

Journey to the West: Trans-Pacific Historical Biogeography of Fringehead Blennies of the Genus *Neoclinus* (Teleostei: Blenniiformes)

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Several temperate marine taxa of the northern hemisphere follow a trans-Pacific biogeographic track with representatives on either side of the intervening boreal waters. Shelter-dwelling blenniiform fishes of the genus *Neoclinus* exhibit this trans-Pacific distribution pattern with three species in the eastern North Pacific and eight species in the western North Pacific. We reconstructed the phylogeny of the Neocliniini (*Neoclinus* and the monotypic *Mccoskerichthys*) using six genetic markers: four mitochondrial genes (*COI*, cytochrome *b*, 12S and 16S), and two nuclear genes (*RAG-1*, *TMO-4C4*). Ancestral state reconstruction and molecular clock dating were used to explore hypothetical ancestral distributions and area relationships, and to estimate divergent times within this group. The monophyly of the genus *Neoclinus*, and the reciprocal monophyly of the

eastern Pacific and western Pacific lineages were supported. Available evidence, including the eastern Pacific and western Atlantic occurrence of a New World clade of blennioid fishes that includes this lineage, supports the origin of the Neocliniini in the eastern Pacific with a single divergence event to the west across the North Pacific by the ancestor of the western Pacific clade. Estimated divergence time of the eastern and western Pacific clades of *Neoclinus* was 24.14 million year ago, which falls during the Oligocene epoch. Estimated times of divergence in other trans-Pacific lineages of marine fishes vary widely from recent Pleistocene events to as early as 34 mya.

Key words: *Mccoskerichthys*, North Pacific, Eastern North Pacific, Western North Pacific, Phylogeny.

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BACKGROUND

Several temperate marine taxa of the northern hemisphere follow a long recognized trans-Pacific biogeographic track, most without representatives in the intervening boreal waters (Andriashev 1939; Ekman 1953; Briggs 1974). It is seen in several groups of marine animals including invertebrates and fishes (brief reviews in Ilves and Taylor 2007; Ellingson et al. 2014). However, the hypothesized timing of divergence of these groups varies widely (Ilves and Taylor 2007) ranging from relatively recent dispersal events during Pleistocene interglacial periods (e.g., Grant and Bowen 1998; Cox et al. 2014) to much older events, dating back as far as 35 mya to the late Eocene/early Oligocene (Ellingson et al. 2014; Thacker 2015).

Fringeheads of the blenniiform genus *Neoclinus* (Fig. 1) are one of the groups exhibiting this distributional track. This lineage of coastal fishes includes species found in warm temperate waters on either side of the otherwise inhospitable cold-water barrier of the North Pacific and, as far as known, does not occur in intervening areas including islands of the western Pacific (Hubbs 1953; Hastings and Springer 2009). As adults, species of *Neoclinus* inhabit shelters including the vacant tests of gastropods and barnacles (Fukao and Okazaki 1987), and empty worm tubes (Stephens and

Springer 1971), as well as artificial shelters such as discarded cans and bottles (McCleneghan and Ames 1976). These shelters provide protection from predators and serve as egg-deposition sites that are guarded by resident males (Lindquist 1981; Murase and Sunobe 2011) and are often the focus of intense territorial disputes (Hongjamrassilp et al. 2018). This cryptobenthic behavior dictates that adults have limited dispersal ability, although, similar to most other blennies, species of *Neoclinus* have a pelagic larval phase (Watson 2009).

The eleven currently recognized species of *Neoclinus* are included in the Neocliniini (*sensu* Lin and Hastings 2013) along with the monotypic genus *Mccoskerichthys* that has a restricted distribution along the tropical eastern Pacific coasts of Costa Rica and Panama (Rosenblatt and Stephens 1978). Three species of *Neoclinus* (*N. blanchardi*, *N. stephensae*, and *N. uninotatus*) are found in coastal waters of the northeastern Pacific from northern California, USA to Baja California, Mexico (Hubbs 1953; Love 2011). The remaining eight species (*N. bryope*, *N. chihioe*, *N. lacunicola*, *N. monogrammus*, *N. nudiceps*, *N. nudus*, *N. okazakii*, and *N. toshimaensis*) inhabit the northwest Pacific including the coastal waters of Japan, the northern part of Taiwan, and Korea (Fukao 1987, 1990; Murase et al. 2015). Hubbs (1953) who described two of the eastern Pacific species proposed that the ancestor of the northwestern Pacific species *Neoclinus bryope* migrated from the northeastern Pacific coast to the western Pacific through the Aleutian Islands during a relatively recent interglacial period. However, at that time only the three eastern Pacific species and a single western Pacific species (*N. bryope*) were known. Subsequently seven more western Pacific species have been described (Stephens and Springer 1971; Fukao 1980; Murase et al. 2010). Fukao and Okazaki (1987) hypothesized an older emigration from the northeastern Pacific during the late Pliocene to early Pleistocene, based on Nishimura's (1980) hypothesis of faunal exchange between these regions. We studied the species-level relationships within *Neoclinus* using DNA sequence data and 1) confirmed the monophyly of the genus *Neoclinus* and the reciprocal monophyly of the eastern and western Pacific clades; 2) determined the ancestral distribution to be in the eastern Pacific, and 3) estimated the divergence time of the eastern and western Pacific clades around 24.14 mya.

MATERIALS AND METHODS

1 Taxon sampling

2
3 Muscle tissues from the three eastern Pacific species of *Neoclinus*, *M. sandae*, and the
4 outgroup species *Alloclinus holderi* (Lin and Hastings 2013) were obtained from the Marine
5 Vertebrate Collection at the Scripps Institution of Oceanography. Muscle tissues of three of the
6 western Pacific species of *Neoclinus* (*N. nudus*, *N. bryope*, and *N. okazaki*) were obtained from A.
7 Murase's personal collection (UMNB-I) (Table S1).

9 Molecular data, sequence assembly and tests of codon saturation

10
11 Novel sequence data from four mitochondrial markers (12S, 16S, cytochrome *c* oxidase
12 subunit I (*COI*), and cytochrome *b*) and two nuclear markers (TMO-4C4 and RAG1) were
13 generated for eight species (Table S1), while comparable data for three species were obtained from
14 GenBank. Total genomic DNA was extracted from muscle tissue with a ZR Genomic DNA-Tissue
15 MiniPrep (Zymo Research, USA) following the manufacturer's instructions. Primers used to
16 amplify the six markers are listed in table S2. The PCR was performed with the conditions listed in
17 table S3. Resulting amplicons were cleaned using ExoSAP-IT (exonuclease I and shrimp alkaline
18 phosphatase) in a specially formulated buffer from USB Corporation (Cleveland, OH) to remove
19 excess primers and dNTP. All PCR products were sequenced in both directions using standard
20 Sanger sequencing methods via Retrogen, Inc (San Diego, CA). All genetic marker sequences were
21 uploaded to the NCBI database (access numbers are provided in Table S1).

22 Sequences were assembled and edited in Geneious 7.1.9. The completed sequences were
23 aligned using MAFFT v. 7 (Katoh and Standley 2013) in Mesquite 3.51 (Maddison and Maddison
24 2018). All protein-coding genes (*COI*, cytochrome *b*, RAG-1, and TMO-4C4) were assigned with
25 codon position under the minimizing stop codon algorithm and translated to amino acids in
26 Mesquite 3.51 to ensure the absence of stop codons associated with pseudogenes.

27 Fishes in the order Blennioformes have been reported to have a high rate of molecular
28 evolution especially in protein-coding genes (Lin and Hastings 2011; Eytan et al. 2012). To address
29 the problem regarding 3rd codon saturation, four protein-coding gene loci were tested for saturation
30 using Xia's test in DAMBE7 (Xia and Kumar 2018). The protein-coding genes were partitioned by
31 1st, 2nd and 3rd codon positions in MEGA 7 (Kumar et al. 2016) before implementation of Xia's

test in DAMBE7. Saturation plots of transitions and transversions were generated against corrected genetic distance with the General Time Reversible (GTR) model of evolution and Xia's test was used to interpret codon saturation. Codon saturation is indicated when the transition versus transversion plot shows a plateau, however, Xia's test of saturation is more conservative requiring "substantial saturation" as indicative of a level unsuitable for phylogenetic analysis.

Phylogenetic analyses, molecular dating and ancestral state reconstruction

Maximum Parsimony (MP), Maximum likelihood (ML), and Bayesian inference (BI) analyses were used to reconstruct the phylogenetic relationships. Each gene dataset was used to reconstruct gene trees under ML (Felsenstein 1981) in RAxML v.7.4.2 (Stamatakis 2006) via RaxMLGUI v1.3 (Silvestro and Michalak 2012) with the rapid bootstrapping algorithm. The General Time Reversible model with gamma rate of heterogeneity model (GTR + G) was applied and replicate bootstrap was set at 1,000 replicates. For the species tree analysis, each gene dataset was analyzed with jModelTest (Posada 2008) to find the best-fit evolutionary model, after which all genetic marker datasets were concatenated in Mesquite 3.51 (Maddison and Maddison 2018). Based on results from Xia's test of saturation (see RESULTS section for interpretation), only the 3rd codon of cytochrome *b* was found to be saturated, consequently it was excluded from the concatenated dataset. The concatenated dataset was analyzed under MP in PAUP4.0 (Swofford 1998) with the heuristic search tree bisection reconnection (TBR) and branch-swapping from 1,000 random-addition-sequence replicates. Gaps between nucleotides were set as missing characters. Bootstrap node support was estimated with 10,000 heuristic searches with maxTree = 1,000. For the ML analysis, the concatenated dataset was analyzed in RAxML. Each gene was partitioned before analyzing with the rapid bootstrap algorithm using 1,000 bootstrap replicates with the GTR + G model. Finally, for the BI analysis, the concatenated dataset was analyzed using Bayesian Metropolis-coupled Markov chain Monte Carlo (MCMC) in MrBayes v. 3.2.2 (Ronquist and Huelsenbeck 2003). Each gene partition was applied with different models according to jModelTest results. The model parameter values were "unlinked" among each partition. Before the BI analysis, the algorithm was set to run with three hot and one cold chains for 10 million generations and was sampled every 1,000 generations. The first 10% of the BI results from the MCMC analysis was discarded as burn-in.

The divergence times were estimated using BEAST v.1.7 and the BEAUti package (Drummond et al. 2012). The concatenated dataset of all genes was imported into BEAST and the substitution model parameter was set as unlinked. Clock model and trees were set as linked. Relaxed molecular clock analysis, uncorrelated lognormal model (UCLN), and calibrated Yule-process-speciation priors were set (Drummond et al. 2006). No reliably dated fossil blenniiforms are known, thus we used the secondary calibration from Lin and Hastings (2013) which provided the divergent time of *A. holderi* from the ancestor of *M. sandae* and *Neoclinus* (38 mya with 95% HPD interval ranging from 23.25 to 54.37) that was based on 21 molecular markers from 1,410 fish taxa (Betancur-R et al. 2013). Then, three independent MCMC analyses were run for 40 million generations and sampled every 1,000 generations. 10% of the sample was discarded from the analysis. Log files were summarized on a maximum clade credibility tree with TreeAnnotator (Drummond et al. 2012).

The historical biogeography within the Neocliniini was studied using data on the current distribution of species (either in the eastern or western North Pacific). Three species of *Neoclinus* are restricted to warm temperate waters of the eastern Pacific from the central Baja peninsula northward to Bodega Bay, California (Love et al. 2005), while *Mccoskerichthys sandae* is restricted to the tropical eastern Pacific coasts of Costa Rica and Panama (Rosenblatt and Stephens 1978). The remaining species of *Neoclinus* are found in the western Pacific in both warm temperate and subtropical waters from northern Japan southward to Okinawa, Korea and Taiwan (Stephens 1961; Fukao 1980; Fukao and Okazaki 1987). No members of the Neocliniini have been found in boreal waters and none in waters above 38 degrees north latitude. Ancestral distributions were reconstructed in Mesquite 3.51 (Maddison and Maddison 2018) under MP.

RESULTS

We obtained a total of 3,717 base pairs (bp) from six genetic markers (Table S4). This included: 1) 651 bp in *COI* (73.51% parsimony informative sites (PI); 2) 462 bp in cytochrome *b* (58.44% PI); 3) 316 bp in 12S (24.37 % PI); 4) 490 bp in 16S (26.73% PI); 5) 364 bp in TMO-4C4 (13.13% PI); and 6) 1,434 bp in RAG-1 (3.35% PI). Results from the transition versus transversion plots (Fig. S1) indicated that the 3rd codon position of cytochrome *b* and *COI* might be saturated.

However, Xia's test of saturation only shows that the 3rd codon of cytochrome *b* is significantly saturated (Table S5). Thus, only the 3rd codon position of cytochrome *b* was excluded from the phylogenetic analysis. This resulted in 3,563 bp total in the final dataset. Among the amplified sequences, only the cytochrome *b* gene from *M. sandae* was not able to be amplified and consequently treated as missing data in all phylogenetic analyses.

Although the gene tree obtained from cytochrome *b* was not well supported with bootstrap values and the tree topology was poorly resolved, the gene tree from 12S + 16S provided strong bootstrap support for most nodes (Fig. 2). While the tree from 12S + 16S is well-resolved, the topology differs somewhat from the gene tree of *COI*, TMO-4C4, and RAG-1 (Fig. 2). The tree topologies based on *COI*, TMO-4C4, and RAG-1 were similar to the tree topology obtained using all combined sequence data reconstructed using MP, ML, and BI methods (Fig. 3). This well-resolved tree topology supports the monophyly of the genus *Neoclinus*. Within *Neoclinus*, two well-resolved clades were obtained reflecting a monophyletic western Pacific clade and a monophyletic eastern Pacific clade (Fig. 4). Reconstruction of the ancestral distributions (Fig. 5) indicates an eastern Pacific origin of the Neocliniini with a single vicariant or dispersal event across the North Pacific by the ancestor of the western Pacific clade of *Neoclinus* and subsequent diversification of this clade within the western Pacific (Fig. 6).

The molecular dating results from secondary calibration indicated that the ancestor of *Neoclinus* and *M. sandae* diverged around 35.50 million years ago (95% HPD interval: 22.41 to 48.89) which falls in the late-Eocene to early-Oligocene period (Fig. 7). Moreover, the western and eastern Pacific clades of *Neoclinus* diverged around 24.14 million years ago (95% HPD interval: 13.03 to 35.06), which falls during the late Oligocene period.

DISCUSSION

Our phylogenetic analysis of the Neocliniini supports the monophyly of the trans-Pacific genus *Neoclinus* consistent with the finding of Lin and Hastings (2013). Although not all western Pacific species were included in our study, the consensus topology strongly supports two reciprocally monophyletic clades within *Neoclinus* that correspond to the three eastern Pacific species and the three western Pacific species included in this study. Within the eastern Pacific clade,

the two largest species in the genus, *N. uninotatus* and *N. blanchardi*, that reach 250 mm and 305 mm maximum standard length (SL), respectively, are sister species. All other species in the genus, including the eastern Pacific species *N. stephensae*, grow no larger than 100 mm SL (Stephens and Springer 1971; Fukao 1980; Love 2011). Relationships within the western Pacific species included in this study are congruent with the two groups identified based on morphological characters in that *N. bryope* and *N. okazakii*, both members of the *bryope* group (Fukao 1987), are sister species (Fig. 3).

We were unable to obtain tissue samples for DNA extraction for five of the 11 species of *Neoclinus*. The five unsampled species are restricted to the western Pacific and three of these are similar to other western Pacific species included in this study. *Neoclinus chihiroe* is morphologically and genetically similar to *N. bryope* and *N. okazakii*, while *N. lacunicola* and *N. toshimaensis* are morphologically and genetically similar to *N. nudus* (Fukao 1987, 1990; Fukao and Okazaki 1987). The genetics of two recently described species, *N. monogrammus* and *N. nudiceps* (Murase et al. 2010), have not been studied. Morphologically they are similar to one another in having a single pore in the lateral line (double pores in all others) and two supraorbital cirri (more than two in others). While it would be ideal to have sampled all species, available evidence supports the monophyly of the western Pacific lineage of *Neoclinus*.

The trans-Pacific distribution characteristic of *Neoclinus* (Fig. 4) is seen in a variety of other marine organisms from several groups (Andriashev 1939), including mollusks (Amano and Vermeij 1998, 2003; Cox et al. 2014), polychaetes (Uschakov 1971), crustaceans (Schweitzer 2001), pinniped mammals (Deméré et al. 2003), and a variety of fishes. These include most notably the surfperches (Embiotocidae; Bernardi and Bucciarelli 1999), greenlings (Hexagrammidae; Crow et al. 2004), rockfishes (Sebastidae; Barsukov 1981; Hyde and Vetter 2007; Ingram and Kai 2014) and gobies (Gobiidae; Ellingson et al. 2014; Thacker 2015). This pattern is also apparent in a variety of other fishes in which similar species are found on opposite sides of the North Pacific. This includes the smelts (Osmeridae; McAllister 1963; Ilves and Taylor 2007), sardines (Clupeidae; Bowen and Grant 1997; Grant and Bowen 1998), sculpins (Cottidae; Knope 2013), thornyhead rockfishes (Sebastidae; Stepien et al. 2000), pholids (Pholidae; Radchenko et al. 2012) and pricklebacks (Stichaeidae; Hastings and Walker 2003; Markevich and Kharin 2011).

The Neocliniini may, however, be unique in that the sister group of the North Pacific clade is found in shallow coastal waters of the tropical eastern Pacific (Fig. 4). *Mccoskerichthys sandae*

has a restricted range along the Pacific coast of Costa Rica and Panama where it occurs in holes within corals (Rosenblatt and Stephens 1978). To our knowledge this sister-group relationship between a temperate North Pacific clade and a neotropical lineage is unique among trans-Pacific lineages of temperate marine organisms.

The outgroup relationships of the Neocliniini were incompletely resolved in a recent study of the phylogeny of the Blenniiformes (Lin and Hastings 2013). The Neocliniini was included in a clade with temperate eastern Pacific cryptotremine labrisomids (*Alloclinus* and *Auchenionchus*; Stephens and Springer 1974) and a large Neotropical clade that includes over 240 species of labrisomid blennies, chaenopsid blennies and sand stargazers (Dactyloscopidae). With the exception of the western Pacific clade of *Neoclinus* and three species of labrisomids that occur in the eastern Atlantic (*Labrisomus nuchipinnis*, *Malacoctenus africanus* and *M. carrowi*), this entire lineage is restricted to the eastern Pacific and western Atlantic (Hastings 2009). Consequently, all evidence, including our phylogenetic analysis, supports an eastern (Pacific) origin of the Neocliniini (Fig. 5). This result supports a scenario in which *Mccoskerichthys* and *Neoclinus* diverged, the former in the tropical eastern Pacific and the latter in the warm-temperate waters of the eastern Pacific. The province of the ancestor of this lineage, either the tropical or warm temperate portion of the eastern Pacific is unresolved (Fig. 6; Lin and Hastings 2013, fig. 5). This was followed by the divergence of the eastern and western Pacific lineages of *Neoclinus*, probably via dispersal event across the North Pacific, and subsequent speciation within each of these clades (Fig. 6).

The hypothesized ancestral distribution of other temperate trans-Pacific groups and thus the region of origin, whether the eastern or western North Pacific, varies across taxa. Hypotheses of an east to west track among fishes include greenlings of the Hexagrammidae (Crow et al. 2004), surfperches of the Embiotocidae (Bernardi and Bucciarelli 1999), sardines of the Clupeidae (Bowen and Grant 1997) and “bay gobies” of the Gobiidae (Ellingson et al. 2014), although Thacker (2015) implied a west to east track for the latter clade. A west to east track was hypothesized for rockfishes of the genus *Sebastes* (Baruskov 1981; Hyde and Vetter 2007) and possibly for osmerid fishes (Ilves and Taylor 2007).

The estimated time of divergence of the trans-Pacific clades of *Neoclinus* (24.14 mya, HPD: 13.03 to 35.06; Fig. 7) should be considered tentative given both the wide confidence intervals and the fact that five of the western Pacific species were not included in this study. However, it implies a much older origin of the trans-Pacific divergence event than assumed by earlier authors. Hubbs

(1953) understandably assumed a Pleistocene interglacial dispersal by the ancestor of *N. bryope*, at that time the only known western Pacific species. This assumption was followed by subsequent authors (Fukao 1980), and has been hypothesized for other groups of trans-Pacific fishes (e.g., *Sardinops*, Grant and Bowen 1997). Our hypothesis of an older divergence corresponds with that reported for some other groups of fishes, but estimates of timing of these events vary widely. Trans-Pacific divergence times were estimated at 5 mya for embiotocids (Bernardi and Bucciarelli 1999) and rockfishes of the genus *Sebastolobus* (Stepien et al. 2000). Estimates for a trans-Pacific divergence event within rockfishes of the genus *Sebastes* range from 8.35 mya (Ingram and Kai 2014) to 15-17 mya (Baruskov 1981; Hyde and Vetter 2007). Trans-Pacific divergence of osmerid fishes was estimated at 15-25 mya (Ilves and Taylor 2007). More recent studies on the lineage of bay gobies of the Gobionellinae (Ellingson et al. 2014) found reciprocally monophyletic clades in the eastern and western Pacific with an even older estimated divergence time of about 34 mya (late-Eocene to early-Oligocene) that was corroborated as 34.2 mya by Thacker (2015). This range of estimated trans-Pacific divergence times is not surprising given the variety of study organisms, the various techniques used in these studies, some of which are outdated, based on single genes, or have incomplete taxon sampling, as well as the wide confidence intervals ascribed to recent methods (Rutschmann 2006).

The North Pacific experienced a relatively long interval of warm global oceans from 15 to 25 mya that may have provided suitable habitat for members of these temperate lineages in the intervening areas and/or may have facilitated dispersal of pelagic larvae across intervening gaps for a relatively long time (Ilves and Taylor 2007). This appears to have been cut off by cooling in the mid-Miocene (Zachos et al. 2001; Ilves and Taylor 2007) interrupting distributions and/or limiting opportunities for trans-Pacific dispersal events until more recent interglacial periods in the Pleistocene for pelagic lineages (e.g., Bowen and Grant 1997) and more boreal lineages (e.g., Cox et al. 2014). The trans-Pacific lineages of fishes that have been studied exhibit an array of dispersal potentials that may have affected their ability to cross the North Pacific in the past. The relatively recent estimated divergence of pelagic sardines may be expected given their relatively high dispersal potential as pelagic schooling adults (Bowen and Grant 1997). Lineages with older estimated divergence times such as rockfishes (*Sebastes*) and surfperches (Embiotocidae) retain developing embryos in females reducing their potential for dispersal during early life history (Hyde and Vetter 2007; Bernardi and Bucciarelli 1999). Similarly, gobies and blennies lay demersal eggs,

reducing their dispersal potential as larvae, and adults of both groups are small, benthic species that move little as adults reducing their dispersal potential even further. Application of modern methods and greater taxon sampling is needed to clarify the evolutionary history of these and other temperate marine clades transitioning the North Pacific.

Supplemental information is available at <https://www>.

CONCLUSIONS

Fringeheads of the blenniiform genus *Neoclinus* are one of several groups of coastal fishes with representatives on opposite sides of the North Pacific but not in intervening waters. Available evidence indicates a single dispersal or vicariant event across the North Pacific from this group's origin in the eastern Pacific, followed by speciation in both the western Pacific and eastern Pacific clades. Published accounts of other lineages of fishes exhibiting this trans-Pacific biogeographic track indicate an array of divergence times. However, these studies lack a consistent methodology and level of taxonomic coverage, limiting generalization of the underlying drivers of this biogeographic pattern.

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Supplementary materials

Fig. S1. Plots of transitions (blue) vs. transversions (green) for four protein coding DNA markers against corrected genetic distance (GTR), created in DAMBE5. Plots of protein coding genes show the 1st + 2nd and 3rd codon positions. (download)

Table S1. List of specimens, locality, and Genbank accession numbers for 8 terminal taxa used in this phylogenetic analysis. SIO: Marine Vertebrate Collection at Scripps Institution of Oceanography and UMN: A. Murase's personal collection. Bold accession numbers are the novel sequences generated in this study. (download)

Table S2. List of primers used in this study. (download)

Table S3. List of PCR conditions for each genetic marker used in this study. (download)

Table S4. Number of characters contributed by each genetic marker. Protein coding genes were partitioned by 1st + 2nd and 3rd codon position. P-U = Parsimony uninformative, P-I = Parsimony informative. (download)

Table S5. Results and interpretation of Xia's test of saturation, given either a symmetrical (Sym) or asymmetrical (Asym) tree (Xia, X., Z. Xie, M. Salemi, L. Chen, and Y. Wang. 2003. An index of substitution saturation and its application. *Molecular Phylogenetics and Evolution* 26: 1–7). Results suggesting saturation from the transitions vs. transversions plots (see Fig. S1) are in bold. Iss refers

to the index of substitution saturation and Iss.c refers to the critical index of substitution saturation,
respectively. (download)

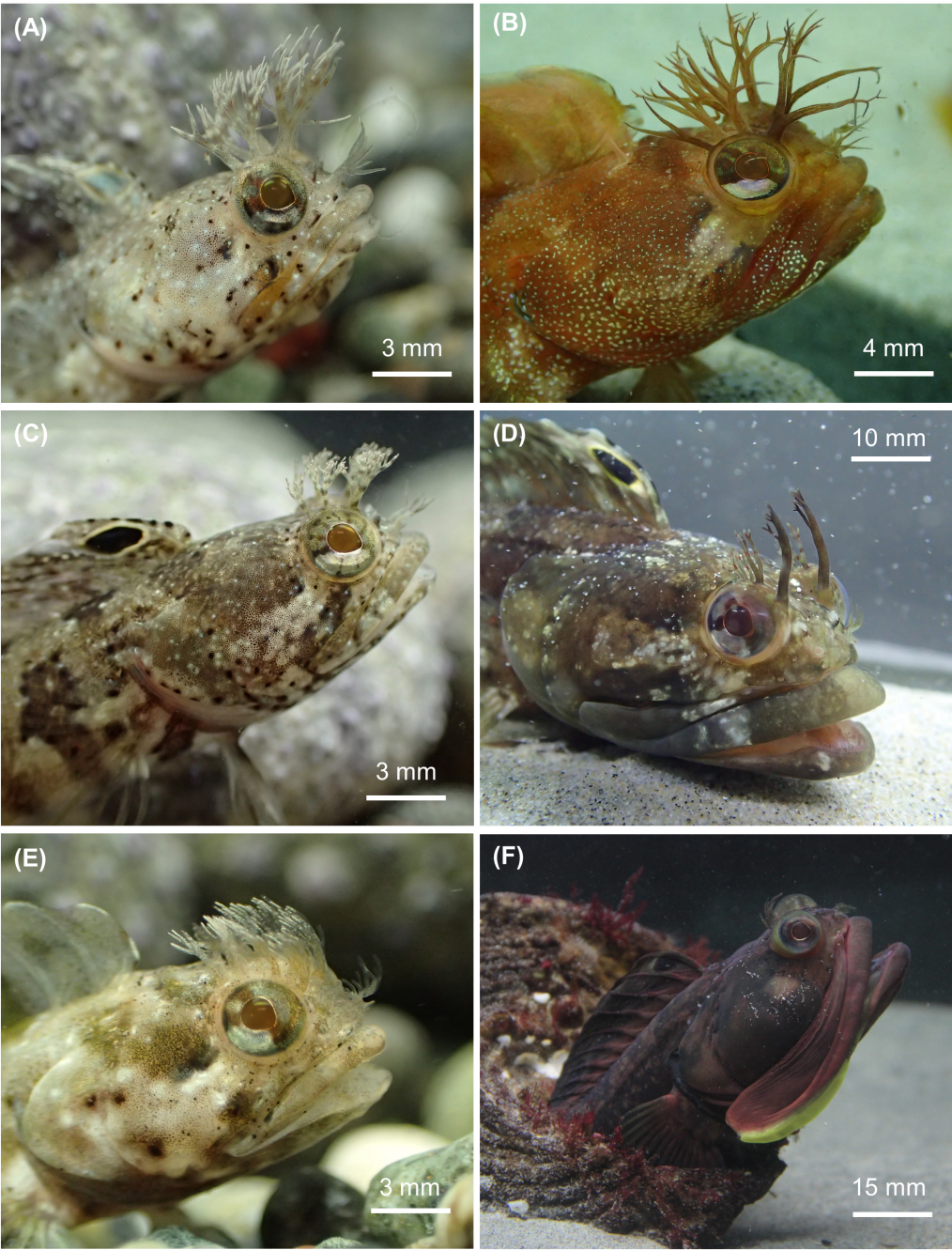


Fig. 1. Representatives of fringehead blennies included in this study. (a) *Neoclinus okazakii*, (b) *N. stephensae*, (c) *N. bryope*, (d) *N. uninotatus*, (e) *N. nudus*, (f) *N. blanchardi*. A, C, E are western Pacific species, while B, D, F are eastern Pacific species. Photos by W. Hongjamrassilp.

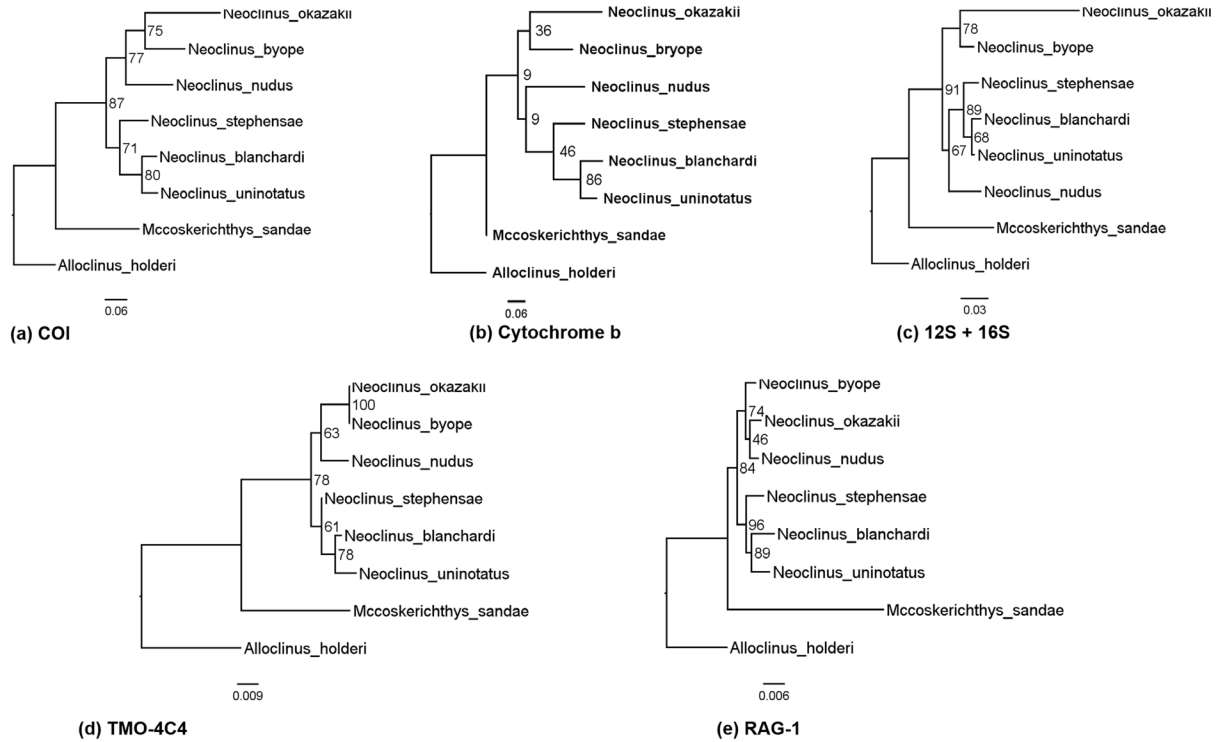


Fig. 2. Individual gene trees of the Neocliniini and its outgroup, *Alloclinus holderi*, implemented under Maximum likelihood analysis in RAXML: (a) *COI*, (b) cytochrome b, (c) 12S + 16S, (d) TMO-4C4, (e) RAG-1.

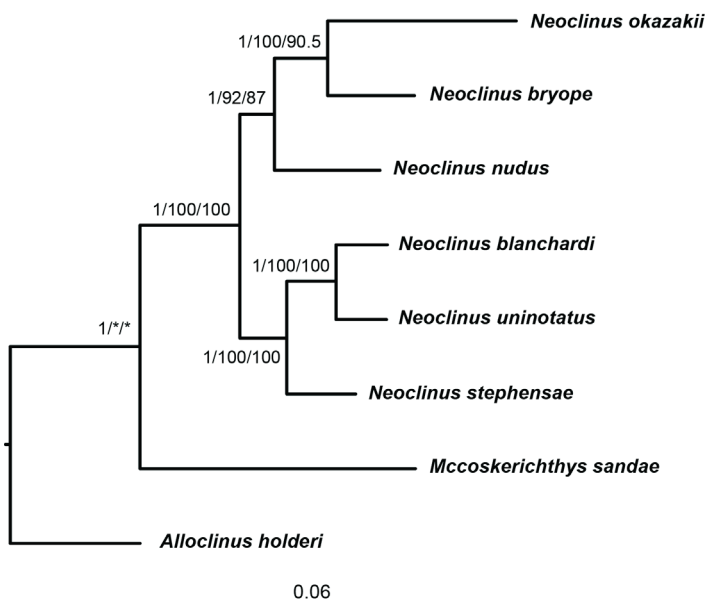


Fig. 3. Species tree for the Neocliniini from concatenated six genetic markers dataset analyzed using Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BI). Numbers on each node on the tree from left to right are: posterior probability from BI, bootstrap value from ML, and bootstrap value from MP. Asterisk means no support value was shown in the result.

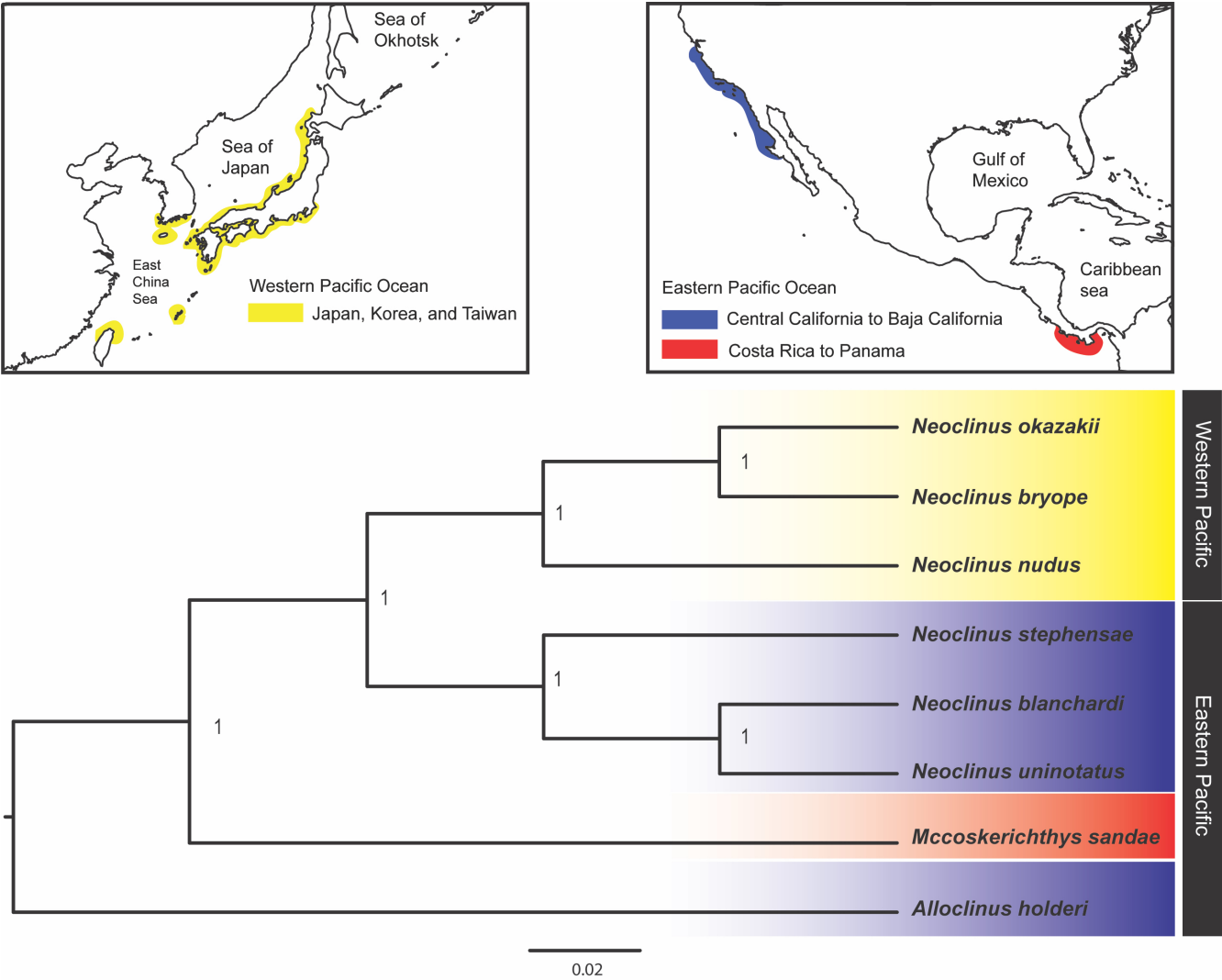


Fig. 4. General distributions of western Pacific *Neoclinus* species (yellow), eastern Pacific *Neoclinus* species and *Alloclinus holderi* (blue), and *Mccoskerichthys sandae* (red), mapped on the phylogeny from Bayesian inference analysis. Numbers on each node represent posterior probability values.

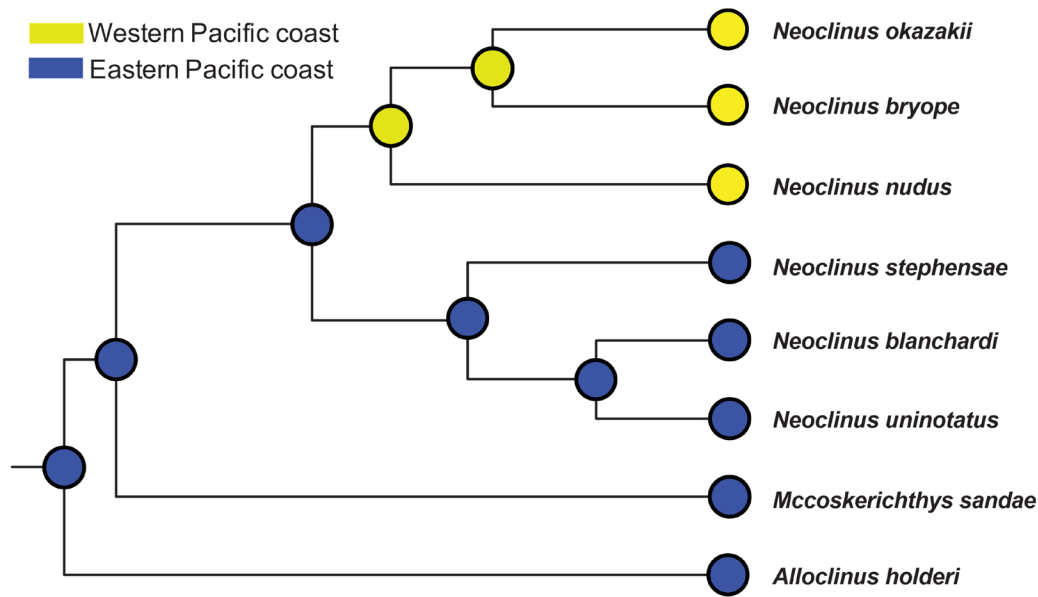


Fig. 5. Reconstructed ancestral distributions of the Neocliniini. Blue = eastern Pacific; yellow = western Pacific.



Fig. 6. Reconstruction of hypothetical diversification within the Neocliniini inferred from maximum parsimony. The distribution of the ancestor of *Mccoskerichthys* and *Neoclinus*, whether temperate or tropical eastern Pacific, is unresolved. WP (yellow) = western Pacific; WTEP (blue) = warm temperate eastern Pacific; TEP (red) = tropical eastern Pacific.

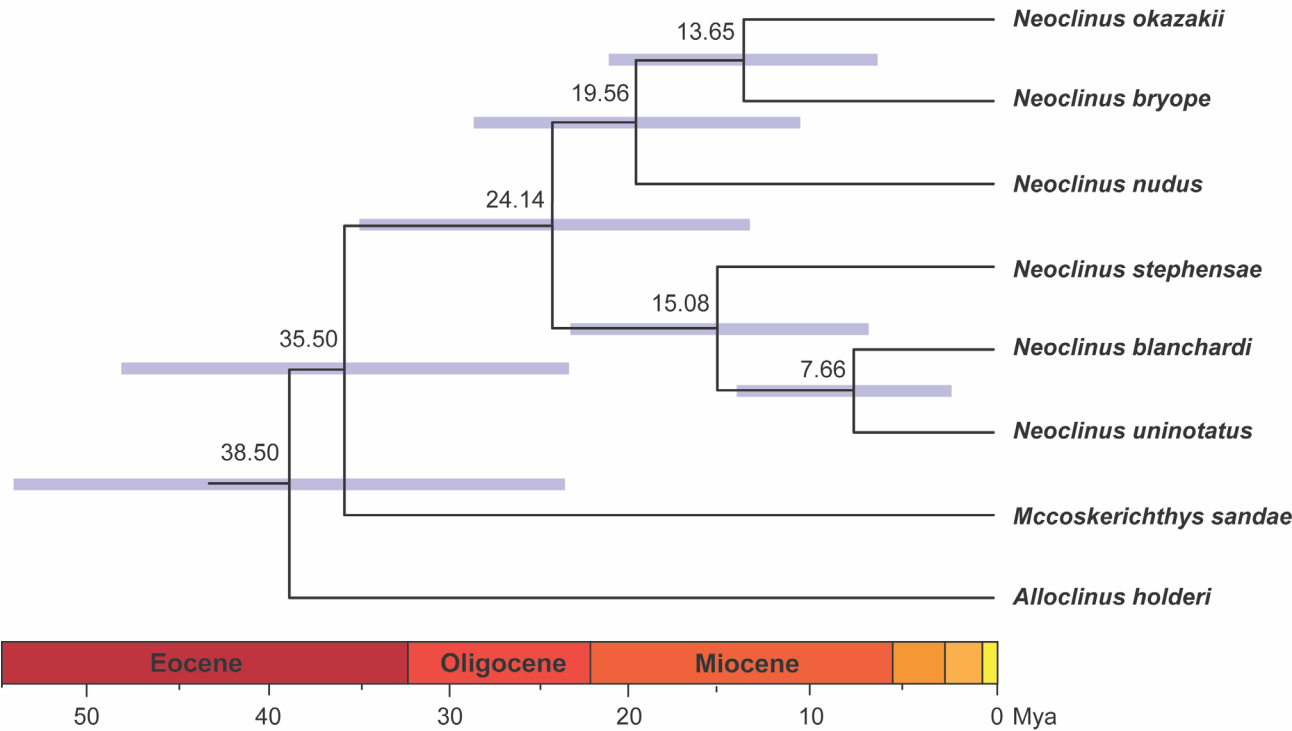


Fig. 7. Time-calibrated phylogenetic tree of the Neocliniini inferred from Bayesian relaxed molecular clock analysis. The bar at the bottom of the figure is the geological time scale in million-year units. Grey bars on the tree show range of divergent times with the means (in Mya) shown as number above each node.