



Phylogenetics and geography of speciation in New World *Halichoeres* wrasses

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ABSTRACT

The New World *Halichoeres* comprises about 30 small to medium sized wrasse species that are prominent members of reef communities throughout the tropical Western Atlantic and Eastern Pacific. We conducted a phylogenetic analysis of this group and related lineages using new and previously published sequence data. We estimated divergence times, evaluated the monophyly of this group, their relationship to other labrids, as well as the time-course and geography of speciation. These analyses show that all members of New World *Halichoeres* form a monophyletic group that includes *Oxyjulis* and *Sagittalarva*. New World *Halichoeres* is one of numerous labrid groups that appear to have radiated rapidly about 32 Ma and form a large polytomy within the julidine wrasses. We reconstruct the tropical Western Atlantic to be the ancestral area of New World *Halichoeres*, with four invasions of the Eastern Pacific and one reversal from East Pacific to Western Atlantic. These five speciation events were spread across the history of the group, with none corresponding closely to the time of the closure of the Isthmus of Panama. Three speciation events within the Atlantic occurred across the Orinoco-Amazon outflow and within the Pacific, five involve splits between lineages that occupy coastal reef systems and offshore islands. Of eight sister species pairs, seven show complete allopatry and one is fully sympatric.

1. Introduction

Labrid fishes of the tropical Eastern Pacific and Western Atlantic are a polyphyletic assemblage that includes members of several lineages that appear to have colonized the region from both the east and west (Choat et al., 2012; Westneat and Alfaro, 2005). Only three of these lineages have undergone multiple subsequent speciation events in this region, and now contribute substantially to regional biodiversity. These include a lineage of 10 *Scarus* species (Choat et al., 2012), 17 species in a monophyletic group of parrotfish formed by *Sparisoma*, *Nicholsina* and *Cryptotomus* (Robertson et al., 2006; Smith et al., 2008) and about 30 species in the New World *Halichoeres* (Barber and Bellwood, 2005; Westneat and Alfaro, 2005).

Although considerable attention has been directed at the potential role of the closure of the Isthmus of Panama as an agent causing speciation in this region, past work with other labrid lineages; *Scarus* (Choat et al., 2012) and *Sparisoma* & *Nicholsina* (Robertson et al., 2006),

estimated a combined total of two speciation events that could be attributed to the Isthmus of Panama, thus implying a rather minor role for the Isthmus in labrid speciation. Within the tropical East Pacific and Western Atlantic, the 30 small to intermediate sized (range of adult size is 45–500 mm Total Length) labrid species of the genus *Halichoeres*, and their close relatives, represent the largest potentially monophyletic labrid radiation in this region and they are therefore an excellent system for studying the geography of speciation and particularly the role of the Isthmus of Panama in reef fish diversification and biogeographic patterns.

Previous molecular phylogenetic studies with this group included five (Westneat and Alfaro, 2005) and 10 species (Barber and Bellwood, 2005), and cast doubt on its monophyly (Barber and Bellwood, 2005). Both studies supported an origin of the group in the Indo-Australian Archipelago. Other key inferences were that New World *Halichoeres* speciation events within the East Pacific and Atlantic have been more common than speciation across the Isthmus of Panama (Barber and

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Table 1
Specimens of New World *Halichoeres* and *Thalassoma* included in this analysis with Genbank accession numbers.

Species	Cox1	Cytb	12s	16s	CR	Rhod	TMO4C4	Rag2
<i>Thalassoma bifasciatum</i>	JQ842343	AY328863	MG665314	MG665338	MG665289	MG665239	MG665362	MG665264
<i>Halichoeres adustus</i>	JQ839442		MG665315	MG665339	MG665291	MG665241	MG665364	MG665266
<i>Halichoeres bathyphilus</i>	JQ839445	EF185124	MG665316	MG665340	MG665292	MG665242	MG665365	MG665267
<i>Halichoeres bivittatus</i>	JQ842511	AY823563	MG665317	MG665341	MG665293	MG665243	MG665366	MG665268
<i>Halichoeres brasiliensis</i>		AY823577	MG665318	MG665342	MG665294	MG665244	MG665367	MG665269
<i>Halichoeres burekai</i>		EF185121			MG665295	MG665245	MG665368	MG665270
<i>Halichoeres chierchiae</i>	JQ839456	GU938857	MG665319	MG665343	MG665296	MG665246	MG665369	MG665271
<i>Halichoeres cyanocephalus</i>	JQ839462	AY591382	MG665320	MG665344	MG665297	MG665247	MG665370	MG665272
<i>Halichoeres dimidiatus</i>			MG665321	MG665345	MG665298	MG665248	MG665371	MG665273
<i>Halichoeres discolor</i>	JQ839464		MG665322	MG665346	MG665299	MG665249	MG665372	MG665274
<i>Halichoeres dispilus</i>	JQ839468	EF185122	MG665323	MG665347	MG665300	MG665250	MG665373	MG665275
<i>Halichoeres garnoti</i>	JQ842887	AY591367	MG665324	MG665348	MG665301	MG665251	MG665374	MG665276
<i>Halichoeres insularis</i>	JQ839475	GU938863						
<i>Halichoeres maculipinna</i>	JQ842159	AY591359	MG665325	MG665349	MG665302	MG665252	MG665375	MG665277
<i>Halichoeres melanotis</i>	JQ839487	GU938864	MG665326	MG665350	MG665303	MG665253	MG665376	MG665278
<i>Halichoeres nicholsi</i>	JQ839496	EF185125	MG665327	MG665351	MG665304	MG665254	MG665377	MG665279
<i>Halichoeres notospilus</i>	AY850759	GU938856	MG665328	MG665352	MG665305	MG665255	MG665378	MG665280
<i>Halichoeres penrosei</i>			MG665329	MG665353	MG665306	MG665256	MG665379	MG665281
<i>Halichoeres pictus</i>	JQ839789	EF185123	MG665330	MG665354	MG665307	MG665257	MG665380	MG665282
<i>Halichoeres poeyi</i>	JQ839506	AY823579	MG665331	MG665355	MG665308	MG665258	MG665381	MG665283
<i>Halichoeres radiatus</i>	JQ842891	AY823575	MG665332	MG665356	MG665309	MG665259	MG665382	MG665284
<i>Halichoeres rubrovirens</i>		GU938858	MG665333	MG665357	MG665310	MG665260	MG665383	MG665285
<i>Halichoeres salmofasciatum</i>	JQ839527		MG665334	MG665358	MG665311	MG665261	MG665384	MG665286
<i>Halichoeres semicinctus</i>	KF929956	AY328859	MG665335	MG665359	MG665312	MG665262	MG665385	MG665287
<i>Halichoeres socialis</i>		EF185120	MG665336	MG665360	MG665312	MG665262	MG665384	MG665287
<i>Oxyjulis californicus</i>	JN582151	DQ132499	MG665337	MG665361	MG665290	MG665240	MG665363	MG665265
<i>Sagittalarva inornata</i>	JQ952620		JX684105	JX684106			JX684104	JX684103

Bellwood, 2005; Rocha et al., 2005; Westneat and Alfaro, 2005).

In the present study, we reconstruct the phylogenetic relationships among 26 of the 30 described species using a sequence data set from mitochondrial and nuclear loci. We use the resulting phylogenetic hypotheses to test for monophyly of the group, explore relationships of the group to other lineages of labrids, and reconstruct the biogeographic history of the group in order to identify the geographic barriers that have been important in speciation. We also discuss some of the ecological diversity found within this group.

2. Materials and methods

2.1. New World *Halichoeres* sequence data

Tissue samples were secured for 24 species of *Halichoeres* that inhabit the Eastern Pacific or the Western Atlantic, as well as the Eastern Pacific *Oxyjulis californicus*, which previous studies have shown to fall within the clade of New World *Halichoeres* (Alfaro et al., 2009; Cowman et al., 2009; Kazancıoğlu et al., 2009; Westneat and Alfaro, 2005), and *Thalassoma bifasciatum*, which served as outgroup (Table 1). There are four additional species not included in our analysis that are expected to fall within the NW *Halichoeres* group. These include from the East Pacific *Halichoeres malpelo*, and *H. aestuaricola* (Robertson and Allen, 1994), and from the Western Atlantic *H. caudalis* (Snyder and Burgess, 2016) and *H. sazimai* (Luiz et al., 2009).

DNA was extracted from fin clips or muscle tissue samples that had previously been stored in 70–90% ethanol using either Chelex (Bio-rad, Hercules, CA) or the Qiagen DNasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA) following the protocols suggested by the manufacturer. Four genes already commonly used in labrid phylogenetics, the mitochondrial ribosomal 12s and 16s and the nuclear *Rag2* and *Tmo-4C4*, as well as the nuclear marker rhodopsin (*Rh*) and the mitochondrial control region (*CR*) were amplified using the polymerase chain reaction (PCR); primers and PCR conditions were obtained from the literature (Chen et al., 2003; Westneat and Alfaro, 2005).

PCR products were cleaned of the unincorporated primers and the excess dNTPs after the PCR reactions using ExoSap (Amersham

Biosciences), and the purified products were then cycle-sequenced using the BigDye Terminator v.3.1 cycle sequencing kit (1/8th reaction) (Applied Biosystems) with each gene's original or additional internal primers used for amplification. The cycle sequencing protocol consisted of 25 cycles with a 10-s 94 °C denaturation, 5-s of 50 °C annealing, and a 4-min 60 °C extension stage. Sequencing was conducted at the Yale University DNA Analysis Facility using an ABI 3730xl DNA Genetic Analyzer (Applied Biosystems). In addition to the new sequences produced, sequence data were downloaded from GenBank for the mitochondrial loci cytochrome oxidase I (*coxI*) and cytochrome oxidase b (*cytb*), which were available for the majority of New World *Halichoeres* species and *Sagittalarva inornata* (see Table 2).

Geneious 5 (Drummond et al., 2011) was used to inspect the sequence chromatograms and assemble them into contigs; the consensus sequences for each individual gene were then aligned using MUSCLE (Edgar, 2004). After the alignments had been inspected by eye for accuracy, the 3' and 5' ends of the sequences were trimmed to minimize missing characters. The sequences of the ribosomal loci 12s and 16s were aligned to the sequences of the same loci used in the Westneat and Alfaro (2005), the Alfaro et al. (2009) and the Victor et al. (2013) studies, and the loop regions were also removed in order to minimize the risk of poor homology assessment. The final data matrix consisted of 457 bp for *Rag2*, 443 bp for *Tmo-4C4*, 297 bp for 12s, and 376 bp for 16s, 320 bp for *CR*, 506 bp for *Rh*, 612 bp for *coxI* and 513 bp for *Cytb*, a total of 3529 nucleotides used in the concatenated analyses. All the sequences that were generated for this study have been deposited in GenBank (accession numbers listed in Table 1).

2.2. Phylogenetic position of New World *Halichoeres*

To determine whether species of the New World *Halichoeres* form a monophyletic group we combined the newly generated sequences for *Rag2*, *Tmo-4C4*, 12s, and 16s, as well as *coxI* available in GenBank for species of New World *Halichoeres* to the combined dataset used in the Victor et al. (2013) study. This combined dataset was trimmed to reduce the number of non-*Halichoeres* species and was subjected to phylogenetic analyses using both maximum likelihood and Bayesian inference methods.

Table 2

Biogeographic distribution of NW *Halichoeres* species and closely related taxa included in tree shown in Fig. 3. Abbreviations are: Rev. = Revillagigedo; Gal. = Galapagos; Gua. = Guadalupe; E. Pac. = Eastern Pacific; Malp. = Malpelo; Car. = Caribbean + Bermuda; G.Mex. = Gulf of Mexico; Braz. = Brazilian Province + Trindade + Martin Vaz; Fern. = Fernando de Noronha/Rocas + St Peter and St Paul Archipelago.

Areas: Area code (Fig. 3)	Rev. A	Coc. B	Gal. C	Mal. D	Gua. E	E. Pac. F	Car. G	Gulf Mex. H	Brazil I	Fern. J
<i>H. adustus</i>	1	1	1	0	0	0	0	0	0	0
<i>H. bathyphilus</i>	0	0	0	0	0	0	1	1	0	0
<i>H. bivittatus</i>	0	0	0	0	0	0	1	1	1	0
<i>H. brasiliensis</i>	0	0	0	0	0	0	0	0	1	0
<i>H. burekae</i>	0	0	0	0	0	0	0	1	0	0
<i>H. chierchiaie</i>	0	0	1	0	0	1	0	0	0	0
<i>H. cyanocephalus</i>	0	0	0	0	0	0	1	1	1	0
<i>H. dimidiatus</i>	0	0	0	0	0	0	0	0	1	0
<i>H. discolor</i>	0	1	0	1	0	0	0	0	0	0
<i>H. dispilus</i>	0	1	1	0	0	1	0	0	0	0
<i>H. garnoti</i>	0	0	0	0	0	0	1	1	0	0
<i>H. insularis</i>	1	0	0	0	1	0	0	0	0	0
<i>H. maculipinna</i>	0	0	0	0	0	0	1	1	1	0
<i>H. melanotis</i>	0	0	0	0	0	1	0	0	0	0
<i>H. nicholsi</i>	1	0	1	1	1	1	0	0	0	0
<i>H. notospilus</i>	1	0	0	1	0	1	0	0	0	0
<i>H. penrosei</i>	0	0	0	0	0	0	0	0	1	0
<i>H. pictus</i>	0	0	0	0	0	0	1	1	0	0
<i>H. poeyi</i>	0	0	0	0	0	0	1	1	1	0
<i>H. radiatus</i>	0	0	0	0	0	0	1	1	1	1
<i>H. rubrovirens</i>	0	0	0	0	0	0	0	0	1	0
<i>H. salmofasciatus</i>	0	1	0	0	0	0	0	0	0	0
<i>H. semicinctus</i>	0	0	0	0	1	1	0	0	0	0
<i>H. socialis</i>	0	0	0	0	0	0	1	0	0	0
<i>Oxyjulis californica</i>	0	0	0	0	0	1	0	0	0	0
<i>Sagittalarva</i>	0	1	1	1	0	1	0	0	0	0

We used jModelTest (Posada, 2008) to select the best fitting model of sequence evolution from the candidate pool of models that can be utilized in MrBayes 3.2 (Ronquist et al., 2012) using corrected Akaike information criterion (AICc; Akaike, 1973), but did not include the proportion of invariant sites parameter in the candidate pool, as this parameter is already taken into consideration by the gamma parameter (Yang, 2006). jModelTest selected HKY + G as the best model for *Rag2*, *16s*, and *cox1* and GTR + G for *12S* and *TmoC4C*.

We concatenated the five individual gene datasets in Mesquite 3 (Maddison and Maddison, 2014), and ran maximum likelihood analyses using RAxML (Stamatakis, 2006), with each individual gene partition assigned a GTR + G model, the model implemented in RAxML that is the most similar to the models selected by jModeltest.

We ran 500 fast bootstrap replicates. We also used MrBayes 3.2 (Ronquist et al., 2012) run on the Cipres Science Gateway V. 3.3 (Miller et al., 2010) to perform Bayesian analyses. We partitioned the concatenated dataset by locus and assigned the HKY + G or GTR + G model according to the jModeltest results. We ran multiple replicates with two analyses of 10 million generations each, with four chains (one cold, three heated) sampling every 1000 generations. We used Tracer 1.6 (Drummond and Rambaut, 2007) to check the trace files and ensure that the chains had reached convergence, and discarded the first 25% of trees as burnin. We combined the post-burnin trees to obtain a 50% majority rule consensus tree and compared the topologies of the different replicates to each other to assess support for the results of the analyses.

To generate a timetree that could provide an estimate of the time of origin of the NW *Halichoeres* clade we analyzed the concatenated alignment as five unlinked gene partitions, after having assigned HKY + G or GTR + G models to the appropriate loci. We used uncorrelated lognormal priors in BEAST 1.8 (Drummond and Rambaut, 2007) and assigned an incompletely sampled birth-death prior to the rates of cladogenesis. We ran two analyses of 50 million generations each, with sampling every 5000 generations. We used Tracer 1.6 (Drummond and Rambaut, 2007) to inspect the trace files, ensuring that the chains had reached convergence and the ESS values for all

parameters were greater than 200. We removed the first 10% of the trees from each analysis as burnin, used LogCombiner to merge the files with the remaining trees, and TreeAnnotator (Drummond and Rambaut, 2007) to obtain a timetree.

Six calibration points were used to convert the molecular tree into a time-calibrated phylogeny; five of these calibrations were based on the labrid fossil record and had an exponential distribution, while the sixth calibration was a secondary calibration point taken from previously published literature and had a normal distribution. Following Alfaro et al. (2009) we used the oldest putative stem hypsigenyine, *Phyllopharyngodon longipinnis* from the Middle Eocene of Monte Bolca (Bellwood, 1990), to provide a minimum estimate of the age of crown labrids, and the age of the acanthomorph-rich fossil deposits of Nardo, which have a confirmed minimum age of at least 83 Ma (Schlüter et al., 2008) to provide a soft upper boundary. The Bolca deposits have been dated between 48.9 and 50.7 Ma, with the Pesciara site thought to be close to 50 (Papazzoni and Trevisani, 2006; Papazzoni et al., 2017). The exponential prior on this node had an offset of 50 and a mean of 11.

The fossil *Trigonodon jugleri* known from the Early Miocene (20 Ma), a stem chiseltooth wrasse, was used to provide a minimum age for the crown of the Hypsigenines (minus *Lachnolaimus*) (Bellwood and Schultz, 1991; Carnevale, 2015; Schultz & Bellwood, 2004); the age of the Monte Bolca deposits (50 Ma) was used to provide a soft upper bound. The exponential prior on this node had an offset of 14 and a mean of 12.

The fossil parrotfish *Calotomus preisli* is known from the same Middle Miocene deposits as *Trigonodon* (14 Ma) and is used to date the group that includes the seagrass – associated clade of parrotfishes (Bellwood and Schultz, 1991; Carnevale, 2015); the age of the Monte Bolca deposits (50 Ma) was again used to provide a soft upper bound. The exponential prior on this node had an offset of 14 and a mean of 12.

We used fossil remains assigned to an unnamed taxon of the genus *Bolbometopon*, known from the Late Miocene (5.33 Ma) to provide a minimum age for the split between *Bolbometopon* and *Cetoscarus* (Bellwood and Schultz, 1991; Carnevale, 2015); the age of the origin of the Miocene (23 Ma) was used to provide a soft upper bound. The

exponential prior on this node had an offset of 5.3 and a mean of 6.

We also used a fossil identified as *Tautoga* sp. from the Miocene of the Calvert Formation, Maryland, USA (15 Ma, Carnevale, 2015; Carnevale and Godfrey (2017)). As *Tautoga* is a generalized taxon with no recognized morphological synapomorphies, we conservatively used this fossil as a minimum age estimate of the clade comprising *Centrolabrus*, *Tautoga* and *Tautogolabrus*. The age of the Monte Bolca deposits (50 Ma) was again used to provide a soft upper bound. The exponential prior on this node had an offset of 15 and a mean of 11.5.

Finally, we used the 95% highest posterior density (HPD) inferred by the Near et al. (2013) study of the acanthomorph radiation for the split between the lineages that represent the outgroups in our study. The normal prior on this calibration point has a mean of 61 Ma for the separation between *Epinephelus* and *Kyphosus* and a standard deviation of 2.5.

2.3. Phylogenetic relationships of New World *Halichoeres*

To obtain a more robust estimate of relationships within NW *Halichoeres*, we added to the five loci used in the labrid analyses the new sequences of *Rhod* and *CR* that had been generated, as well as *Cytb* sequences downloaded from GenBank. As sequences for these loci are not available for the majority of labrid taxa, only NW *Halichoeres*, as well as very closely related species were included (*Thalassoma bifasciatum*, used as an outgroup, *Sagittalarva inornata*, and *Oxyjulis californica*).

We used jModelTest (Posada, 2008) to select the best fitting model of sequence evolution from the candidate pool of models that can be utilized in MrBayes 3.2 (Ronquist et al., 2012) using corrected Akaike information criterion (AICc; Akaike, 1973), but again did not include the proportion of invariant sites parameter in the candidate pool.

To generate a time calibrated phylogeny, we analyzed the concatenated alignment as eight unlinked gene partitions, after having assigned HKY + G or GTR + G models to the appropriate loci according to the jModeltest results. We used uncorrelated lognormal priors in BEAST 1.8 (Drummond and Rambaut, 2007) and assigned a birth-death prior to the rates of cladogenesis. We ran two analyses of 10 million generations each, with sampling every 1000 generations. We used Tracer 1.6 (Drummond and Rambaut, 2007) to inspect the trace files, ensuring that the chains had reached convergence and the ESS values for all parameters were greater than 200. We removed the first 10% of the trees from each analysis as burnin, used LogCombiner to merge the files with the remaining trees, and TreeAnnotator (Drummond and Rambaut, 2007) to obtain a timetree. As no fossils can currently be assigned to the clade of NW *Halichoeres*, we used the age recovered in the analysis of the larger labrid dataset for the split between the lineage that includes *Thalassoma bifasciatum* and the NW *Halichoeres* group as a secondary calibration point. We assigned a normal prior with a mean of 24.5 and a standard deviation of 3.5, which generates a 95% distribution that overlaps with the 95% HPD for the corresponding node in the 5 loci analyses.

2.4. Historical biogeography

We used the R package BioGeoBEARS (Matzke, 2013) in combination with our timetree to investigate the biogeographic history of the NW *Halichoeres* group. We first delimited 10 geographic areas based on the distribution of the species included in this study, their pattern of endemism, and the known marine biogeographic regions of the Eastern Pacific and Western Atlantic Ocean (Briggs and Bowen, 2012). Some of these areas are individual oceanic islands or archipelagos, and are of easy delimitation; these are the Revillagigedo islands; Cocos Island; the Galapagos islands; Malpelo Island, the island of Guadalupe in the Pacific Ocean and the islands of Fernando de Noronha and Rocas plus the St Peter and St Paul Archipelago in the Atlantic Ocean; as well as larger water bodies that include large tracts of coastal areas such as the

tropical Eastern Pacific, which spans from Central California to Panama; the Caribbean region, spanning the Northern coast of South America and the Eastern coast of Central America up to the Southern part of the Yucatan peninsula, all Caribbean islands as far North as the Bahamas, and including Bermuda; the Gulf of Mexico, from the Northern coast of the Yucatan peninsula all the way to Western Florida; and the Brazilian Province, which includes the entire coast of Brazil as well as the islands of Trindade and Martin Vaz.

We then used BioGeoBEARS (Matzke, 2013) to fit three different models of reconstructed biogeographical history: the dispersal–extinction–cladogenesis model (DEC; Ree et al., 2005), the dispersal–vicariance model (DIVA; Ronquist, 1997) and the BayArea model (Landis et al., 2013). These models can be compared to one another and to versions of each model that also include a founder-event speciation (indicated as +J). The fit of each of the different models are assessed using the Akaike information criterion (AIC) (Matzke, 2013). We did not constrain the directionality or timing of dispersal in our analyses and we set the maximum number of ancestral areas to five, as this is the current maximum number of areas inhabited by a single species of NW *Halichoeres*. In our analyses we did not enforce constraints on the adjacency of the areas, that is lineages were free to disperse from one area to any other area without having to disperse to neighboring areas first. We chose this approach, which has been used in many other biogeographic analyses of marine organisms (i.e., Litsios et al., 2012; Santini et al., 2016), due to the fact that many marine fishes with pelagic larvae have high potential for long range dispersal, potentially allowing them to bypass geographically closer areas that may, at the time of the successful dispersal event, not have had appropriate ecological conditions for colonization. Additionally, it is possible that colonization of geographically proximate areas did occur and was followed by extinction of local populations.

2.5. Patterns of habitat use

We surveyed patterns of habitat use and feeding behavior in NW *Halichoeres* from direct observations of fish in the field. Patterns of habitat use were quantified on the barrier reef of Belize where eight of the species occur. Divers swam 20 min transects along rough depth gradients in a variety of back reef, reef crest, and reef slope habitats recording and identifying all the *Halichoeres* that were encountered in a 3 m wide corridor. Results are reported from 32 transects all made within 5 km of Carrie Bow Cay.

3. Results

3.1. Phylogenetic position of NW *Halichoeres*

The RAxML and MrBayes analyses of the five-loci labrid dataset (Figs. 1, S1) are broadly congruent with the previous molecular hypotheses of relationships, as could be expected from the fact that the sequence datasets are overlapping. We only comment on the major results that have relevance for the phylogenetic position of the NW *Halichoeres*, and refer the readers to the work of Westneat and Alfaro (2005), Alfaro et al. (2009), Cowman et al. (2009), Kazancioglu et al. (2009) and Victor et al. (2013) for a more in-depth discussion of labrid interrelationships.

Our topologies (Figs. 1, S1) show high support, both in Bayesian posterior probabilities (PP) over 0.95 and maximum likelihood bootstrap proportions (BSP) over 90% for a number of key nodes. All analyses (Figs. 1, S1) support a clade formed by all species of New World *Halichoeres* plus *Sagittalarva inornata* and *Oxyjulis californica*. This group is one of several substantial lineages of julidines that appear to have diverged rapidly and form a large polytomy deeply nested within the julidines. Monophyly of the NW *Halichoeres* clade is supported by a PP of 1 and BSP of 87%. The phylogenetic placement of this clade is however poorly supported. In the MrBayes analyses (Fig. 1) it appears in a polytomy with

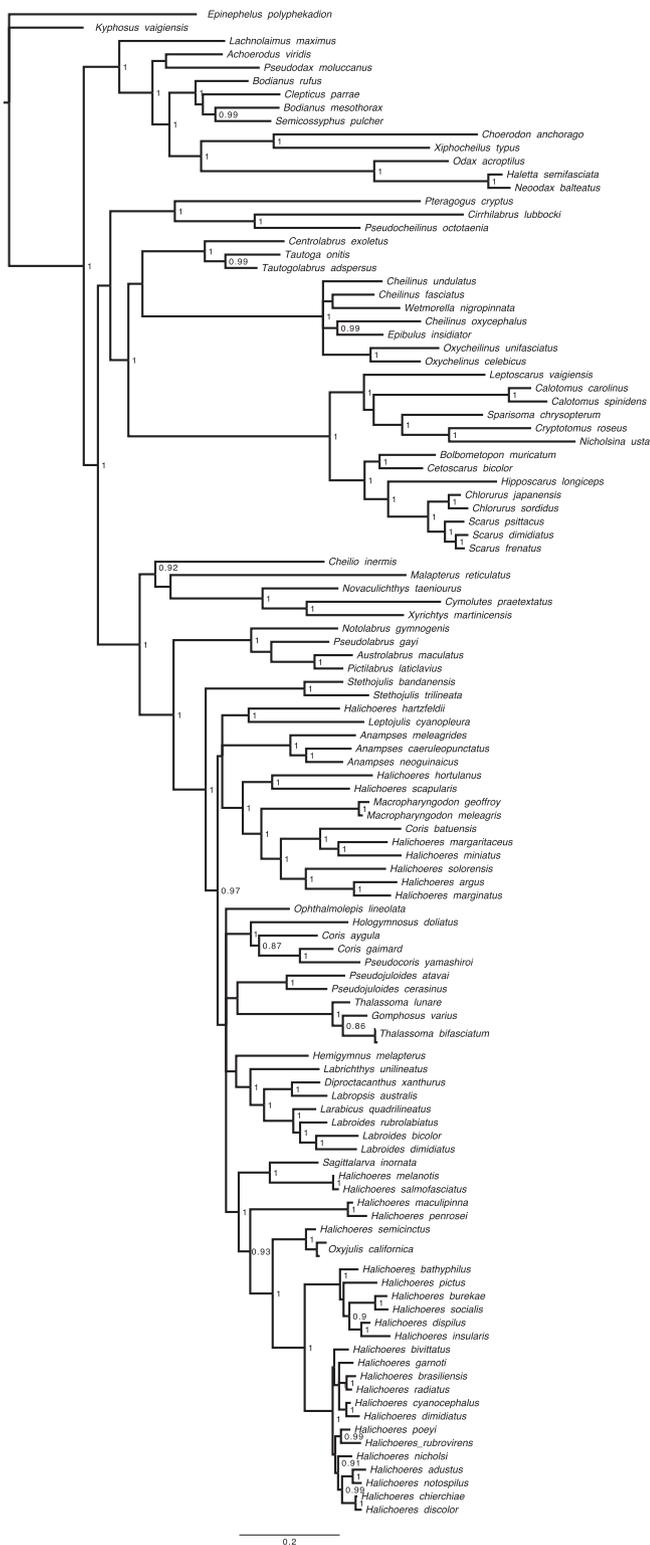


Fig. 1. Phylogenetic tree of labrids based on the Bayesian analysis of the five-loci dataset performed in MrBayes 3.2. Values next to nodes indicate PP support.

several additional julidine lineages, including *Ophthalmolepis lineolata*; a clade formed by *Hologymnosus*, several species of *Coris* as well as *Pseudocoris yamashiroi*; a *Pseudojuloides* + *Thalassoma* + *Gomphosus varius* group; a clade formed by several species of *Labroides* + *Hemigymnus melapterus* + *Labrichthys unilineatus* + *Diproctacanthus xanthurus* + *Labropsis australis* + *Larabicus quadrilineatus*. In the RAXML tree (Fig. S1) the clade formed by *Hologymnosus* + *Coris* + *Pseudocoris yamashiroi* appears as the

sister group to the NW *Halichoeres*, but the BSP support is below 50%.

Within the NW *Halichoeres* group several lineages can be identified; the monophyly of all these groups is highly supported and the backbone of the groups has high PP/BSP support. The first lineage to branch off includes *Sagittalarya* + *Halichoeres melanotis* + *H. salmofasciatus*; the second lineage to branch off includes *H. maculipinna* + *H. penrosei*; the third lineage includes *Oxyjulius californica* + *H. semicinctus*. The two remaining lineages include the bulk of NW *Halichoeres* diversity; monophyly of these two groups is strongly supported, but the relationships within them are not. The first group includes *Halichoeres bathyphilus*, *H. pictus*, *H. burekai* + *H. socialis*, and *H. dispilus* + *H. insularis*. The last *Halichoeres* subclade includes *H. bivittatus*, *H. garnoti*, *H. brasiliensis* + *H. radiatus*, *H. cyanocephalus* + *H. dimidiatus*, *H. poeyi* + *H. rubrovirens*, *H. nicholsi*, *H. adustus* + *H. notospilus*, *H. chierchiai* + *H. discolor*.

The BEAST analyses recover a very similar topology to these found by MrBayes and RAXML. The NW *Halichoeres* clade appears as sister to a group formed by all the lineages that are closely related in the non-dated analyses, but the support for such groups is very weak (0.35 PP). As in the previously described analyses, monophyly of the NW *Halichoeres* is high, and the pattern of branching of the various *Halichoeres* lineages is the same as in the ML and non-dated bayesian analyses. The age of the split between the NW *Halichoeres* group and its sister taxon is ~32 Ma (25–41 Ma 95% HPD), while the crown age of the NW *Halichoeres* is ~26 Ma (21–36 Ma 95% HPD).

3.2. NW *Halichoeres* interrelationships

Adding to the five loci used in previous analyses three additional markers, and performing a time-calibrated phylogenetic analysis in BEAST results in a slightly different topology for the NW *Halichoeres*. The first lineage to branch off from the remaining members of this clade now includes *Halichoeres maculipinna* + *H. penrosei* sister to a group formed by *Sagittalarya* and *H. insularis* + (*H. melanotis* + *salvofasciatus*). Most nodes in this subclade have PP support below 0.8, with only *H. maculipinna* + *H. penrosei*, *H. melanotis* + *salvofasciatus* and the split between *Sagittalarya* and *H. insularis*, *H. melanotis* and *H. salvofasciatus* receiving a high PP of 1. The next lineage to branch off from the *Halichoeres* group includes *Oxyjulius californica* and *H. semicinctus*; monophyly of this group is supported by a PP of 1, as is the sister group relationships between this lineage and its sister taxon, that includes all remaining species. The first of the two remaining subclades includes *Halichoeres bathyphilus*, *H. dispilus*, *H. pictus*, and *H. burekai* + *H. socialis*. The last subclade includes the remaining 13 species of NW *Halichoeres* sampled for our study, among which is the recently discovered *H. rubrovirens* (Rocha et al., 2010), which appears to be the sister group of *H. adustus* + *H. notospilus*.

The age of the NW *Halichoeres* clade is ~23 Ma (16–30 Ma 95% HPD), while the crown age of the first subclade is 20 Ma (16–27 Ma 95% HPD). The split between *Sagittalarya* and the *H. insularis*, *H. melanotis* and *H. salmofasciatus* is 13 Ma (8–19 Ma 95% HPD). The split between *Oxyjulius* + *H. semicinctus* and the remaining *Halichoeres* is dated at ~17 Ma (11–23 Ma 95%HPD), while the crown age of the two most species-rich subclades is respectively ~9 Ma (6–13 Ma 95% HPD) and 8 Ma (5–11 Ma 95% HPD).

3.3. Biogeographic reconstruction

The BioGeoBear analysis indicates that inclusion of the founder-event speciation (+J) in the various models significantly improves their score (Table S1), even though the scores of the DEC + J, DIVA + J and BayArea-like + J are very similar to one another. For this reason, we present the results BayArea-like + J model (Fig. 3), which has slightly better scores than all the others. We also include the results of the DEC + J and DEC models in the supplementary material (Figs. S3 and S4). We chose the DEC model as this has the best score among the models that did not include the +J parameter.



Fig. 3. Preferred model of biogeographic reconstruction (BayArea like + J) according to BioGeoBear analysis of NW *Halichoeres* species distribution.

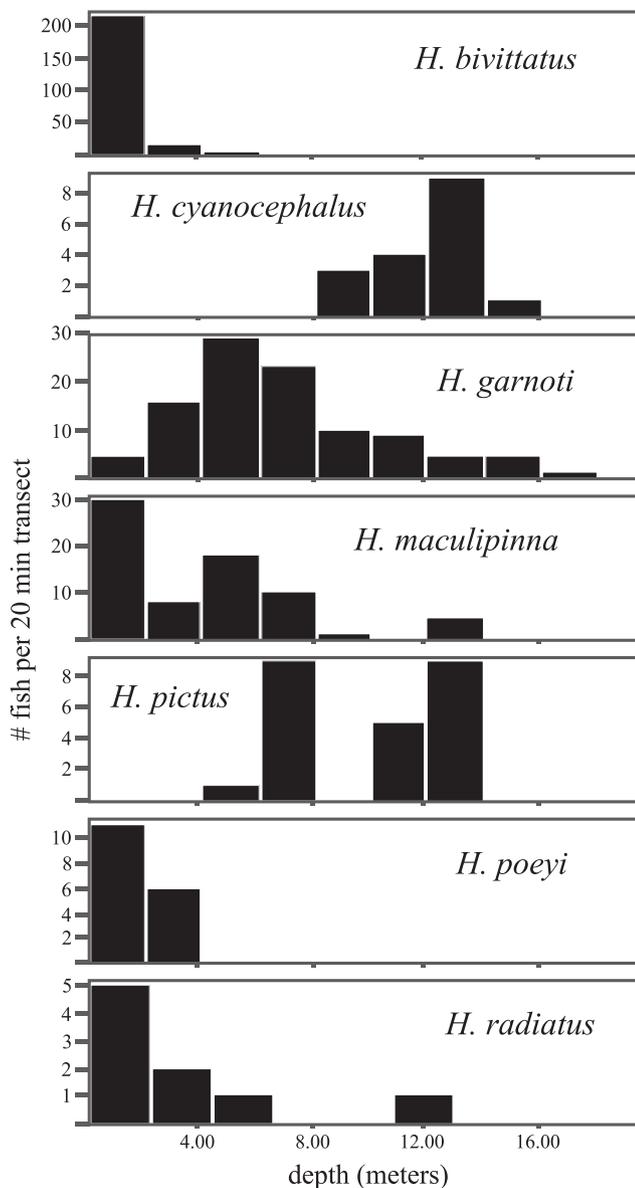


Fig. 4. Abundance of seven species of NW *Halichoeres* along a depth gradient from back reef to fore reef habitats on the Belizean barrier reef. Data are presented as average per 20 min transect swim.

4. Discussion

4.1. Inter- and intra-relationships of NW *Halichoeres*.

Our analyses confirm the non-monophyly of the genus *Halichoeres*, corroborating the findings of previous studies (Alfaro et al., 2009; Barber and Bellwood, 2005; Cowman et al., 2009; Kazancioglu et al., 2009; Westneat and Alfaro, 2005). In our analyses (Fig. 1, S1) the genus *Halichoeres* appears to be composed by at least four lineages, three of which are found in the Indo-Western Pacific (IWP): *Halichoeres hartzfeldii*, *H. hortulanus* + *H. scapularis*, and a larger clade formed by *Halichoeres margaritaceus*, *H. miniatus*, *H. solorensis*, *H. argus* and *H. marginatus*, in addition to *Coris batuensis*. This result is similar to that of Barber and Bellwood (2005) study of the IWP *Halichoeres*, in which two major IWP clades are identified.

In our analyses, however, all Atlantic and Eastern Pacific species of *Halichoeres* appear to form a monophyletic group that includes *Oxyjulis californica* and the recently described *Sagittalarva*. This conflicts with the Barber and Bellwood (2005) results that inferred a polyphyletic NW

Halichoeres. Their study, based on three mitochondrial loci (*cox1*, *16s*, and *12s*) found *Halichoeres maculipinna* to be more closely related to *Thalassoma* than to other NW *Halichoeres*. That result may be caused by the use of only mitochondrial loci; the analysis of the individual loci used for our study also indicates that some of the mitochondrial genes tend to place *H. maculipinna* outside of the NW clade, but analyses of the nuclear loci alone, or of the concatenated five and eight loci data-sets always recovered a monophyletic NW *Halichoeres* (monophyletic when *Oxyjulis* and *Sagittalarva* are also included).

Within the NW *Halichoeres* clade a number of groups can be identified, even though some of the internal nodes are not well supported in any of the analyses. While the presence within the NW *Halichoeres* of *Oxyjulis californica* had been suggested by previous studies (Alfaro et al., 2009; Cowman et al., 2009; Kazancioglu et al., 2009), a novel result of our work is the inclusion in this group of the recently described *Sagittalarva* (Victor et al., 2013). This species, characterized by a unique larval morphology, was found to occur in deep water (30–100 m) in the Eastern Pacific and is known both from an adult caught off the southernmost tip of Baja California as well as larvae collected several thousand kilometers to the south over the Galápagos Rift hydrothermal vents (Victor et al., 2013). Victor et al. (2013) performed some preliminary phylogenetic analyses of *cox1*, *12s*, *16s*, *Rag2* and *Tmo4C4*, and suggested that *Sagittalarva* did not group with the other julidine labrids of the New World or *Pseudojuloides* or *Halichoeres* of the Indo-Pacific. In contrast, our results strongly support that *Sagittalarva* belongs within the NW *Halichoeres*, and always appears to be closely related to other Eastern Pacific species, including *H. melanotis* and *sal-mofasciatus*.

4.2. NW *Halichoeres* biogeographic history

Our reconstructions of the biogeographic history of NW *Halichoeres* provides support for an Atlantic origin for the group. This broadly agrees with earlier studies which suggested that the NW *Halichoeres* lineage separated from Indo-Pacific lineages at the beginning of, or prior to, the Miocene and that subsequent diversification was restricted to the west Tethys regions (East Pacific, Caribbean and Atlantic) (Barber and Bellwood, 2005; Cowman and Bellwood, 2013b; Westneat and Alfaro, 2005). Our study provides further detail, with the suggestion that diversification in specific NW *Halichoeres* clades also occurred within specific regions reflecting, and possibly laying the foundations for, divisions among modern New World reef fish faunas (Kulbicki et al., 2013; Cowman et al., 2017). At a deeper level, reconstructions have suggested that the origins of the *Halichoeres* clade sensu lato was in the Indo-Australian Archipelago (Barber and Bellwood, 2005; Cowman and Bellwood, 2013a,b; Westneat and Alfaro, 2005). However, as noted by Cowman and Bellwood (2013a) these reconstructions may be strongly influenced by patterns of extinction and survival, with the survival of lineages within the Indo-Australian Archipelago reflecting its role as refuge, rather than a site of origin (cf. Pellissier et al., 2014). Thus, while an Atlantic origin of the group was favored in all of our reconstructions, the lack of a clear sister group to the NW *Halichoeres*, together with Atlantic-Pacific transitions at two of the three deepest nodes in the phylogeny, suggest that considerable caution should be used in viewing this result. Regardless of the original immigration route, the evidence is strong that the current NW *Halichoeres* lineage occupied both the Atlantic and East Pacific by 20 Ma.

The NW *Halichoeres* clade appears to have originated during the Late Oligocene/Early Miocene (21–36 Ma, mean age 26 Ma), a time that saw major changes in tropical coastal ecosystems worldwide (Alfaro et al., 2007; Bellwood et al., 2017; Cowman and Bellwood, 2011; Williams and Duda, 2008). This period was marked by a functional restructuring of rocky and coral reef ecosystems and the diversification of many major reef fish families (Bellwood et al., 2017). However, the major regions differed markedly in their patterns of diversification. In the Indian and Pacific Oceans increased diversity appears to have arisen

primarily from the migration of lineages from the Indo-Australian Archipelago (Bellwood et al., 2015; Cowman and Bellwood, 2013a). In marked contrast, over the last 23 Myr the Atlantic and the post-Miocene East Pacific have been characterized by regional diversification. The NW *Halichoeres* appear to have been a major component of this regional diversification with their isolation in the west Tethys following the Terminal Tethyan Event being followed by a prolonged period of diversification.

In our analysis of the biogeographic history of 26 species from the NW *Halichoeres* group, 5 of the 25 inferred speciation events are reconstructed to have occurred across the Isthmus of Panama (Fig. 3). None of these five speciation events corresponds to a time consistent with the closure of the Isthmus around 3.2–3.5 Ma, suggesting that the restricted connection between the Atlantic and Pacific in this region of Panama was a potent barrier to gene flow for a long period of time preceding the ultimate formation of the isthmus, a pattern seen in a number of reef fish groups (Lessios, 2008; Cowman and Bellwood, 2013a). Interestingly, these five speciation events are distributed fairly evenly across the depth of the tree, with three events occurring between 5 and 7 Ma, one event at about 16 Ma, and one event at about 21 Ma (Fig. 3). We note that the events around 5–7 Ma and 21 Ma correspond closely with the timing of two periods of extensive faunal dispersal between South and Central America (Bacon et al., 2015). Even if these periods of elevated rates of terrestrial dispersal did not coincide with a closure of the Isthmus (O’Dea et al., 2016) they align with speciation events in the NW *Halichoeres* group. In sum, while the Isthmus has had a significant impact on diversification in this clade, the temporal range of its impact covers most of the history of the group. Our results suggest reinterpretation of some previous assumptions about the identity of geminate sister species across the Isthmus of Panama, including *H. dispilus* & *H. pictus* and *H. bivittatus* & *H. nichlosi* (Lessios, 2008). However, until all 30 known species of NW *Halichoeres* are included in a phylogenetic analysis, all such assumptions about sister species remain tentative.

Among the remaining 20 speciation events in the phylogeny, 12 were reconstructed as having taken place within the tropical Western Atlantic and eight within the East Pacific. In the Atlantic, the Amazon-Orinoco outflow is implicated in three speciation events, the oldest being the split between *H. maculipinna* and *H. penrosei* at about 6 Ma and the other two being more recent (Fig. 3). Nine speciation events appear to have taken place within the Caribbean and Gulf of Mexico. As in a previous study of parrotfishes (Robertson et al., 2006), most of these do not reflect recent sister-species splits and are found deeper in the phylogeny. The one exception is the split between *H. socialis*, which is known only from mangrove island reef systems inside the barrier reef of Belize (Randall and Lobel, 2003), and *H. burekae*, which is found in the North-Western Gulf of Mexico at the Texas Flower Gardens Bank and in the South-Western Gulf of Mexico, on reefs off the coast of Veracruz, Mexico (Weaver and Rocha, 2007).

The patterns observed in the alternative models (DEC + J and DEC; Figs. S3, S4) are largely congruent; one important difference is that the DEC model (Fig. S4) supports vicariance as having had an important role in the separation of several lineages, such as that of *Halichoeres rubrovirens* from its sister clade of *H. adustus* and *H. notospilus*; that of *H. burekae* from *H. socialis*; and *H. melanotis* from *H. salmofasciatus*. In the analyses performed under the DEC + J and Bayarea + J models these splits could have been interpreted as due to long range dispersal, due to the ancestral node having been present in only one of the areas occupied by one of the descendant lineages. Under DEC the ancestral distribution appears to have been composed of both areas occupied by the daughter lineages, thus more closely matching the traditional vicariant scenarios in historical biogeography.

The specific geography and general isolation of tropical marine habitats in this part of the world does not seem to have produced an exceptional history of speciation. The median age of species in the NW *Halichoeres* is 4.2 Myr which is only slightly older than the 3.4 Myr

found in a broad survey of Indo-Pacific lineages (Hodge and Bellwood, 2015).

Among the eight speciation events that occurred within the Eastern Pacific, five involve splits between widespread species that occur on coastal reefs from Baja to Northern South America and offshore islands of varying levels of isolation, including Cocos, Socorro, Malpelo, and the Revillagigedo Islands. Unlike the angelfish endemics on these islands (Alva-Campbell et al., 2010), the NW *Halichoeres* colonization events appear to have occurred over a prolonged period and to have involved multiple lineages. The remaining three speciation events include two events within the large, mostly East Pacific *H. nichlosi* group, and the split between *H. semicinctus* and *Oxyjulis californica*. This last sister-species pair is the only one of eight sister species pairs in the entire radiation that shows almost complete range sympatry.

It is noteworthy that the majority of speciation events in NW *Halichoeres* appear to reflect isolation though either vicariance or long range dispersal as a predominant speciation mechanism, despite the potential for ecological speciation within this group (Rocha et al., 2005). This may reflect a broader issue underpinning the success of this group in the New World. The exceptional ability of NW *Halichoeres* to colonize a broad range of habitats, from seagrasses to rocky or coral reefs, appears to have underpinned their evolutionary and ecological success covering a period of 20 Myr in some of the world’s most isolated and depauperate tropical coastal ecosystems.

4.3. Morphological and ecological diversity in NW *Halichoeres*

The low rate of sympatry among sister species and the large number of island endemics among NW *Halichoeres* imply a strong role for speciation under allopatry. Nevertheless, there is considerable ecological diversity within the radiation, particularly among the eight species that are sympatric in the Caribbean. Most species of NW *Halichoeres* are generalist predators on an exceptionally wide range of benthic invertebrates, including polychaetes, brachyuran crabs and shrimp, echinoderms, gastropod and bivalve mollusks, amphipods, isopods, chitons, sipunculans, stomatopods, and fish (Clifton and Motta, 1998; Randall, 1967; Wainwright, 1988). An important axis of trophic diversity is the extent to which species are strong-jawed and feed on hard-shelled prey (Clifton and Motta, 1998; Wainwright, 1988). Within the Caribbean *H. maculipinna* feeds mostly on polychaetes while *H. bivittatus*, *H. garnoti*, *H. poeyi*, and *H. radiatus* feed mostly on crabs, gastropods and echinoderms (Randall, 1967; Wainwright, 1988). One species in the radiation, *H. pictus*, is a specialized zooplanktivore, and several other species are facultative planktivores (*H. socialis*, *H. burekae*, *H. dispilus*) or feed extensively on plankton as juveniles (*Oxyjulis*). We note that *H. pictus* and the three facultative planktivores form a clade (Fig. 2) while *H. bathyphilus*, which is sister to this group, appears to be a generalist benthic feeder (Sazima et al., 2007).

All species in the radiation are strongly reef associated with the exception of *H. aestuaricola* which is found mostly over sand and mud bottoms of mangroves and coastal estuaries. In the East Pacific coral reefs are not as well developed as they are in the Caribbean and the species there are found in a range of living, rocky habitats. Some species in NW *Halichoeres* also have substantial populations that live in seagrass, particularly *H. bivittatus* and *H. poeyi*. In the Caribbean, where as many as eight species can be geographically sympatric and several species can be seen in micro-sympatry, there are distinct patterns of habitat preferences. On the barrier reef in Belize, for example (Fig. 4), *H. bivittatus* and *H. poeyi*, reach their highest densities in about 1 m depth, while *H. garnoti* has a broader depth range and is most abundant at about 6 m, and *H. cyanocephalus* at 14 m depth. *H. bathyphilus* is found deeper still, between 50 and 80 m. While *H. pictus*, *H. maculipinna* and *H. radiatus* overlap in depth with other species, the feeding ecology of the first two species is quite different from *H. bivittatus*, *H. garnoti*, and *H. poeyi*, and *H. radiatus* is much larger than other species in the group, factors that possibly reduce competition. In the East Pacific, *H.*

adustus and *H. notospilus* are most common in very shallow water along wave-swept rocky shores, while *H. chierchaie*, *H. nicholsi*, and *H. dispilus* all seem to show wider depth preferences.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.jympev.2017.12.028>.

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