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Phylogeny of *Parablennius* Miranda Ribeiro, 1915 reveals a paraphyletic genus and recent Indo-Pacific diversification from an Atlantic ancestor

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ABSTRACT

A molecular phylogeny of 15 (out of 26 recognized) species of *Parablennius* Miranda Ribeiro, 1915 was constructed based on two mitochondrial and two nuclear gene fragments, and using maximum parsimony, maximum likelihood and Bayesian approaches. The closely related genera *Hypleurochilus*, *Salaria* and *Scartella* were also studied to ascertain their relationship with *Parablennius*. Phylogenetic analyses were compared with morphology-based taxonomical studies. *Hypleurochilus*, *Salaria* and *Scartella* appear well supported within a clade including all *Parablennius*, indicating that this genus is paraphyletic. The species pairs *P. parvicornis–P. sanguinolentus* and *P. gattorugine–P. ruber* are well-supported and relatively distant from remaining *Parablennius*. Remaining *Parablennius* form two distinct well-supported groups: (1) a clade of Atlantic-Mediterranean *Parablennius* (*P. pilicornis*, *P. marmoreus*, *P. rouxi*, *P. salensis* and *P. tentacularis*); (2) a clade including Hypleurochilus, the Indo-Western Pacific Parablennius (*P. cornutus*, *P. intermedius*, *P. tasmanianus* and *P. yatabei*) and the Atlantic-Mediterranean *P. incognitus* and *P. zvonimiri*. Use of a relaxed molecular clock suggests that Indo-Pacific *Parablennius* originated recently from an Atlantic *Parablennius* that may have dispersed via southern Africa, rather than via the Tethys seaway.

1. Introduction

The Blenniidae are present in most oceans, including tropical and cool and warm temperate waters, but absent from polar waters. Given their wide distribution they are an interesting system to study historical relationship between oceans, and the relation between an ocean's eastern and western boundaries (Almada et al., 2009a). In this study we focus on the genera *Parablennius* and closely related genera.

The genus *Parablennius* Miranda Ribeiro, 1915 presently includes 26 valid species (Patzner et al., 2009), all of which are marine and demersal. The systematics of these species have largely been based on morphological characters, in particular meristic and osteological characters (Bath, 1977, 1981, 1982, 1989, 1990, 1996, 2008; Bock and Zander, 1986; Norman, 1943; Springer, 1968; Zander, 1978). The use of different putative diagnostic characters has led to several revisions. For instance, Bath (1977) proposed a division between the genera *Parablennius* and *Pictiblennius* Whitley, 1930, based on the presence of teeth on the vomer, but later dismissed the validity of this genus given the intra-specific variation in this character (Bath, 1982). Additionally, teeth on the vomer are present in *Parablennius ruber* (Valenciennes, 1836), implying its inclusion within *Pictiblennius*. But this species is otherwise morphologically close to *P. gattorugine* (Linnaeus, 1758), which lacks that character, further undermining the validity of the genus *Pictiblennius*.

The relationship between *Parablennius* and other blenniid genera has also been subject to several revisions. A review of the osteology of European Blenniidae (Bock and Zander, 1986; Zander, 1978) suggested a close affiliation among *Aidablennius*, *Parablennius* and *Scartella*, based on elements of the neurocranium, with these genera in turn separated from *Lipophrys–Coryphoblennius*, based on the morphology of the supratemporal canal. The cladogram, as described by Bock and Zander (1986), joined these two groups with the generic pair *Hypleurochilus–Hypsoblennius*, distinguished by its restricted gill openings, based on the presence of the anterodorsal wall of the premaxillary.

Molecular data provided additional information and novel hypotheses regarding the systematics of *Parablennius* and its relation with other genera. Almada et al. (2005), using mitochondrial DNA of north-eastern Atlantic and Mediterranean blenniids, found no evidence for an association between *Aidablennius* and *Parablennius*, and did not recover *Parablennius* as a monophyletic clade, but rather a clade containing this genus together with *Scartella* and *Salaria*. A more extensive molecular study of the Blenniidae (in

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prep.), confirmed that these 3 genera, together with *Hypleurochilus*, form a well-supported clade. The present study aims to focus on the phylogeny of the genus *Parablennius* and its relationships with closely related genera.

The genus *Parablennius* is most diverse in the temperate waters of the Northeast Atlantic and Mediterranean (Floeter et al., 2008), where nine species occur (seven endemic specie, the amphi-Atlantic *P. pilicornis*, and *P. parvicornis* that exists across all Macronesian islands, including Cape Verde, extending its distribution north to the Azores). The next most diverse regions are the Eastern Central Atlantic, with seven species, and the Australian region, with five endemic species. Only two species occur in the Western Atlantic: the endemic *P. marmoreus* (Poey, 1876) and the amphi-Atlantic *P. pilicornis*, which occurs along the Southwestern Atlantic, all along the Eastern Atlantic and on the Eastern Coast of South Africa (Luiz et al., 2004). The Indo-Western Pacific region, with only three species, is relatively species poor given its area and when contrasted with Eastern Atlantic regions (see Fig. 1).

This distribution pattern suggests that the ancestor of *Parablennius* had an Atlantic distribution and the genus subsequently colonized the Indo-Pacific region. This is further reinforced by the Atlantic-Mediterranean distribution of the more closely related genera, namely *Coryphoblennius*, *Hypleurochilus*, *Lipophrys*, *Microlipophrys*, *Salaria* and *Scartella*. We propose two hypotheses of colonization of the Indo-Pacific region: (1) migration from the Mediterranean eastward to the Indic Ocean via the Tethyan seaway, which closed in the lower Miocene, approximately 18–20 Ma, resulting in the formation of the Red Sea (Coleman, 1993; Hrbek and Meyer, 2003; Ruggieri, 1967); (2) migration from the south-eastern Atlantic via southern Africa into the Indian Ocean and subsequently the Pacific Ocean.

Using both mitochondrial DNA sequences (partial 12S and 16S rRNA regions) and two nuclear regions (the first intron of the S7 gene and a portion of the rhodopsin gene), we address the following issues: (a) the monophyly of *Parablennius*; (b) the relationship between *Parablennius* species and *Hypleurochilus*, *Salaria* and *Scartella*; (c) biogeographic patterns among *Parablennius* species from phylogenetic results; (d) alternate hypotheses of dispersal between the Atlantic and Indo-Pacific Ocean, by application of a molecular clock; and (e) relationship between molecular phylogenetics and diagnostic morphological characters.

2. Material and methods

2.1. Sampling

We included in our analysis 15 of the 26 species of the genus *Parablennius* Miranda Ribeiro, 1915, including 11 Atlantic-Mediterranean species: *P. incognitus*, *P. gattorugine*, *P, marmoreus*, *P. parvicornis*, *P. pilicornis*, *P. rouxi*, *P. ruber*, *P. salensis*, *P. sanguinolentus*, *P. tentacularis*, and *P. zvonimiri*; a South African species, *P. cornutus*; and four Indo-Pacific species: *P. intermedius*, *P. tasmanianus*, *P. thysanius* and *P. yatabei*. In order to perform a preliminary investigation of the geographic structure of the amphi-Atlantic *P. pilicornis*, we analyzed samples from Europe, Brazil and South Africa. We also included four of the eleven species of *Hypleurochilus*: the Atlantic *H. aequipinnis* (Günther, 1861), *H. fissicornis* (Quoy and Gaimard, 1824), *H. pseudoaequipinnis* (Bath, 1994), and a new species from Trindade Island (Brazil); two of the four species of the Atlantic-Mediterranean *Salaria*: *S. pavo* (Risso, 1810) and *S. fluviatilis* (Asso, 1801); and three of the seven species of *Scartella*: the Atlantic Ocean *Sc. cristata* (Linnaeus, 1758), *Sc. caboverdiana* (Bath, 1990), and the Indian Ocean *Sc. emarginata* (Günther, 1861). We included, as outgroup species, *Coryphoblennius galerita* (Linnaeus, 1758), *Lipophrys pholis* (Linnaeus, 1758) and *L. trigloides* (Valenciennes, 1836). These three Atlantic species form a well supported clade and are part of a larger group that is sister to a *Parablennius–Scartella–Salaria* clade (Almada et al., 2005).

For species sampled, collection site locations and GenBank Accession Numbers see Table 1.

2.2. DNA extraction, amplification and sequencing

Total genomic DNA was extracted from fin or muscle samples preserved in 96% ethanol with the REDExtract-N-Ampkit (Sigma–Aldrich) following the manufacturer's instructions. Voucher

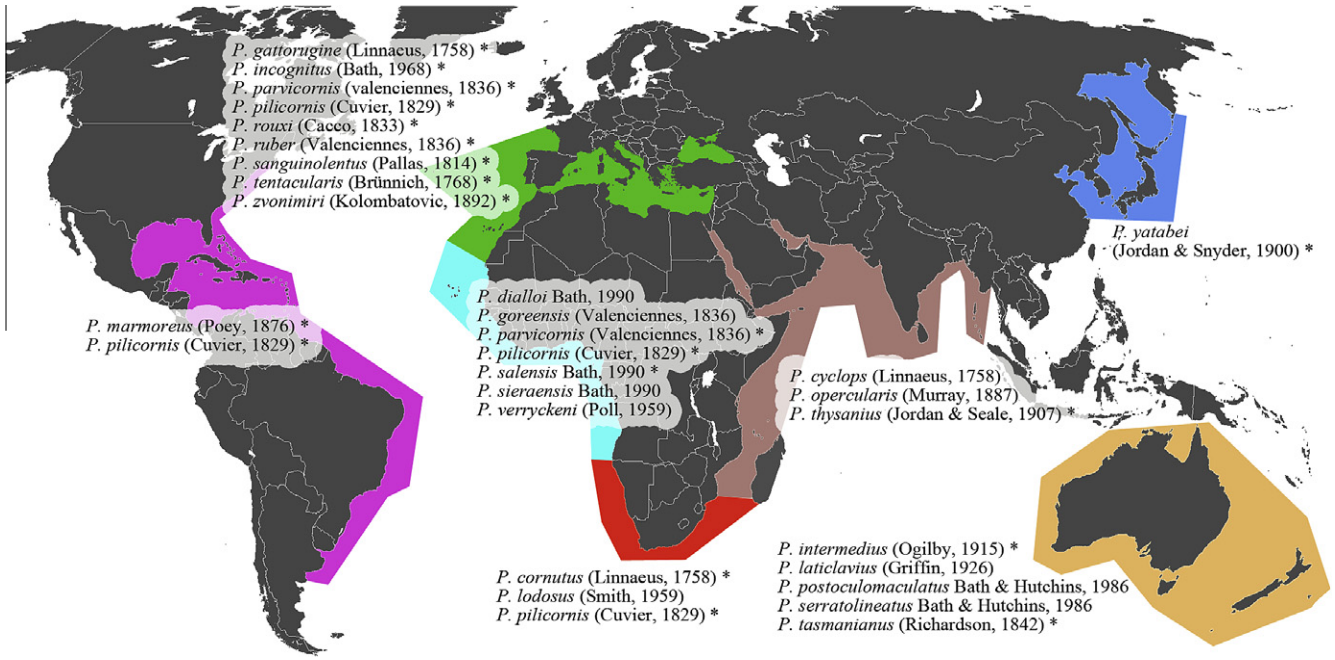

Fig. 1. Distribution of the genus *Parablennius* in major marine biogeographic regions. Most of the *Parablennius* species are exclusive to a single region, with the exception of the amphi-Atlantic *P. pilicornis* and *P. parvicornis*. Species included in the analyses are indicated with an asterisk.

Table 1
Species analyzed, location and sequence accession numbers.

Species	Locality	12S	16S	S7 (1st intron)	Rhodopsin
Coryphoblennius galerita	United Kingdom	AY098753	EF521665	EF527744	HM630110
Hypleurochilus aequipinnis	Cameroon	JQ697234	JQ697263	JQ697294	JQ697335
Hypleurochilus fissicornis	Brazil	JQ697235, JQ697332	JQ697264	JQ697295	JQ697336
Hypleurochilus pseudoaequipinnis	São Tomé & Príncipe	JQ697236	JQ697265	JQ697296	–
Hypleurochilus n sp.	Trindade Island, Brazil	JQ697333	JQ697276	JQ697297	JQ697337
Hypsoblennius brevipinnis	Pacific coast of Panama	JX843402–JX843403	JX843406–JX843407	JX843410–JX843411	–
Hypsoblennius invemar	Gulf of Mexico	JX843404–JX843405	JX843408–JX843409	JX843412–JX843413	–
Lipophrys pholis	Portugal	AY987012	AY987017	FJ465584	HM630123
Lipophrys trigloides	Portugal	EF521811	AY098827	JQ697298	HM630119
Parablennius cornutus	South Africa	JQ697237	JQ697266	JQ697299, JQ697300	JQ697338–JQ697339
Parablennius gattorugine	Portugal	AY098777	DQ160200	JQ697301	JQ697340–JQ697341
Parablennius incognitus	Portugal	AY098787	AY098829	JQ697302	JQ697343
Parablennius intermedius	Australia	JQ697238	JQ697267	–	JQ697342
Parablennius marmoreus	Panama	JQ697239	JQ697268	JQ697303	–
Parablennius parvicornis	Portugal	AF414712	AY345196	JQ697316–JQ697318	HM630108
Parablennius pilicornis	Brazil	JQ697247–JQ697249	JQ697289–JQ697291	JQ697307–JQ697315	JQ697356–JQ697358
	Portugal	AY098796	AY098831, JQ697292	JQ697306	JQ697359
	South Africa	JQ697250–JQ697262	JQ697277–JQ697288	JQ697304–JQ697305	JQ697344–JQ697355
Parablennius rouxi	Spain	AY098782	AY098833	–	JQ697360
Parablennius ruber	Portugal	AY098779	AY098834	FJ465582	JQ697361
Parablennius salensis	Cape Verde	AY098789	AY098836	JQ697327–JQ697328	JQ697362
Parablennius sanguinolentus	Portugal	AF414705	AF324193	JQ697319	HM630109, JQ697363
Parablennius tasmanianus	Australia	JQ697240	JQ697269	JQ697320	JQ697364
Parablennius tentacularis	Spain	JQ697241–JQ697242	JQ697270–JQ697271	JQ697321–JQ697322	JQ697365–JQ697366
Parablennius thysanius	Hawaii	JQ697334	JQ697293	–	–
Parablennius yatabei	Japan	JQ697243 JQ697244	JQ697273	JQ697323	JQ697367
Parablennius zvonimiri	Italy	AY098790	AY098840	JQ697324	JQ697368
Salaria fluviatilis	Portugal	FJ465653	FJ465743	JQ697329	JQ697369
Salaria pavo	Portugal	FJ465669	FJ465754	JQ697330	JQ697370
Scartella caboverdiana	Cape Verde	JQ697245	JQ697274	JQ697331	JQ697372
Scartella cristata	Spain	AY098803	AY098845	JQ697325	JQ697371
Scartella emarginata	South Africa	JQ697246	JQ697275	JQ697326	JQ697373

specimens are deposited, as ethanol preserved tissues, in the Australian Museum-Sydney (*P. intermedius*), Mie University (*P. yatabei*), Museum Victoria (*P. tasmanianus*) and ISPA-IU (remaining species). PCR amplification of mitochondrial (12S and 16S) and nuclear fragments (the first intron of the nuclear S7 ribosomal protein gene and part of the rhodopsin gene) were performed with the following pairs of primers: 12s rDNA — 12SFor and 12SRev (Almada et al., 2005); 16s rDNA — 16SFor and 16SRev (Almada et al., 2005); the first intron of the S7 ribosomal protein gene — S7RPEX1F and S7RPEX2R (Chow and Hazama, 1998); and the rhodopsin gene – RhodF and RhodR (Taylor and Hellberg, 2005). Except in the few cases when amplification or sequencing failed, all these gene fragments were sequenced for each species. For all genes, each sample was sequenced in both directions using the PCR primers. Sequencing reactions were performed by Stabvida (Oeiras, Portugal; http://www.stabvida.com).

2.3. DNA analyses, phylogenetic inference and testing

All sequences were aligned using Clustal X (Thompson et al., 1994) and alignments were deposited in TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S12485). We computed net average genetic distances between *P. pilicornis* from Brazil (2–3 samples, depending on the genetic marker), Europe (2–3 samples) and South Africa (9–12 samples), applying a likelihood distance with parameters selected using the AIC criteria as implemented in jModelTest v0.1.1 (Posada, 2009), and implemented with MEGA v.4 (Tamura et al., 2007). Congruence among all data sets was tested by the incongruence-length difference test (ILD) (Farris et al., 1985), as implemented in Paup* v.4.0b10 (Swofford, 2003). ILD tests did not reveal significant heterogeneity among the two mitochondrial markers ($p = 0.31$).

The alignment of the mitochondrial markers and of each nuclear marker was analyzed by three phylogenetic inference methods. Maximum parsimony-based (MP) phylogenetic relationships were estimated using PAUP*, with 100 heuristic searches using random additions of sequences and implementing the TBR algorithm. Branch support values for each note were tested by bootstrap analysis, with 1000 resamplings (Felsenstein, 1985). Maximum likelihood (ML) phylogenetic trees were inferred using RAxML BlackBox (Stamatakis et al., 2008). Branch support was tested by 100 rapid bootstrap inferences. Bayesian analysis (BY) was performed, using MRBAYES 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003), with two independent runs of four simultaneous MCMC chains for 2 million generations (sampling parameters every 100 generations), with the heating parameter set to 0.15. In all cases, the average standard deviation of split frequencies indicated convergence among independent runs. Majority-rule consensus trees were estimated combining results from duplicated analyses, after discarding the first 2000 samples.

In addition, we performed two Bayesian approaches on a concatenated alignment of mitochondrial and nuclear markers: a mixed model Bayesian analysis in MRBAYES, wherein substitution models were allowed to differ among genes but topology is based on the signal from the combined data; and a Bayesian Estimation of Species Trees (BEST) analysis (Liu, 2008) wherein genealogies are estimated for each marker and then probabilities for species trees that best harbor the individual genealogies. For the BEST analysis, two independent runs of 5 MCMC chains were run for 10 million generations, with independent GTR + Γ models for each of the 3 partitions.

Alternative specific phylogenetic hypotheses were tested under the likelihood criterion (with ML trees) using the Shimodaira–Hasegawa (S–H; Shimodaira and Hasegawa, 1999) test as implemented in PAUP*.

2.4. Molecular dating

In order to examine whether our data were compatible with dispersal between the Atlantic and Indo-Pacific Ocean via the Tethyan seaway, we performed a molecular clock analysis. To examine whether the data set of each genetic region evolved at equal rates (strict molecular clock), we conducted a likelihood ratio test comparing topologies obtained with and without a molecular clock constraint, using PAUP*. We calibrated molecular clocks specific for each mitochondrial fragment and for the first intron of S7, based on the sequence divergence between *Hypsoblennius invemar* Smith-Vaniz and Acero, 1980, from the Gulf of Mexico, Texas, and *H. brevipinnis* (Günther, 1861), from the Pacific coast of Panama (for sequence accession numbers see Table 1), assuming they diverged before the formation of the Isthmus of Panama (ca. 3.5 Ma). A preliminary study of several *Hypsoblennius* species using these three markers indicate that *H. brevipinnis* and *H. invemar* are sister species (unpublished data). Mean net genetic divergence between *H. invemar* and *H. brevipinnis* was calculated with MEGA v5. (Tamura et al., 2011), using the genetic distance most adequate for each marker, under the BIC criterion: Jukes–Cantor (12S and S7) and Kimura-2 (16S). The net average distance between this pair of species was: 0.045 (12S), 0.056 (16S), and 0.083 substitutions/site (S7). Substitution rates (half the divergence estimates) divided by 3.5 Ma gave us the following estimates of clock rates: 0.006 (12S); 0.008 (16S); and 0.011 subst. per site per Ma (S7). When analyzing the concatenated mitochondrial fragment, we used the more conservative value between the two rRNAs. Divergence times were estimated in a Bayesian framework, using BEAST v1.7.2 (Drummond and Rambaut, 2007) assuming an uncorrelated log-normal relaxed molecular clock, a normally distributed prior of the ucld.mean with mean equal to the clock estimate, a GTR + Γ substitution model with 6 gamma categories, and a Yule process prior. Each such analysis was run for 20 million generations, sampling every 1000 generations, and discarding the first 10% of samples.

3. Results

3.1. Molecular data

The concatenated mitochondrial DNA fragment, including 12S and 16S fragments, comprised a total of 921 bases, including 224 parsimony informative sites. The model selected by jModelTest, using the AIC criterion, was TIM2 + Γ + I, with a gamma shape parameter of 0.317% and 19.3% invariant sites. The first intron of the nuclear gene S7 comprised a total of 694 bases, including 306 parsimony informative sites. The model selected by jModelTest, using the AIC criterion, was HKY + Γ, with a gamma shape parameter of 1.752. The fragment of the rhodopsin nuclear gene comprised a total of 821 bases, including 108 parsimony informative sites. The model selected by jModelTest, using the AIC criterion, was TPM1uf + Γ, with a gamma shape parameter of 0.207.

3.2. Paraphyly of Parablennius

All phylogenetic analyses, for each DNA region and each inference method, strongly supported an ingroup including the genera *Hypleurochilus*, *Parablennius*, *Salaria* and *Scartella*. Most combinations of regions and methods recovered internal structure in the backbone of the ingroup, except MP analysis of the concatenated mitochondrial fragment (Fig. S1). No phylogenetic result supported the monophyly of *Parablennius*. Trees constrained to form a monophyletic *Parablennius* clade were statistically significantly different from unconstrained trees, indicating that our data did not support a monophyletic *Parablennius* (12S + 16S: S–H $p < 0.001$; S7: S–H $p < 0.000$; rhodopsin: S–H $p < 0.001$).

The mixed-model Bayesian and BEST approach, using the concatenated alignment of mitochondrial and nuclear markers, yielded the most structured phylogenetic hypothesis for the ingroup (see Fig. 2). Most trees obtained using only the mitochondrial or either nuclear alignment, with any of the inference methods, albeit exhibiting lack of support for some nodes, were compatible with the mixed-model Bayesian tree.

All analyses identified two well-supported species pairs, *P. parvicornis–P. sanguinolentus* and *P. gattorugine–P. ruber*. Most analyses identified the species pair *P. parvicornis–P. sanguinolentus* as sister to the remaining ingroup species. All analyses of the S7 fragment, however, identified the species pair *P. gattorugine–P. ruber* as sister to the remaining ingroup species (Fig. S3). Tests between these trees and ML topologies constrained to place the *P. parvicornis–P. sanguinolentus* as basal to the remaining ingroup were non-significant for all fragments (S–H tests, $p > 0.05$). Thus, overall, our evidence suggests an initial separation of the species pair *P. parvicornis–P. sanguinolentus*, followed by the separation of *P. gattorugine–P. ruber* from a sister clade containing *Hypleurochilus*, *Salaria*, *Scartella* and the remaining *Parablennius*.

All analyses revealed a well-supported *Scartella* clade. The monophyly of the two *Salaria* species was only recovered in the BY and ML analysis of the S7 fragment, and in the mixed-model Bayesian tree. The monophyly of this genus was supported by a previous study with a more extensive sampling of *Salaria* (Almada et al., 2009b). The mixed-model Bayesian tree supports a *Salaria–Scartella* clade, sister to a well supported clade formed by *Hypleurochilus* and the remaining *Parablennius* species, i.e., excluding the formerly referred to species pairs. Therefore, there is strong evidence that the two species pairs are distant and distinct from the remaining *Parablennius*.

3.3. Relationships among Parablennius

Mixed-model Bayesian and BEST analyses reveals an internal structure of the *Hypleurochilus–Parablennius* clade that is compatible or not statistically different from results from remaining analyses, and that contains: (i) a clade including several, but not all, Atlantic-Mediterranean *Parablennius* (*P. pilicornis*, *P. marmoreus*, *P. rouxi*, *P. salensis* and *P. tentacularis*; dark gray box in Fig. 2); (ii) the *Hypleurochilus* clade; (iii) *P. zvonimiri* sister to the *Hypleurochilus–Parablennius* subclade, wherein *P. incognitus* is sister to the Indo-Western Pacific *Parablennius* (*P. cornutus*, *P. intermedius*, *P. tasmanianus*, *P. thysanius* and *P. yatabei*; light gray box in Fig. 2). We were only able to sequence the mitochondrial markers of *P. thysanius*, but analyses of these markers consistently places this species within the Indo-Pacific *Parablennius*, close to *P. cornutus* (see Fig. S1). This precise internal topology of the *Hypleurochilus–Parablennius* clade, however, is not recovered in any of the analyses of individual markers. For instance, using rhodopsin we recovered a polytomy with an isolated *P. zvonimiri*, the clade of most Atlantic-Mediterranean species – referred to above as (i) – the *Hypleurochilus* clade, and a clade with *P. incognitus* is sister to the Indo-Western Pacific *Parablennius*. Using the mitochondrial concatenation, we recovered a similar polytomy, but with support for the union of *Hypleurochilus* and the *P. incognitus*-Indo-Pacific *Parablennius* clade.

Preliminary analysis among *P. pilicornis* samples from Brazil, Europe and South Africa indicated very low genetic variation and no geographic signal. Average net distances among the three geographic regions was lower than 0.5%, for 12S, 16S, and rhodopsin. The S7I indicated a larger distance between Europe and the other two regions (c.a. 2%), and a small distance between Brazil and South Africa (0.2%).

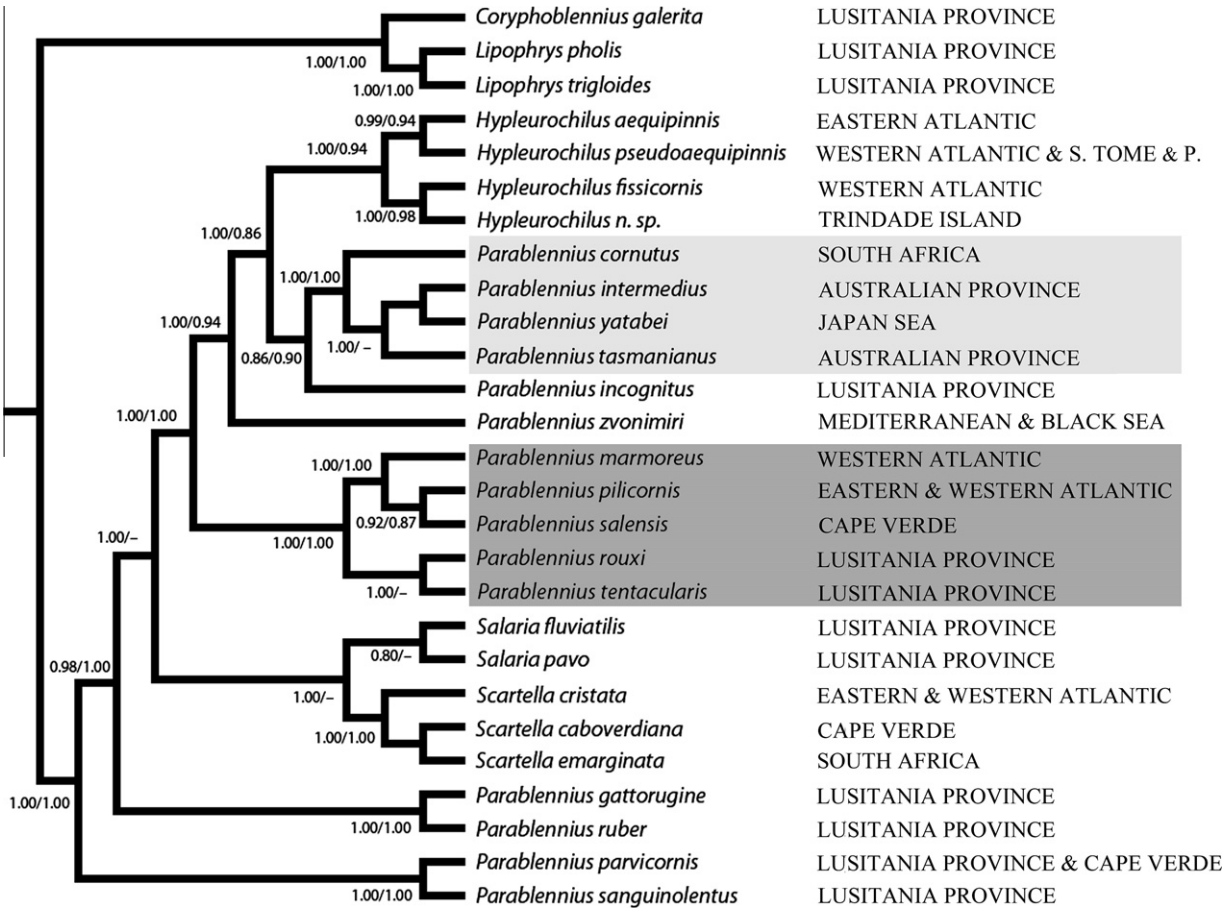

Fig. 2. Phylogenetic relationships based on a mixed-model Bayesian analysis of the concatenated data set, with independent models for the mitochondrial 12S and 16S rRNA sequences, and for the nuclear S7l intron and rhodopsin. Support values near each node correspond to posterior probabilities of the mixed-model Bayesian analysis and of a BEST analysis based on three independent estimates of mitochondrial, S7 and rhodopsin genealogies. Dark gray box corresponds to clade including several, but not all, Atlantic-Mediterranean *Parablennius*; light gray box corresponds to the Indo-Western Pacific clade.

3.4. Molecular dating

A strict molecular clock was rejected for mitochondrial markers and the nuclear S7 fragment. Bayesian estimates using a relaxed molecular clock estimated the divergence of the Indo-Pacific *Parablennius* clade from the Atlantic *P. incognitus–Hypleurochilus* as having occurred after to the closure of the Tethys seaway (18–20 Ma): 12S + 16S – 5.808 Ma [1.742–13.020]; S7 – 3.190 Ma [1.148–6.467]. Divergence among the sampled Indo-Pacific *Parablennius* appears to have occurred relatively rapidly, as suggested by the estimate of the age of their most recent common ancestor: 12S + 16S – 3.668 Ma [1.030–8.371]; S7 – 1.132 Ma [0.219–2.679].

4. Discussion

4.1. Phylogenetic analysis

Our phylogenetic analysis of three independent DNA markers, with varying rates of evolution, clearly supports the lack of monophyly of the genus *Parablennius* Miranda-Ribeiro 1915. Overall, the species pairs *P. gattorugine–P. ruber* and *P. parvicornis–P. sanguinolentus* appear as well supported clades that are separate from the remaining *Parablennius*. The position of these species pairs varies according to which combination of method and molecular fragment was used, but they tend to appear in a more basal position relative to a strongly supported clade containing *Salaria*, *Scartella*, *Hypleurochilus* and the remaining *Parablennius*. Among the main group of *Parablennius* there appears to be a separation among a clade consisting of largely Atlantic-Mediterranean species (*P. marmoreus*, *P. pilicornis*, *P. rouxi*, *P. salensis*, and *P. tentacularis*) and a clade which includes Indo-Pacific species (*P. cornutus*, *P. intermedius*, *P. tasmanianus* and *P. yatabei*) together *P. incognitus* and *P. zvonimiri*, present in the Mediterranean and adjoining Atlantic waters.

The four *Hypleurochilus* species form a well supported monophyletic clade that is consistently more closely related to the Indo-Western Pacific *Parablennius* and *P. incognitus* than these species are to the Atlantic-Mediterranean *Parablennius*, clearly suggesting the lack of monophyly of even this reduced group of *Parablennius* as a genus, i.e., excluding the two species pairs *P. gattorugine–P. ruber* and *P. parvicornis–P. sanguinolentus*. Although this study is not conclusive regarding the relationship between *Hypleurochilus*, *Salaria*, *Scartella* and *Parablennius*, as we did not include samples of several species from each genus, it clearly demonstrates that these genera are closely related and calls for further examination of the taxonomic status of *Parablennius*.

In this study, we lacked material to address the relationship of *Hypsoblennius* and *Chasmodes*, for which there are few available DNA sequences, with *Hypleurochilus*, *Parablennius*, *Salaria* and *Scartella*. Analysis of the available mitochondrial sequences of those genera did not reveal sufficient phylogenetic signal to group these genera with remaining *Parablenniini* (results not shown). However, results in this study to not conform to the cladogram proposed by Bock and Zander (1986), which suggests, based on osteological characters, a closer relationship between *Hypsoblennius* and *Hypleurochilus* than that of this genus with *Parablennius*.

4.2. Morphological analysis

The molecular phylogenetic results reveal some parallels with morphological systematics. Bath (1990, 1996) distinguished five *Parablennius* groups, based on the number of mandibular pores, the form of ventral rays, the number of pectoral rays, the type of *canalis lateralis*, among other characters. The two species pairs, *P. gattorugine–P. ruber* and *P. parvicornis–P. sanguinolentus*, correspond to Bath's groups 2 and 5, respectively. These groups contain no additional species, which together with our phylogenetic results, suggest these species pairs are also morphologically distinct from the remaining *Parablennius*.

Bath's group 1 includes all and only the Indo-Pacific *Parablennius* and the southern African *P. cornutus*. The remaining *Parablennius* are Atlantic-Mediterranean and are classified in Bath's groups 3 and 4. Group 3 is further divided among three subgroups: the east central African *P. dialloi* (not included in our molecular analyses); those with teeth in the vomer (*P. incognitus* and *P. zvonimiri*); and those without such teeth and with only one pore in the canalis orbitalis (*P. rouxi* and *P. tentacularis*). Group 4 includes the amphi-Atlanic *P. pilicornis*, and east central African species (*P. goreensis*, *P. salensis*, *P. sierraensis* and *P. verryckeni*).

A comparison of the dorsal neurocranial bones, particularly their shape, position and relation to the median supratemporal pore shows similarities among *Hypleurochilus*, *Hypsoblennius* (except *Hb. striatus*) and *Parablennius* (except for *P. parvicornis* and *P. sanguinolentus*) (Bath, 1996). In contrast, *Hb. striatus*, *P. parvicornis*, *P. sanguinolentus* and some species of the genera *Chasmodes* and *Scartella* share a distinct pattern of the head lateral line channels. These contrasts further suggest the distinctness of the species pair *P. parvicornis–P. sanguinolentus*, and the relationship between the remaining *Parablennius* and *Hypleurochilus*.

4.3. Biogeography and timing of colonization events

The structure of our phylogenetic trees suggests the ancestral distribution of the group to be the Atlantic Ocean. The morphological similarity among Indo-Pacific *Parablennius* (Bath, 1996) in conjunction with their well supported monophyletic clade suggests that these species radiated after a single colonization. The relaxed molecular clock analyses suggest that the Indo-Pacific *Parablennius* clade diverged less than 10 Ma, i.e., after the closing of the Tethys seaway, ca. 18–20 Ma. We cannot rule out the possible effect of including the remaining Indo-Pacific species on the estimated time of divergence of this clade from its Atlantic ancestor. The present results are most consistent with an origination of the Indo-Pacific clade from an Atlantic-Mediterranean ancestor that colonized that region via southern Africa, and dispersed and diverged within the last ca. 10 Ma, rather than an older colonization via the Tethys seaway. There are strong relationships between reef fishes from the eastern Atlantic and the Indian Ocean, with a number of these being recent colonisations via southern Africa (Floeter et al., 2008). The direction is usually inferred to be from the Indian Ocean to the Atlantic, for example, population genetic surveys support the direct recent (post Benguela-establishment) colonization of the Atlantic by an Indo-Pacific goby, genus *Gnatholepis* (Rocha et al., 2005), and an angelfish, genus *Centropyge* (Bowen et al., 2006). There is also an assumed invasion of the Atlantic by *Chaetodon sedentarius* from the western Indian Ocean *C. dolosus* clade at around 1.6 Ma (Fessler and Westneat, 2007). Alva-Campbell et al. (2010) indicate that the genus *Holacanthus* likely originated approximately 10.2–7.6 mya. Since the basal species is found in Western Africa, *Holacanthus* may have originated from an Indian Ocean invasion.

The genus *Salaria* is largely restricted to the Mediterranean Sea and adjoining Atlantic waters. Few species in the closely related *Scartella*, *Hypleurochilus* and *Parablennius* are present on both sides of the Atlantic (only *P. pilicornis* and *Sc. cristata*). *Parablennius marmoreus* is endemic to the western Atlantic. One *Scartella* species is endemic to Cape Verde and four *Scartella* are endemic to Southwest Atlantic oceanic islands: *Sc. itajobi* (Rangel and Mendes, 2009); *Sc. nuchifilis* (Valenciennes, 1836); *Sc. poiti* (Rangel, Gasparini and Guimarães, 2004); and, *Sc. springeri* (Bauchot, 1967). The genus *Hypleurochilus* is most diverse along the Northwest Atlantic (*H. bermudensis*, *H. caudivittatus*, *H. geminatus*, *H. multifilis and H. springeri*), with two species in the Southwest Atlantic (*H. fissicornis* and *H. pseudoaequipinnis*) – a third species from Trinidade Island is being formally described at the moment – and four in the Eastern Central Atlantic (*H. aequipinnis*, *H. bananensis*, *H. langi and H. pseudoaequipinnis*). Despite the presence of these genera on both sides of the Atlantic, it is noteworthy that none of them reached the Eastern Pacific coast: *Hypleurochilus* is confined to the Atlantic; *Scartella* is largely distributed in the Atlantic, with only a single species, *S. emarginata* (Günther, 1861), present in the Southeast Atlantic and the Western Indian Ocean; and *Parablennius* is most diverse in the Atlantic, with some species in the Indo-Pacific Ocean, but none present in the Eastern Pacific Coast. This contrasts with other Blenniidae genera distributed on both sides of the Isthmus of Panama (e.g., *Hypsoblennius* and *Ophioblennius*), which must have colonized the Eastern Pacific before the formation of the isthmus of Panama (3.5 Ma). *Scartella*, *Hypleurochilus* and *Parablennius* may have reached the Western Atlantic only after the initial shoaling of the Isthmus region or the final closure of the Isthmus of Panama. After this event, dispersal across the Atlantic may have intensified, possibly as a consequence of an increase in trans-oceanic currents (Haug and Tiedemann, 1998). The low level of divergence among *P. pilicornis* from Europe, Brazil and South Africa, as indicated by preliminary molecular analyses, and the absence of this species from Atlantic Islands, is consistent with high levels of trans-Atlantic long-distance dispersal. The report of the tropical Eastern Central Atlantic *Chromis limbata* in the South Western Atlantic is an additional illustration of recent dispersal from the Eastern to Western Atlantic (Leite et al., 2009).

Overall, this suggests an Eastern Atlantic origin of this group, with Western Atlantic ancestors migrating recently westward via the North Equatorial Current and the ancestors of the Indo-Pacific *Parablennius* migrating from the south-eastern Atlantic around the southern African coast into the Indian Ocean. This probably took place before the late Pliocene cooling of the south Atlantic, when they dispersed and radiated throughout the Indian Ocean, Australian region, and the Pacific Ocean (Springer, 1991). The absence of *Parablennius* from the Eastern Pacific is consistent with post-Tethyan dispersal from the Atlantic around Africa: an older arrival in the Indian Ocean would increase the likelihood that a lineage could cross the trans-Pacific barrier.

Finally, as suggested by Almada et al. (2001), the Eastern Atlantic-Mediterranean waters could have acted both as an area preserving old Atlantic lineages and generating opportunities for their diversification. It is the only area where all major clades are present, several of them with endemic species. The exception is the Indo-Pacific *Parablennius* clade, which seems however to be closely related to the Atlantic-Mediterranean *P. incognitus*, *P. zvonimiri* and *Hypleurochilus*.

4.4. Taxonomy and systematics

The systematics of the Blenniidae and, in particular, the tribe Parablenniini, which include, among others, the genera *Coryphob-*

lennius, *Hypleurochilus*, *Lipophrys*, *Parablennius*, *Salaria* and *Scartella* included in this study, has been subject the several revisions (Hastings and Springer, 2009). The lack of morphological synapomorphies and a high degree of homoplasy in osteologic features has been a recurring issue. Osteological characters such as the neurocranium and the canalis lateralis have proven to be of taxonomic value at different hierarchical levels (e.g., Bath, 1996; Bock and Zander, 1986).

In this paper, we provide molecular evidence confirming that the genus *Parablennius* is paraphyletic. This is the case even if we were to exclude the two morphologically distinct basal species pairs *P. gattorugine–P. ruber* and *P. parvicornis–P. sanguinolentus*, given the support for a closer phylogenetic relationship between, on the one hand, *Hypleurochilus* and, on the other, *P. zvonimiri*, *P. incognitus* and Indo-Pacific *Parablennius*. The genus still warrants further revision, which should be based on a more comprehensive analysis of *Parablennius* species, namely including the east central Atlantic species and the remaining Indo-Pacific species, as well as additional species of *Hypleurochilus*.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2012.12.010.

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