

# Tethyan closure drove tropical marine biodiversity: Vicariant diversification of intertidal crustaceans

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## Funding information

National Natural Science Foundation of China, Grant Number: NSFC-31422048/31372156/31772417/31530067; Southeast Asia Biodiversity Research Institute; Chinese Academy of Sciences, Grant Number: Y4ZK111B01

Editor: Jonathan Waters

## Abstract

**Aim:** The mid-Miocene closure of the Tethys (Terminal Tethyan Event, TTE) induced by the collision of the African–Arabian plate with the Eurasian plate is considered to be a major driver of tropical marine biodiversity. We used intertidal talitrids of *Talorchestia sensu lato* to test the hypothesis that the TTE can best explain the current disjunct distribution of this genus along Mediterranean–East Atlantic and Indo–West Pacific coastlines.

**Location:** Mediterranean–East Atlantic and Indo–West Pacific.

**Methods:** We sequenced four nuclear and two mitochondrial loci for 87 samples across the Mediterranean–East Atlantic to Indo–Pacific areas. We examined phylogenetic relationships, estimated divergence times and reconstructed ancestral geographical distributions of *Talorchestia sensu lato* within a wide geographical context.

**Results:** The phylogenetic analyses revealed that *Talorchestia sensu lato* is monophyletic. Divergence time estimation and ancestral range reconstruction show that the genus originated from the Tethys during the Miocene and split into Mediterranean–East Atlantic and Indo–West Pacific lineages c. 15 Ma. The Mediterranean–East Atlantic lineage further diverged into west and east Mediterranean clades, and the Indo–West Pacific lineage contains Indian Ocean and west Pacific clades.

**Main conclusions:** The evolutionary history of *Talorchestia sensu lato* corresponds well with the geological history of Tethyan closure, suggesting that the Tethyan closure drove the vicariant diversification of *Talorchestia sensu lato* into Mediterranean–East Atlantic and Indo–West Pacific lineages. This indicates that the intertidal environment may be the last to be separated by the TTE, and the diversification pattern of intertidal crustaceans can best reflect the vicariance effect of Tethyan closure. Deep divergences within clades were related to geological isolation in the Mediterranean–East Atlantic region and across the Indo–Pacific boundary.

## KEYWORDS

Amphipoda, biogeography, disjunct distribution, evolution, Indo–West Pacific, Mediterranean–East Atlantic

## 1 | INTRODUCTION

The closure of the Tethys Seaway (Terminal Tethyan Event, TTE) in the mid-Miocene (18–12 Ma) connected Eurasia and Africa and separated the Atlantic Ocean from the Indian Ocean (Adams, Gentry, & Whybrow, 1983). The historical process associated with TTE has been identified as a key driver in tropical marine biodiversity (Cowman & Bellwood, 2013; Floeter et al., 2008; Harzhauser et al., 2007; Malaquias & Reid, 2009; Portik & Papenfuss, 2015), as best demonstrated in the fossil record (Renema et al., 2008). The TTE, preceded by the northward movement of the African–Arabian plates and simultaneous counter clockwise rotation of the Arabian plate, formed the so-called *Gomphotherium* land bridge which permitted terrestrial interchange between Eurasia and Africa during the early Miocene (Harzhauser et al., 2007; Kapli et al., 2015). Concurrently, the TTE also caused the emergence of geographical barriers for marine species, fragmenting previously continuous Tethyan distribution ranges into Mediterranean–East Atlantic (MS–EA) and Indo–West Pacific (IWP) and leading to vicariant speciation (Hou & Li, 2017). Despite the broad importance of the final closure, its effect on marine biodiversity during this complex and prolonged process remains contentious, and the temporal patterns of vicariant events associated with TTE among various tropical marine taxa are poorly studied (Floeter et al., 2008). Only limited biological evidence supports the geological date of the closure and directly reflects the physical barrier of the Tethyan closure (Barber & Bellwood, 2005; Bellwood, van Herwerden, & Konow, 2004; Cowman & Bellwood, 2013). Therefore, an appropriate choice of organism in which to address these questions is one whose evolutionary history is expected to parallel the geological development of the TTE. Recent research suggests that the phylogeography of Amphipoda taxa often reflects historical events of geological nature (Bauzà-Ribot et al., 2012; Copilaş-Ciocianu & Petrussek, 2017; Grabowski, Mamos, Băcela-Spychalska, Rewicz, & Wattier, 2017; Hou, Sket, Fiser, & Li, 2011). *Talorchestia sensu lato* talitrids (Crustacea, Amphipoda) may serve as an excellent model group in which to explore the historical biogeography of the TTE, given the animal's poor overland dispersal ability as well as broad distribution in both MS–EA and IWP.

The genus *Talorchestia sensu lato* was described as an assemblage that includes *Africorchestia* from western Africa and south-western Europe (Lowry & Coleman, 2011), *Britorchestia*, *Deshayesorchestia* and *Sardorchestia* from western Europe, the Mediterranean Sea and the East Atlantic (Lowry & Bopiah, 2013; Tafani, Ugolini, Bazzicalupo, Mengoni, & Ruffo, 2004), and *Talorchestia sensu stricto* with a broad range throughout the western Pacific Ocean and across the northern Indian Ocean to the Persian Gulf (Lowry & Momtazi, 2015). All species of *Talorchestia sensu lato* inhabit intertidal niches, the area between tide marks, which were likely to be the last marine habitats separated by the TTE. The current disjunct intertidal distribution of *Talorchestia sensu lato* on the eastern and western sides of the Arabian plate indicates that the evolutionary history of this group may be an accurate marker of past geological events, such as the TTE.

The Tethys Seaway between Eurasia and Africa began narrowing and shallowing at the boundary of the Oligocene and Miocene with African rifting and the opening of the Red Sea (Chorowicz, 2005; Dibattista & Bowen, 2013). The progressive continental collision resulted in the formation of the *Gomphotherium* land bridge at 18–16 Ma (Popov et al., 2004; Rögl, 1998), which disrupted the exchange between MS–EA and IWP. By 12 Ma, the Tethys closure was nearly complete, driven by the uplift of the Zagros Mountains (Mouthereau, Lacombe, & Vergés, 2012), and the climate became dryer over the Arabian plate and north-eastern Africa (Ramstein, Fluteau, Besse, & Joussaume, 1997). The Tethyan closure and associated climatic changes promoted further vicariance for marine and coastal faunas. Based on this scenario, we hypothesize that the diversification pattern of *Talorchestia sensu lato* may be revealed via congruence of the divergence time estimates with the TTE: (1) pre-TTE hypothesis (Figure 1a), an earlier divergence between MS–EA and IWP than the geological date of TTE, (2) TTE hypothesis (Figure 1b), compatible timing with TTE and (3) post-TTE hypothesis (Figure 1c), dispersal occurring after the TTE between MS–EA and IWP.

Here, we test whether TTE vicariance can explain the present disjunct distribution of *Talorchestia sensu lato* along MS–EA and IWP coastlines. We reconstructed a phylogeny using nuclear and mitochondrial DNA (mtDNA) sequence data. We further estimated the divergence times of major lineages and conducted a biogeographic analysis to assess the spatiotemporal diversification mode associated with the TTE.

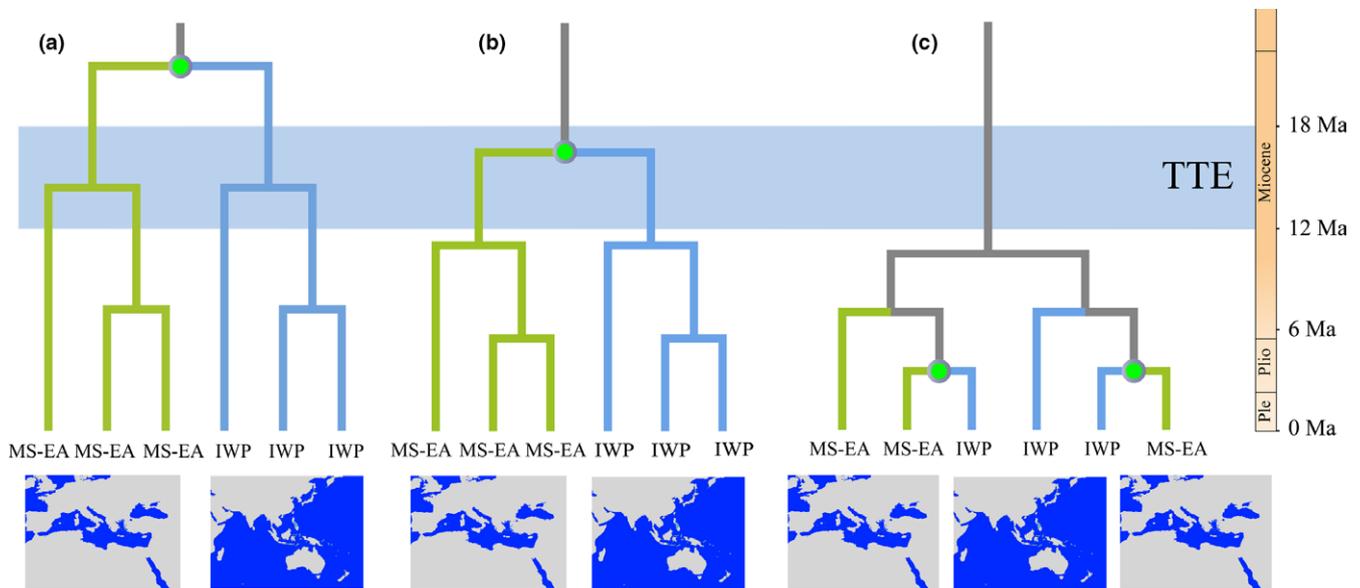
## 2 | MATERIALS AND METHODS

### 2.1 | Sample collection

Samples of *Talorchestia sensu lato* were collected from 39 sites covering most of the known distribution along the East Atlantic coastline, Mediterranean Basin and IWP intertidal zones. A total of 87 specimens of eight species were examined, including *T. martensii*, *T. mindorensis*, *T. qeshm*, *T. spinipalma*, *T. ugolinii* (also known as *Britorchestia ugolinii*), *T. deshayesii* (as *Deshayesorchestia deshayesii*), *T. pelecaniformis* (as *Sardorchestia pelecaniformis*), *T. triconuta* (as *Africorchestia triconuta*) and four undescribed species with distinct morphology. Two species from the genus *Platorchestia* (*P. japonica* and *P. pacifica*) and one species from *Talitroides* (*T. topitotum*) were collected as outgroups. Voucher specimens were deposited in the Institute of Zoology, Chinese Academy of Sciences (IZCAS). Detailed sample information is presented in the supplementary material, Table S1.

### 2.2 | Molecular techniques and phylogenetic analysis

Total genomic DNA was extracted from the heads of specimens using the Tiangen Genomic DNA kit. Two mitochondrial fragments



**FIGURE 1** Vicariant hypothesis for the genus *Talorchestia sensu lato* between Indo-West Pacific (IWP) and Mediterranean Sea-East Atlantic (MS-EA) regions. Green circles indicate divergence of major clades. Blue branches represent IWP lineages, and green branches indicate MS-EA lineages. The horizontal blue bar represents the timing of the TTE (Terminal Tethyan Event, 18–12 Ma). (a) pre-TTE hypothesis; (b) TTE hypothesis; (c) post-TTE hypothesis

of cytochrome oxidase subunit I (COI) and 16S, and four nuclear regions of 18S rRNA, 28S rRNA, histone 3 (H3) and sodium-potassium ATPase  $\alpha$ -subunit (NaK) were amplified with annealing temperatures optimized at 45–50°C. Details on primers are provided in supplementary material Table S2, and laboratory protocols were performed as in previous studies (Hou, Fu, & Li, 2007; Hou et al., 2011). Sixteen additional sequences for *T. martensii*, *T. mindorensis* and *T. deshayesii* were acquired from GenBank (Table S1).

All newly generated sequences were proofed and edited using SEQUENCHER 4.5 (demo edition, Gene Codes Corporation). Sequences were aligned using MAFFT 7 with default parameters (<http://mafft.cbrc.jp/alignment/server/>). Protein-coding gene sequences were translated to amino acids in MEGA 7.0.21 (Kumar, Stecher, & Tamura, 2016) to check for the presence of pseudogenes. The COI sequences were translated using the invertebrate mitochondrial genetic code, while H3 and NaK fragments were translated using the standard genetic code. Ribosomal RNA genes were realigned to check for ambiguous regions according to the guide tree generated by the protein-coding genes in MEGA. The number of variable sites and parsimony informative sites was calculated using MEGA. The COI, 16S, 28S, 18S, H3 and NaK sequences were concatenated using SEQUENCEMATRIX 1.7.8 (Vaidya, Lohman, & Meier, 2011).

The best-fit partitioning scheme and nucleotide substitution models were selected using PARTITIONFINDER 1.1.1 (Lanfear, Calcott, Ho, & Guindon, 2012) using the Akaike information criterion (AIC) (Table S3). Phylogenetic relationships were inferred using maximum likelihood (ML) and Bayesian inference (BI) for single and concatenated gene datasets. ML analysis was implemented with RAXML 8.2.9 (Stamatakis, 2014), starting with 1000 rapid bootstrap replicates followed by a thorough optimization of the best-scoring ML tree. The GTRGAMMA model of rate heterogeneity was applied. Bayesian

analysis was conducted using MRBAYES 3.2.2 (Ronquist et al., 2012), with the best-fit substitution model. Two independent runs were carried out with four Monte Carlo Markov chains (MCMCs) for 20 million generations, sampling every 1,000 generations with a burn-in of 25%. Convergence of the runs was investigated by the standard deviation of split frequencies (<0.01), and effective sample size value for each parameter was checked with TRACER 1.6 (Rambaut & Drummond, 2014). The last 15,000 trees were used to construct a majority consensus tree and estimate the Bayesian posterior probabilities. Both ML and BI analyses found no conflict among well-supported nodes for single and concatenated gene datasets; therefore, the six genes were combined for the following analyses.

### 2.3 | Divergence time estimation

To specify a designation of taxonomic units when estimating ages of divergence events, single-threshold general mixed Yule coalescent (GMYC) analyses (Pons et al., 2006) were optimized using the 'SPLITS' package (Ezard, Fujisawa, & Barraclough, 2009) in R 3.3.2 (R Core Development Team 2014). An ultrametric tree was constructed under an uncorrelated lognormal relaxed molecular clock model using BEAST 2.4.3 (Bouckaert et al., 2014), with COI and 16S sequences concatenated as a single locus. The clock rate was set to one, and a coalescent constant prior was used to invoke the GMYC null model. We ran two independent analyses of 30 million generations each, sampling every 1,000 generations; results were combined using LOGCOMBINER 2.4.3 (Bouckaert et al., 2014), with the first 25% of each run discarded as burn-in. The stationarity of each run was examined using TRACER with the effective sampling size of each parameter (ESS > 200).

Due to the scarcity of palaeontological records for talitrids, we used two methods to estimate divergence times. First, the

divergence times were inferred under a gene tree framework from the full mtDNA data COI+16S in BEAST, with unlinked substitution models for COI and 16S. The Yule process was used for the tree prior model. The hypothesis that our data evolved according to a strict molecular clock was rejected by a likelihood ratio test using MRBAYES ( $\ln L_{\text{noclock}} = -13177.82$ ,  $\ln L_{\text{clock}} = -13337.36$ ,  $df = 100$ ,  $p < .001$ ). The mitochondrial clock of 0.0115 substitutions per site per million years was applied as a calibration under an uncorrelated lognormal relaxed molecular clock model (Brower, 1994). This rate has been proven to give the same results in comparison with calibrations based on biogeological events for time estimation of amphipods (Copilaş-Ciocianu & Petrussek, 2015). It has been widely used in linking contemporary diversification patterns to historical process in several amphipod phylogenetic studies (Copilaş-Ciocianu & Petrussek, 2015, 2017; Grabowski et al., 2017; Hou, Li, & Li, 2014; Yang, Hou, & Li, 2013). Considering the tip node variation in topologies between mtDNA and concatenated datasets, we constrained the topology to that generated from ML analysis of the combined mitochondrial and nuclear DNA. Two independent searches of 300 million generations were conducted, sampling every 5,000 generations with the first 25% of samples discarded as burn-in. Acceptable convergence to the stationary distribution was assessed by inspecting posterior samples using TRACER. Because all runs produced the same topology, we combined the independent runs using LOGCOMBINER.

Node ages derived from mtDNA gene trees are expected to overestimate lineage splitting times in contrast to multilocus coalescent approaches (McCormack, Heled, Delaney, Peterson, & Knowles, 2011). We therefore used a Bayesian framework for species tree estimation in the program \*BEAST to obtain divergence times. The analysis will co-estimate gene trees embedded in a shared species tree, based on a multispecies multilocus coalescent model (Heled & Drummond, 2010). The entities delimited with GMYC were designated as taxonomic priors. The dataset was partitioned into mtDNA and nuclear loci, unlinking substitution models, clock models and tree models. As described earlier, we used an uncorrelated lognormal relaxed clock model, with a substitution rate of 0.0115 as the informative prior for mtDNA. For the nuclear partition, we could not find a suitable substitution rate from the literature; however, a classic rule of thumb says it is one order of magnitudes lower than mitochondrial rate (Opatova & Arnedo, 2014; Xu et al., 2015). Therefore, we set a rate of 0.00115 for the nuclear partition with an interval of 0.0001 to 0.0115 under an uncorrelated lognormal relaxed molecular clock model. We applied the Yule tree prior with birth rate variation following a lognormal distribution with a mean of 1 and standard deviation of 0.15. Two independent runs of 200 million generations were conducted, sampling every 5,000 generations and discarding the first 25% as burn-in. A maximum clade credibility tree with median node ages was calculated in TREEANNOTATOR 2.4.3 (Bouckaert et al., 2014).

To test the sensitivity of uneven taxon sampling on time estimation, a pruned dataset consisting of 26 ingroup taxa was used to estimate divergence times, of which one specimen with the least missing data was selected for all unique entities according to the

GMYC delimitations (Figure S2). All remaining prior settings in BEAST were identical to the full data analysis. Moreover, we compared the fast evolving rate for the molecular clock to determine concordance with the TTE, such as 0.0177 which was widely used for arthropods (Papadopoulou, Anastasiou, & Vogler, 2010) and 0.0329 which was inferred for stomatopod crustaceans (Crandall, Sbrocco, DeBoer, Barber, & Carpenter, 2012). Further details on molecular clock rates and dating schemes are given in Table S4.

## 2.4 | Ancestral area reconstruction

To infer the ancestral ranges of *Talorchestia sensu lato*, we used the DEC and DEC+J models implemented in the R package 'BioGeoBEARS' (Matzke, 2013). The DEC model estimates two free parameters describing anagenesis:  $d$ , the rate of dispersal and  $e$ , the rate of extinction. It describes the temporal change in the range of a species and distinguishes anagenetic change from cladogenetic change (Yuan et al., 2016). The DEC+J model includes a third parameter,  $j$ . This additional parameter permits one daughter lineage to disperse to a different area from the direct ancestral range, thereby effectively mimicking the process of founder event speciation (Matzke, 2014). We reconstructed ancestral areas under the DEC and DEC+J models and compared the fit of the two models by evaluating AIC and likelihood values.

We performed the ancestral range reconstruction based on the time-calibrated species tree from \*BEAST, allowing dispersal between all regions at any time. The distributions were categorized into four marine biogeographic ecoregions following the classification proposed by Spalding et al. (2007), western Pacific (P, including South-east Asia and Fiji), Indian Ocean (I, including Indian coast area and Madagascar), east Mediterranean region (E, mainly Balkan Peninsula) and west Mediterranean-East Atlantic region (W, including west Africa and Mediterranean coast in west of Balkans).

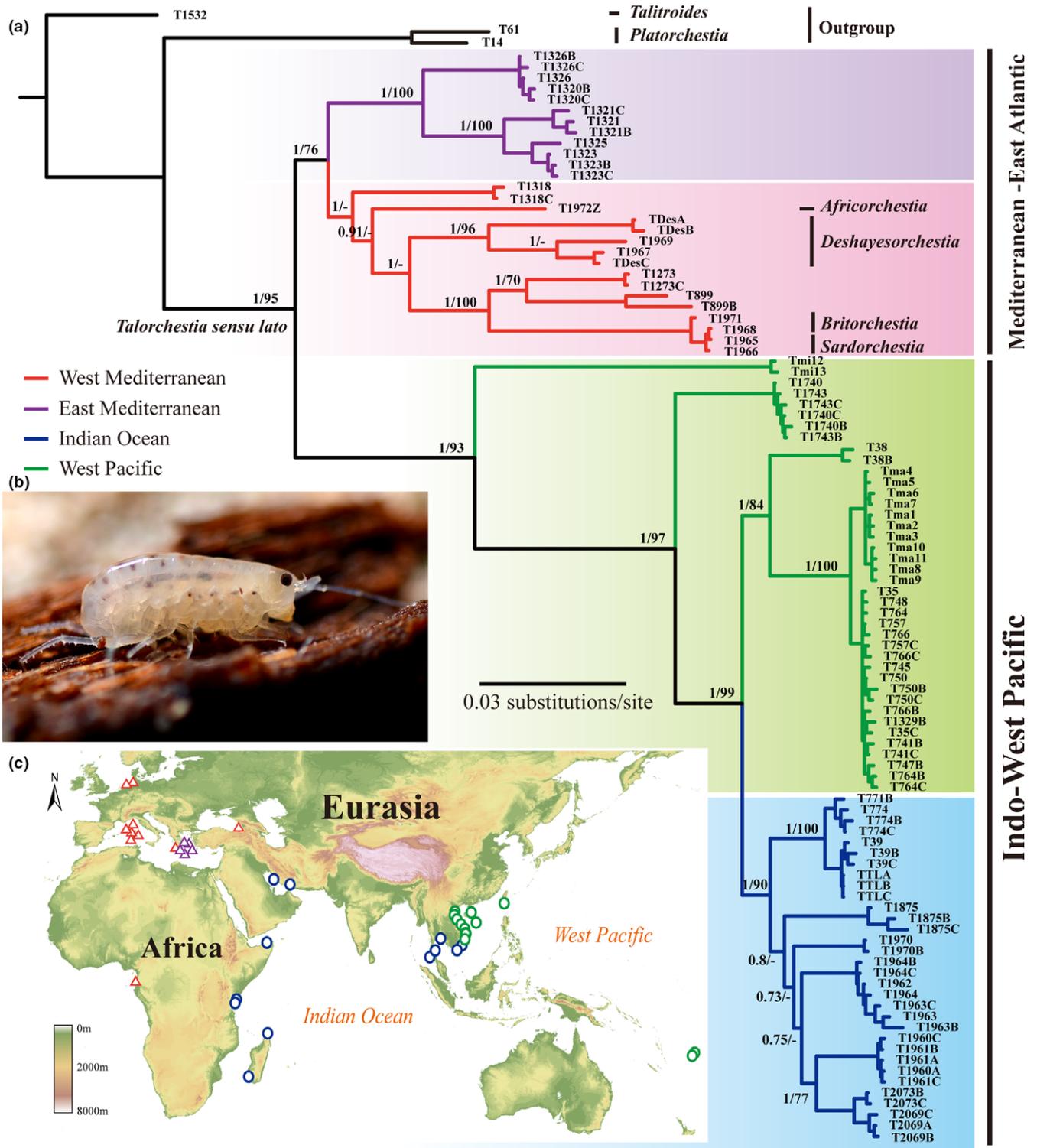
## 3 | RESULTS

### 3.1 | Data characteristics

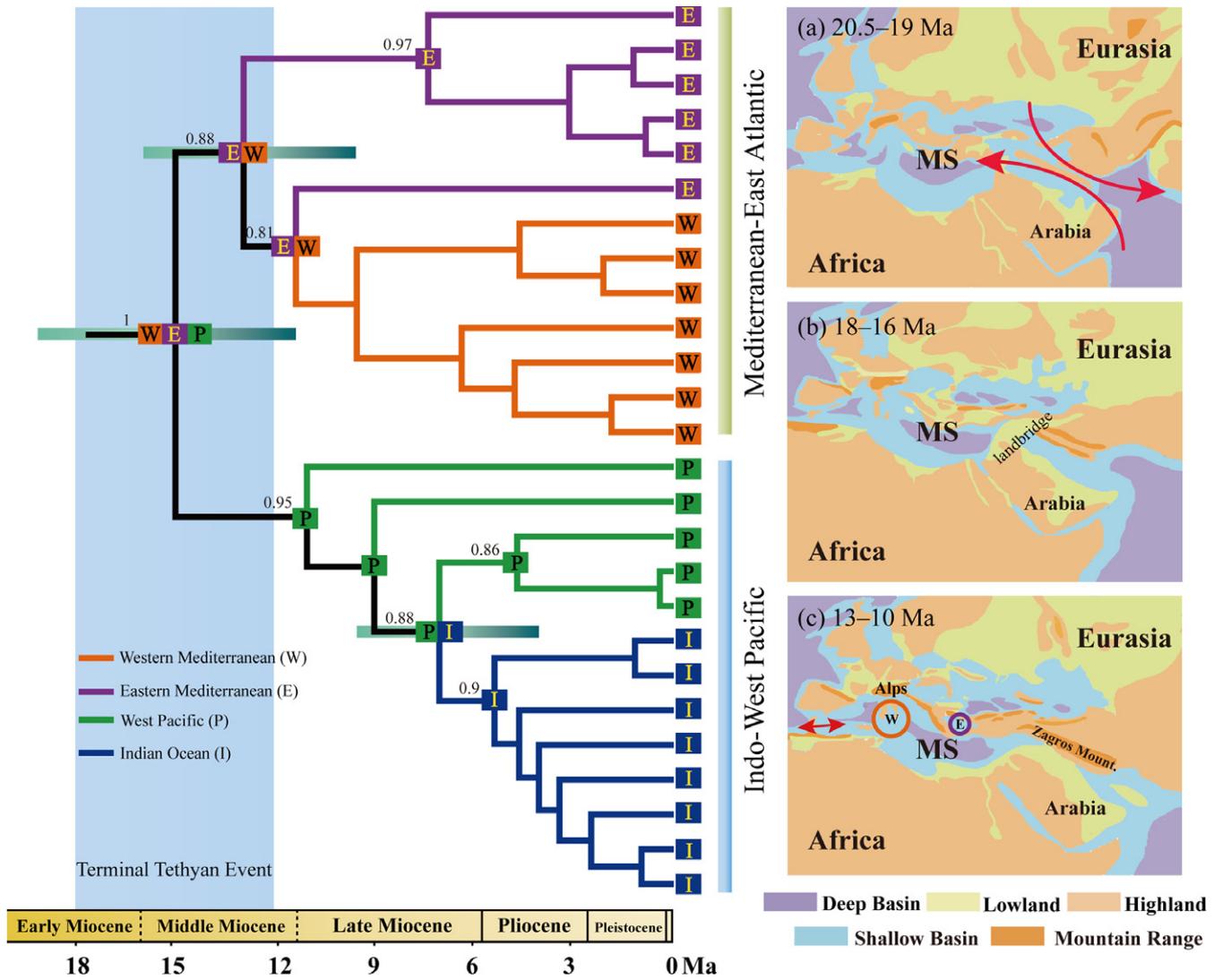
A total of 446 sequences were successfully generated from two mtDNA and four nuclear fragments for 87 specimens in this study. The final alignments contained 99 taxa with 623 bp for COI, 86 taxa with 422 bp for 16S, 84 taxa with 1367 bp for 28S, 83 taxa with 321 bp for H3, 72 taxa with 709 bp for NaK and 38 taxa with 1988 bp for 18S. The protein-coding fragments could be successfully translated into amino acids without stop codons. The concatenated dataset contained 103 taxa with 5430 bp. All new sequences were deposited in GenBank. Detailed information on samples and sequences is presented in Tables S1 and S3.

### 3.2 | Phylogeny reconstruction

Maximum likelihood and BI analyses produced similar trees for single genes and the concatenated dataset (Figure 2 and Figure S1), and most clades are well supported (bootstrap  $\geq 70$ , Bayesian posterior



**FIGURE 2** (a) The Bayesian tree of *Talorchestia sensu lato* derived from the combination of four nuclear and two mitochondrial loci. Support values (Bayesian posterior probabilities  $\geq 0.7$ /maximum likelihood bootstrap proportions  $\geq 70$ ) are shown at nodes. Taxa of the genera *Africorchestia*, *Britorchestia*, *Deshayesorchestia* and *Sardorchestia* are indicated, and the remaining species are attributed to the genus *Talorchestia*. (b) Image of *Talorchestia martensii*, photograph by Zhiyuan Yao. (c) The map shows sampling locations, and detailed sampling information is presented in supplementary material (Table S1). Triangles represent Mediterranean Sea-East Atlantic clades, and circles represent Indo-West Pacific clades. Different colours correspond to clades in the phylogenetic tree: west Mediterranean (purple), east Mediterranean (orange), west Pacific (green) and Indian Ocean (blue)



**FIGURE 3** Biogeographical scenario for the diversification of the genus *Talorchestia sensu lato*. The left part of the figure presents the time-calibrated species tree obtained from \*BEAST analysis and the ancestral range reconstruction of the genus from DEC+j model in BioGEOBEARS. The light blue vertical shading indicates the duration of the TTE (Terminal Tethyan Event, 18–12 Ma). The bars and rectangles on nodes indicate 95% highest posterior density interval of divergence times of major clades and ancestral areas. Bayesian posterior probabilities are presented above the branches for major clades. The right part of the figure depicts the progression of TTE through a series of palaeo-maps. (modified after Rögl, 1998 and Popov et al., 2004). (a) A broad seaway connected Mediterranean-East Atlantic and Indo-West Pacific 20.5–19 Ma. The red arrows show the bidirectional marine faunal dispersal along the marine seaway. (b) Emergence of the ‘*Gomphotherium* land bridge’ 18–16 Ma, separating the Indo-West Pacific clade from the Mediterranean-East Atlantic clade. (c) Uplift of the Alps and Zagros Mountains 13–10 Ma show the near-complete Tethyan closure. The circles indicate the divergence within the Mediterranean clade. W: west Mediterranean clade. E: east Mediterranean clade. The red arrow shows marine faunal dispersal through the Strait of Gibraltar between the east Atlantic Ocean and west Mediterranean Sea

probability  $\geq 0.95$ ). The main conflict occurred in the nuclear markers 18S and H3 and involved a paraphyletic relationship within the MS-EA clade, which might be attributed to these genes providing less informative characters. Therefore, we used the BI tree obtained from the concatenated dataset for our phylogenetic assessment (Figure 2). The genus *Talorchestia sensu lato*, including *Talorchestia sensu stricto*, *Sardorchestia*, *Africorchestia*, *Deshayesorchestia* and *Britorchestia*, formed a monophyletic group. This *Talorchestia* complex was clearly split into two clades, corresponding with the MS-EA and IWP region. The MS-EA lineage was further divided into geographically east and

west Mediterranean clusters: the group of *Britorchestia*, *Sardorchestia*, *Deshayesorchestia* and *Africorchestia* from Italy, Germany, Georgia and Gabon belongs to the west Mediterranean clade, while the east Mediterranean clade includes species from Greece. The IWP lineage was composed of four groups. Three of them are from the west Pacific, including *T. mindorensis* found in China, Taiwan Island and Philippines, *T. spinipalma* from Fiji and *T. martensii* distributed across Southeast Asia. Another group from the Indian Ocean includes the sister groups of *T. martensii* and *T. qeshm*, widespread along the east Africa coastline and the Persian Gulf.

### 3.3 | Divergence times

To generate a designation of taxonomic units when estimating divergence times, we applied a GMYC model to specify independent entities at the interspecific level. The GMYC single-threshold model based on the mitochondrial genes delimited 26 entities (see Figure S2).

We performed divergence time estimations with eight different dating schemes (Table S4). Two dating approaches based on the mitochondrial gene tree and the species tree produced similar estimates, the 95% highest posterior density (HPD) of divergence events almost overlapped, but means derived from the mitochondrial gene tree analysis were slightly younger than those of the species tree (Figure 3 and Figure S3). The analyses using the pruned dataset produced an estimate that was a little older, but all the mean nodal ages were within the confidence intervals calculated from full dataset (Table S4, Figure 3 and Figure S3). Calibrations with three different molecular clocks yielded decreasing time estimates by increasing the clock rates from 0.0115, 0.0177 to 0.0329. The model employing a clock rate of 0.0115 for the species tree analyses on the full dataset had the highest likelihood (Table S5) and was therefore used for subsequent analyses.

From the estimation using the species tree (Figure 3), the basal split between the MS-EA and IWP lineages was estimated to be 15.24 (95% HPD: 11.15–19.42) Ma. The MS-EA lineage started to diversify about 13.14 (9.46–16.8) Ma and radiated along the Mediterranean Sea and Africa coastline forming a west MS-EA clade and an east MS-EA clade. The crown lineage of IWP appeared about 11 Ma and successively diverged into four groups: three inhabiting the west Pacific and another from the Indian Ocean. The most recent common ancestor of the Indian Ocean clade and west Pacific clade was estimated at 7.12 (4.6–9.6) Ma.

### 3.4 | Biogeographic reconstruction

In our BioGEOBEARS analyses, the DEC+J model ( $\ln L = -11.25$ ,  $AIC = 28.5$ ) significantly increased the likelihood of the DEC model ( $\ln L = -13.85$ ,  $AIC = 31.7$ ) (Figure 3 and Figure S4; likelihood ratio test shows  $p$ -value of .023 [ $p < .05$ ]). The ancestral range reconstruction from the DEC+J model showed a broad, ambiguous ancestral origin for *Talorchestia sensu lato*, west Pacific, and west and east Mediterranean Sea. However, the west Pacific, and west and east Mediterranean Sea were covered by the ancient Tethys before the mid-Miocene. Therefore, we suggest that the ancestor of *Talorchestia sensu lato* was widely distributed around the Tethyan margins.

## 4 | DISCUSSION

Our study is the first to use intertidal crustaceans as a model system to explore the biogeographic influence of Tethyan closure. Phylogenetic inferences, molecular dating and biogeographic reconstruction of *Talorchestia sensu lato* support the TTE hypothesis, a pattern that reflects the isolation of MS-EA and IWP during the mid-Miocene. The results show parallels with spatial patterns found in marine fish

and molluscs (Barber & Bellwood, 2005; Bellwood et al., 2004; Bowen, Muss, Rocha, & Grant, 2006; Malaquias & Reid, 2009), but with differences in dating estimation of the splitting between the MS-EA and IWP sister lineages. This discrepancy indicates an incongruent temporal pattern related to TTE and a changing role of the Tethyan closure from an ecological barrier to a physical barrier in driving the current tropical marine biodiversity across the Mediterranean-East Atlantic and Indo-Pacific regions.

### 4.1 | Vicariant diversification driven by the Tethyan closure

Our analyses indicate a Tethyan origin of *Talorchestia sensu lato* during the Miocene and splitting into MS-EA and IWP lineages at c. 15 Ma (Figure 3). This diversification pattern is consistent with the prediction that Tethyan closure drove vicariance between the Atlantic and Indo-Pacific lineages. Before the TTE, the Tethys Seaway connected the Atlantic and Pacific, providing a dispersal route for intertidal talitrids (Figure 3a). Along with the collision between the Arabian and Eurasian plates c. 18–16 Ma, the ancestral Tethyan realm broke into the present-day Mediterranean-East Atlantic and Indo-Pacific provinces, causing the splitting of MS-EA and IWP lineages (Figure 3b). Following the separation, each lineage was restricted *in situ* and evolved independently without distinct gene exchange.

The TTE functioned as an impenetrable dispersal barrier for marine species, fragmenting their previously continuous distributions and leading to vicariant speciation (Hou & Li, 2017). However, it formed the *Gomphotherium* land bridge, allowing terrestrial connections between Africa and Eurasia and providing pathways for transcontinental migration of terrestrial animals and plants (Harzhauser et al., 2007; Koufos, Kostopoulos, & Vlachou, 2005; Pérezgutiérrez et al., 2015; Portik & Papenfuss, 2015; Zhou & Saunders, 2012).

### 4.2 | Deep splits within clades after the Tethyan closure

The MS-EA lineage diverged into the current east Mediterranean clade and west Mediterranean clade at c. 13 Ma. This diversification pattern can be explained by a marine transgression between the Tethys and Paratethys in the east Mediterranean Sea and final structuring of the Alps, which isolated the Balkan block from the rest of the Mediterranean-East Atlantic region during mid-Miocene (Figure 3c) (Oberprieler, 2005). Historical vicariant events leading to similar divergence between Balkan and trans-Mediterranean lineages have been documented in several other groups, such as Compositae, tribe Anthemideae (Oberprieler, 2005) and the beetle subfamily Pachydeminae (Sanmartín, 2003). The species from Gabon nested in the west Mediterranean clade; such a phylogeographical relationship could result from dispersal across the Strait of Gibraltar, which connects the west Mediterranean Sea and east Atlantic.

The IWP lineage is widely distributed along the Indo-West Pacific margins, and the Indian Ocean clade separated from the rest of IWP clade around 7 Ma (Figure 3). This scenario may be driven by the

closure of the Indonesian Seaway. A similar pattern has been reported in reef fish (Read, Bellwood, & Van, 2006) and seahorses (Teske, Hamilton, Matthee, & Barker, 2007), suggesting that the late Miocene tectonic activity in the Indonesian region and associated shifts in circulation patterns during the northward migration of Australia played an important role in the diversification of the Indian Ocean and West Pacific sister groups. A potential alternative explanation exists for diversification of IWP taxa. The separation of the Indian Ocean clade from the IWP lineage could be the result of vicariance during low sea level during the Pleistocene glacial periods (Crandall et al., 2008; Liu, Chang, Borsa, Chen, & Dai, 2014). This explanation seems unlikely though, because the most recent common ancestor of the Indian Ocean *Talorchestia* species far predates the Pleistocene.

### 4.3 | Vicariant patterns related to TTE in tropical marine biodiversity

Vicariant speciation and allopatric divergence derived from the closure of the Tethys has been identified in a diverse set of taxonomic groups (Barber & Bellwood, 2005; Bellwood et al., 2004; Cowman & Bellwood, 2013; Eilertsen & Malaquias, 2015; Hrbek & Meyer, 2003; Reid, Dyal, & Williams, 2010; Teske, Cherry, & Matthee, 2004). The divergence time variation among these cases can be viewed from three stages: pre-TTE, during TTE and post-TTE, which correspond to three different patterns among marine fauna related to TTE. Our results are congruent with the temporal and geographical predictions under TTE (Figure 1b), which means that TTE vicariance can best explain the present disjunct distribution of *Talorchestia sensu lato* along MS-EA and IWP. Similar vicariant divergence has been reported in marine fishes, such as the Atlantic *Holacanthus* and IWP *Pygoplites*, splitting at c. 20 Ma (Bellwood et al., 2004), and the genus *Halichoeres*, diversifying in the Atlantic and Indo-Pacific regions, respectively, about 18 Ma (Barber & Bellwood, 2005). These congruent biogeographic spatiotemporal patterns reflect shared responses by different species to the TTE vicariant event.

The patterns of pre-TTE vicariance are common in marine fishes and molluscs (Figure 1a) (Eilertsen & Malaquias, 2015; Malaquias & Reid, 2009; Reid et al., 2010; Uribe, Williams, Templado, Buge, & Zardoya, 2016), which diverged into IWP and Atlantic groups some 30 Ma. It must be noted that the TTE marks the final physical closure of the seaway, but genetic barriers probably began forming much earlier (Bellwood et al., 2004). The ecological changes induced by a series of tectonic events from Eocene to Miocene have continuously affected diversification in the ancient Tethyan region. The division of Atlantic and IWP clades depends on the ability of populations to maintain genetic connectivity through a narrowing seaway (Bowen et al., 2006; Uribe et al., 2016). Organisms inhabiting different depth ranges and substrate types must have been affected differently, and it is sensible that benthic marine organisms may have been affected earlier than organisms associated with intertidal habitats, and the intertidal taxa may have been the last to be sundered by the TTE (Knowlton & Weigt, 1998; Malaquias & Reid, 2009).

After the final closure of the Tethys, the shortest dispersal route for marine or coastal species between the Mediterranean-East Atlantic and Indo-West Pacific is around southern Africa (Eilertsen & Malaquias, 2015). Some fossils and molecular phylogenetic studies suggest that post-TTE colonization and speciation events from IWP to the Atlantic occurred via the southern tip of Africa during the Pliocene–Pleistocene glacial periods (Figure 1c) (Bowen et al., 2006; Eilertsen & Malaquias, 2015; Gibbons & Thibaultbotha, 2002; Rocha et al., 2005). However, the Benguela cold-water current in southern Africa (Siesser, 1980) acts as a soft biogeographic barrier for shallow-water taxa and tropical/temperate taxa between IWP and Atlantic (Bowen et al., 2006; Gibbons & Thibaultbotha, 2002). In our study, we sampled specimens from both sides of southern Africa, however, the phylogenetic analyses indicated that taxa from east and north-east Africa (Kenya, Djibouti) and Madagascar nested in the IWP clade and species from west Africa (Gabon) nested in the MS-EA clade (Figure 2). This result indicates strong geographical structure, and it appears that no dispersal occurred across South Africa.

The opening of the Suez Canal in 1869 created the current salt-water passage between the Mediterranean Sea and the Red Sea. The new dispersal route has been considered an important pathway for biological invasions, usually from the Red Sea to the Mediterranean Sea (Bernardi, Azzurro, Golani, & Miller, 2016; Golani et al., 2007; Por, 2012). Due to a lack of samples around the Red Sea and east Mediterranean Sea, it is difficult to determine whether migration occurs in *Talorchestia sensu lato* from the current research. Although dispersal around southern Africa and migration via the Suez Canal are possible, the TTE still represents the largest impermeable physical barrier for tropical marine species.

The current study provides a strong case in support of the TTE hypothesis in intertidal ecosystems. The Tethyan closure during the mid-Miocene may have played an important role in vicariant events that have created present-day Mediterranean and Indo-West Pacific biodiversity. Comparisons of various organisms and integrative analyses of phylogenetic and geologic data could shed more light on the biogeographic effects of the Tethyan closure.

### ACKNOWLEDGEMENTS

We thank Prof. Alessio Mengoni and three anonymous reviewers for improvements to the manuscript. We thank Huifeng Zhao and Zhiyuan Yao for their assistance in specimen collection and photography.

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**BIOSKETCH**

**Hongguang Liu** is a Ph.D. Candidate. His scientific interests focus on investigating the influence of Tethyan closure on the present-day distribution patterns of intertidal amphipods. The interests of the research team include the phylogenetic relationships of freshwater amphipods and diversification patterns shaped by Tertiary Tethyan changes.

Author contribution: H.L., S.L. and Z.H. conceived the study and performed part of the fieldwork. A.U. and F.M. led the specimen collection. H.L. carried out the molecular work and analysed the data. H.L., S.L. and Z.H. wrote the manuscript. All authors read and approved the final manuscript.

**SUPPORTING INFORMATION**

Additional Supporting Information may be found online in the supporting information tab for this article.

**How to cite this article:** Liu H, Li S, Ugolini A, Momtazi F, Hou Z. Tethyan closure drove tropical marine biodiversity: Vicariant diversification of intertidal crustaceans. *J Biogeogr.* 2018;00:1–11. <https://doi.org/10.1111/jbi.13183>