacle Chthamalus malayensis: postglacial colonization and recent connectivity across the Pacific and Indian Oceans. Marine Ecology Progress Series. 2008; 364:107–18.

73. Knowlton N. Sibling species in the sea. Annual Review of Ecology and Systematics. 1993; 24(1):189–216.
74. Knowlton N, Weigt LA. New dates and new rates for divergence across the Isthmus of Panama. Proceedings of the Royal Society of London Series B: Biological Sciences. 1998; 265(1412):2257–63.

75. Papadopoulos A, Anastasiou I, Vogler AP. Revisiting the insect mitochondrial molecular clock: the mid-Aegean trench calibration. Molecular Biology and Evolution. 2010; 27(7):1659–72. https://doi.org/10.1093/molbev/msq051 PMID: 20167609

76. Wang J, Tsang LM, Dong Y-W. Causations of phylogeographic barrier of some rocky shore species along the Chinese coastline. BMC Evolutionary Biology. 2015; 15(1):1–15. https://doi.org/10.1186/s12862-015-0387-0 PMID: 26071894

77. Wu TH, Tsang LM, Chan BKK, Chu KH. Cryptic diversity and phylogeography of the island-associated barnacle Chthamalus moro in Asia. Marine Ecology. 2015; 36(3):368–78.

78. Kinnian BP, Gaines SD, Lester SE. Propagule dispersal and the scales of marine community process. Diversity and Distributions. 2005; 11(2):139–48.

79. Horne JB, van Herwerden L, Chat JH, Robertson DR. High population connectivity across the Indo-Pacific: congruent lack of phylogeographic structure in three reef fish congeners. Molecular Phylogenetics and Evolution. 2008; 49(2):629–38. https://doi.org/10.1016/j.ympev.2008.08.023 PMID: 18804542

80. Chang YW, Chan JS, Hayashi R, Shuto T, Tsang LM, Chu KH, et al. Genetic differentiation of the soft barnacle Fistulabalanus albicostatus (Cirripedia: Thoracica: Balanomorpha) in the West Pacific. Marine Ecology. 2017; 38(2):e12422.

81. Shahdadi A, Schubart CD. Taxonomic review of Perisesarma (Decapoda: Brachyura: Sesarmidae) and closely related genera based on morphology and molecular phylogenetics: new classification, two new genera and the questionable phylogenetic value of the epibranchial tooth. Zoological Journal of the Linnean Society. 2017; 182(3):517–48.

82. Shahdadi A, Schubart CD, Mendoza JCE. On the occurrence of Parasesarma cricotum (Rahayu and Davie, 2002 (Decapoda, Brachyura, Sesarmidae) in Palau and Sulawesi. Zootaxa. 2020; 4803(2):zootaxa. 4803.2.12-zootaxa..2.12.

83. Ng PKL, Shih HT, Ho PH, Wang CH. An updated annotated checklist of brachyuran crabs from Taiwan (Crustacea: Decapoda). Journal of National Taiwan Museum. 2017; 70:1–208.

84. Tsang LM, Chan BKK, Williams GA, Chu KH. Who is moving where? Molecular evidence reveals patterns of range shift in the acorn barnacle Hexechamaesipho pilsbryi in Asia. Marine Ecology Progress Series. 2013; 488:187–200.

85. Carpenter KE, Springer VG. The center of the center of marine shore fish biodiversity: the Philippine Islands. Environmental Biology of Fishes. 2005; 72(4):467–80.

86. Carpenter KE, Barber PH, Crandall ED, Ablan-Lagman M, Carmen A, Mahardika GN, et al. Comparative phylogeography of the Coral Triangle and implications for marine management. Journal of Marine Biology. 2011; 2011.

87. Kim SY, Yi CH, Kim JM, Choi WY, Kim HS, Kim M-S. DNA Barcoding of the marine protected species Parasesarma bidens (Decapoda: Sesarmidae) from the Korean waters. Animal Systematics, Evolution and Diversity. 2020; 36(2):159–63.

88. Tsang LM, Chan BKK, Ma KY, Chu KH. Genetic differentiation, hybridization and adaptive divergence in two subspecies of the acorn barnacle Tetractilia japonica in the northwestern Pacific. Molecular Ecology. 2008; 17(18):4151–63. https://doi.org/10.1111/j.1365-294x.2008.03907.x PMID: 19238711

89. Wong KJ, Chan BKK, Shih H-T. Taxonomy of the sand bubbler crabs Scopimera globosa De Haan, 1835, and S. tuberculata Stimpson, 1858 (Crustacea: Decapoda: Dotillidae) in East Asia, with description of a new species from the Ryukyus, Japan. Zootaxa. 2010; 2345(1):43–59.

90. Chen W, Hong W, Chen S, Wang Q, Zhang Q. Population genetic structure and demographic history of the mudskipper Boleophthalmus pectinirostris on the northwestern Pacific coast. Environmental Biology of Fishes. 2015; 98(3):845–56.

91. Cheang CC, Chu KH, Ang J, Put O. Morphological and genetic variation in the populations of Sargassum hemiphyllum (phaeophyceae) in the northwestern Pacific. Journal of Phycology. 2008; 44(4):855–65. https://doi.org/10.1111/j.1529-8817.2008.00532.x PMID: 27041602

92. Dong Y-W, Wang H-S, Han G-D, Ke C-H, Zhan X, Nakano T, et al. The impact of Yangtze River discharge, ocean currents and historical events on the biogeographic pattern of Cellana toreuma along the China coast. PLoS One. 2012; 7(4):e36178. https://doi.org/10.1371/journal.pone.0036178 PMID: 22563446

93. Laurenzano C, Costa TM, Schubart CD. Contrasting patterns of clinal genetic diversity and potential colonization pathways in two species of western Atlantic fiddler crabs. PLoS One. 2016; 11(11):e0166518. https://doi.org/10.1371/journal.pone.0166518 PMID: 27861598
94. Rocha LA. Patterns of distribution and processes of speciation in Brazilian reef fishes. Journal of Biogeography. 2003; 30(8):1161–71.

95. Chan BKK, Tsang L, Chu K. Morphological and genetic differentiation of the acorn barnacle *Tetraclita squamosa* (Crustacea, Cirripedia) in East Asia and description of a new species of *Tetraclita*. Zoologica Scripta. 2007; 36(1):79–91.

96. Shih H-T, Kamrani E, Davie PJ, Liu M-Y. Genetic evidence for the recognition of two fiddler crabs, *Uca iranica* and *U. albimana* (Crustacea: Brachyura: Ocypodidae), from the northwestern Indian Ocean, with notes on the *U. lactea* species-complex. Hydrobiologia. 2009; 635(1):373–82.

97. Shih H-T, Hsu J-W, Wong KJ, Ng NK. Review of the mudflat varunid crab genus *Metaplax* (Crustacea, Brachyura, Varuniidae) from East Asia and northern Vietnam. ZooKeys. 2019; 877:1. https://doi.org/10.3897/zookeys.877.38300 PMID: 31616202

98. Xu S, Song N, Zhao L, Cai S, Han Z, Gao T. Genomic evidence for local adaptation in the ovoviviparous marine fish *Sebastiscus marmoratus* with a background of population homogeneity. Scientific Reports. 2017; 7(1):1–12. https://doi.org/10.1038/s41598-016-0028-x PMID: 28127051

99. Kwok P-W. The ecology of two sesarmine crabs, *Perisesarma bidens* (De Haan) and *Parasesarma plicata* (Latreille) at the Mai Po Marshes Nature Reserve, Hong Kong. Hong Kong: The University of Hong Kong; 1995.

100. Islam MS, Shokita S. Larval development of a mangrove crab (*Perisesarma bidens* De Haan) (Crustacea: Brachyura: Sesarminae). Bangladesh Journal of Fisheries Research. 2000; 4(1):43–56.

101. Poettinger T, Schubart CD. Molecular diversity of freshwater crabs from Sulawesi and the sequential colonization of ancient lakes. Hydrobiologia. 2014; 739(1):73–84.

102. Copilaş-Ciocia D, Petrušek A. The southwestern Carpathians as an ancient centre of diversity of freshwater gammarid amphipods: insights from the *Gammarus fossarum* species complex. Molecular Ecology. 2015; 24(15):3980–92. https://doi.org/10.1111/mec.13286 PMID: 26096651

103. Ryder OA. Species conservation and systematics: the dilemma of subspecies. Trends in Ecology & Evolution 1986; 1:9–10.

104. Crandall KA, Bininda-Emonds OR, Mace GM, Wayne RK. Considering evolutionary processes in conservation biology. Trends in Ecology & Evolution. 2000; 15(7):290–5. https://doi.org/10.1016/s0169-5347(00)01876-0 PMID: 10856956