

Communication

Multi-Locus Phylogenetic Analyses of the Almadablennius Clade Reveals Inconsistencies with the Present Taxonomy of Blenniid Fishes

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Abstract: We used a multi-locus phylogenetic approach (i.e., combining both mitochondrial and nuclear DNA fragments) to address some long-standing taxonomic inconsistencies within the diverse fish clade of Combtooth Blennies (Blenniidae—unranked clade Almadablennius). The obtained phylogenetic trees revealed some major inconsistencies in the current taxonomy of Parablennini, such as the paraphyletic status of the *Salaria* and *Parablennius* genera, casting some doubt regarding their actual phylogenetic relationship. Furthermore, a scarce-to-absent genetic differentiation was observed among the three species belonging to the genus *Chasmodes*. This study provides an updated taxonomy and phylogeny of the former genus *Salaria*, ascribing some species to the new genus *Salariopsis* gen. nov., and emphasizes the need for a revision of the genus *Parablennius*.

Keywords: Blenniidae; phylogeny; *Parablennius*; *Salaria*



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1. Introduction

Combtooth blennies (Blenniidae Rafinesque 1810; herein, blennies) are a diverse clade (>400 species) of nearshore, cryptobenthic fishes that inhabit temperate and tropical marine environments and inland water bodies worldwide [1]. The first major revisions of blenny taxonomy since Norman [2], subdivided the family into six tribes [3,4], each of which was characterized by generic revisions based on morphological characters (Saliariini, [5], Omobranchini [6], Phenablenniini [4]; Nemophini [7], and Parablenniini and Blenniini [8–10]). The specific membership of these tribes, and generic boundaries within, have remained relatively stable, with the exception of Parablenniini and Blenniini, which have been the subject of great disagreement since Zander [9] rejected the new genera set by Bath [8] (See Table 1 in [11] for history of generic revisions).

Our understanding of the taxonomy of this group has changed significantly with the advent of molecular systematics. In particular, the Almadablennius clade (Parablenniini + Blenniini [12]) has received much attention since Almada et al. [11] published a phylogeny where Blenniini *sensu* Williams [13] was nested within Parablennini *sensu* Williams [13] and the genus *Lipophrys* was paraphyletic, demonstrating that the available taxonomy was inconsistent with phylogeny. Subsequent efforts to investigate the relationships and clarify taxonomy within the Almadablennius clade (e.g., [12,14–17]), have led to useful taxonomic changes, such as *Microlipophrys* being split from *Lipophrys* (e.g., [15]) and the resolution of species membership within Blenniini and Parablenniini. Despite these updates and multiple lines of evidence suggesting problems, the taxonomy of the Almadablennius clade remains unresolved (e.g., paraphyly of *Parablennius*, *Hypoleurochilus* and

Salaria, and deep split between Mediterranean and Atlantic specimens of *Scartella cristata*; see Hundt and Simons [16]). In the light of these problems, we re-examined the phylogenetic relationships of the Almadablennius clade using partial sequences of two nuclear and two mitochondrial loci with the explicit aim of testing the monophyly and revising the taxonomy of the genus *Salaria*.

2. Methods

A total of 49 specimens of blennies belonging to 32 morphospecies were collected in the field or obtained as gifts from the colleagues listed in the acknowledgment section (Table 1). Specimens were fixed in 96% ethanol in situ and identified in the laboratory, using the most updated morphological identification keys [18–20].

Table 1. List of species sampled, catalog number, locality, and GenBank accession number for molecular loci sampled. Novel GenBank accession numbers are reported in bold. * *Salariopsis* gen. nov. Roman numbers in brackets refer to analysed specimens shown in Figure 1.

Species	Taxonomical Remarks	Catalog Number	Locality	ENC1	MYH6	16S	Dloop
<i>Aidablennius sphynx</i> (Valenciennes, 1836)	-	MNHN 2012-0219	Balearic Islands, Spain	KF678553	KF678648	MW980003	MZ026013
<i>Blennius ocellaris</i> Linnaeus, 1758	-	MNHN 2012-0221	Balearic Islands, Spain	KF678554	KF678649	MW980004	MZ026014
<i>Chasmodes bosquianus</i> (Lacepède, 1800)	-	JFBM 46472-2	Virginia, USA	KF678501	KF678601	MW980005	MZ026015
<i>Chasmodes longimaxilla</i> Williams, 1983	-	JFBM 46845-1433	Texas, USA	KF678530	KF678627	MW980006	MZ026016
<i>Chasmodes saburrae</i> Jordan and Gilbert, 1882	-	JFBM 46414-2	Florida, USA	KF678500	KF678600	MW980007	MZ026017
<i>Hypleurochilus bananensis</i> (Poll, 1959)	-	EFMM-20-201013	Bacoli, Italy	MZ025976	MZ025994	MW980008	MZ026018
<i>Hypleurochilus fissicornis</i> (Quoy and Gaimard, 1824)	-	-	Chile	MG779097	MG779132	MW980009	MZ026019
<i>Hypleurochilus geminatus</i> (Wood, 1825)	-	JFBM 46839-TX-002	Texas, USA	KF678526	KF678623	MW980010	MZ026020
<i>Parablennius incognitus</i> ^(I) Miranda Ribeiro, 1915	-	MNHN 2012-0237	Balearic Islands, Spain	KF678558	KF678653	MW980011	-
<i>Parablennius incognitus</i> ^(II)	-	EFMM-8-090815	Milazzo, Italy	MZ025977	MZ025995	MW980012	MZ026021
<i>Parablennius incognitus</i> ^(III)	-	EFMM-12-140815	Avola, Italy	MZ025978	MZ025996	MW980013	MZ026022
<i>Parablennius intermedius</i> Miranda Ribeiro, 1915	-	AMS I.45631-021	New South Wales, Australia	KF678474	KF678576	MW980014	MZ026023
<i>Parablennius pilicornis</i> Miranda Ribeiro, 1915	-	MNHN 2012-0240	Banyuls sur Mer, France	KF678560	KF678655	MW980015	MZ026024
<i>Parablennius rouxi</i> Miranda Ribeiro, 1915	-	MNHN 2012-0242	Banyuls sur Mer, France	KF678561	MG779139	MW980016	MZ026025
<i>Parablennius salensis</i> Miranda Ribeiro, 1915	-	JFBM 47280-1	Cape Verde	MG779103	-	MW980017	MZ026026
<i>Parablennius tasmanianus</i> Miranda Ribeiro, 1915	-	SAMAF 12607	Sturt Bay, Australia	MG779104	MG779141	MW980018	MZ026027

Table 1. Cont.

Species	Taxonomical Remarks	Catalog Number	Locality	ENC1	MYH6	16S	Dloop
<i>Parablennius tentacularis</i> Miranda Ribeiro, 1915	-	MNHN 2012-0406-BPS2265	Port-Vendres, France	MG779105	-	MW980019	MZ026028
<i>Parablennius yatabei</i> Miranda Ribeiro, 1915	-	JFBM 47154-1568	Kochi, Japan	KF678540	KF678636	MW980020	MZ026029
<i>Parablennius zvonimiri</i> ^(I) Miranda Ribeiro, 1915	-	EFMM-25-100716	Avola, Italy	MZ025979	MZ025997	MW980021	MZ026030
<i>Parablennius zvonimiri</i> ^(II)	-	MNHN 2012-0247	Banyuls sur Mer, France	KF678564	KF678657	MW980022	MZ026031
<i>Hypsoblennius hentz</i> (Lesueur, 1825)	-	JFBM 46471-VIMS10-78	Virgnia, USA	KF678572	KF678666	MW980023	MZ026032
<i>Hypsoblennius sordidus</i> (Bennett, 1828)	-	-	Chile	MG779098	MG779133	MW980024	MZ026033
<i>Parablennius gattorugine</i> ^(I) Miranda Ribeiro, 1915	-	MNHN 2012-0229	Banyuls sur Mer, France	KF678557	KF678652	MW980025	MZ026034
<i>Parablennius gattorugine</i> ^(II)	-	EFMM-16-060915	Avola, Italy	MZ025980	MZ025998	MW980026	MZ026035
<i>Parablennius parvicornis</i> Miranda Ribeiro, 1915	-	MNHN 2012-0238	Terceira, Azores	KF678559	KF678654	MW980027	MZ026036
<i>Parablennius ruber</i> Miranda Ribeiro, 1915	-	MNHN 2012-0243	Terceira, Azores	KF678562	MG779140	MW980028	MZ026037
<i>Parablennius sanguinolentus</i> ^(I) Miranda Ribeiro, 1915	-	EFMM-23-181115	Ognina, Italy	MZ025981	MZ025999	MW980029	MZ026038
<i>Parablennius sanguinolentus</i> ^(II)	-	EFMM-27-100716	Avola, Italy	MZ025982	MZ026000	MW980030	MZ026039
<i>Parablennius sanguinolentus</i> ^(III)	-	MNHN 2012-0246	Banyuls sur Mer, France	KF678563	KF678656	MW980031	MZ026040
<i>Salaria basilisca</i> ^(I) (Valenciennes, 1836)	-	MZFU-17633	Ghar El Melh, Tunisia	-	-	MH724822	MH715446
<i>Salaria basilisca</i> ^(II)	-	MZFU-17634	Sayeda, Tunisia	-	-	MH724823	MH715447
<i>Salaria pavo</i> ^(I) (Risso, 1810)	-	-	Palermo, Italy	MZ025983	MZ026001	MH724841	MH715465
<i>Salaria pavo</i> ^(II)	-	-	Palermo, Italy	MZ025984	MZ026002	MH724842	MH715466
<i>Salaria pavo</i> ^(III)	-	MNHN 2003-1994	Ile-Tudy, France	KF678551	KF678646	MW980032	MZ026041
<i>Salariopsis</i> * <i>atlantica</i> ^(I) Doadrio, Perea & Yahyaoui, 2011	Previously the genus referred to <i>Salaria</i> Forsskål, 1775	MNCN 279641-279660	Ouerrha R. Morocco	-	-	FJ465736	FJ465527
<i>Salariopsis</i> * <i>atlantica</i> ^(II)	Previously the genus referred to <i>Salaria</i> Forsskål, 1775	MNCN 279641-279660	Ouerrha R. Morocco	-	-	FJ465737	FJ465526
<i>Salariopsis</i> * <i>economidisi</i> ^(I) (Kottelat, 2004)	Previously the genus referred to <i>Salaria</i> Forsskål, 1775	-	Lake Trichonis, Greece	MZ025985	MZ026003	MW980033	MZ026042
<i>Salariopsis</i> * <i>economidisi</i> ^(II)	Previously the genus referred to <i>Salaria</i> Forsskål, 1775	-	Lake Trichonis, Greece	MZ025986	MZ026004	MW980034	MZ026043
<i>Salariopsis</i> * <i>economidisi</i> ^(III)	Previously the genus referred to <i>Salaria</i> Forsskål, 1775	-	Lake Trichonis, Greece	MZ025987	MZ026005	MW980035	MZ026044

Table 1. Cont.

Species	Taxonomical Remarks	Catalog Number	Locality	ENC1	MYH6	16S	Dloop
<i>Salariopsis</i> * <i>fluviatilis</i> ^(I) (Asso, 1801)	Previously the genus referred to <i>Salaria</i> Forsskål, 1775	MZFU-17635	Stream Frattina, Italy	MZ025988	MZ026006	MH724847	MH715471
<i>Salariopsis</i> * <i>fluviatilis</i> ^(II)	Previously the genus referred to <i>Salaria</i> Forsskål, 1775	MZFU-17636	Lake Garda, Italy	MZ025989	MZ026007	MH724848	MH715472
<i>Salariopsis</i> * <i>fluviatilis</i> ^(III)	Previously the genus referred to <i>Salaria</i> Forsskål, 1775	-	-	HM050017	HM050075	-	-
<i>Scartella caboverdiana</i> (Bath, 1990)	-	JFBM 47282	Cape Verde	MG779110	MG779147	MW980036	MZ026045
<i>Scartella cristata</i> ^(I) (Linnaeus, 1758)	-	TIUFRN3520	-	MZ025990	MZ026008	MW980037	MZ026046
<i>Scartella cristata</i> ^(II)	-	BPS3411	Eastern Atlantic	MZ025991	MZ026009	MW980038	MZ026047
<i>Scartella cristata</i> ^(III)	-	EFMM-4-060815	Avola, Italy	MZ025992	MZ026010	MW980039	MZ026048
<i>Scartella cristata</i> ^(IV)	-	EFMM-24-090716	Avola, Italy	MZ025993	MZ026011	MW980040	MZ026049
<i>Scartella emarginata</i> (Günther, 1861)	-	JFBM 47159-1576	Kochi, Japan	KF678541	KF678637	MW980041	MZ026050
<i>Scartella itajobi</i> (Rangel and Mendes, 2009)	-	TIUFRN3508	-	-	MZ026012	MW980042	MZ026051
<i>Diademichthys lineatus</i> (Sauvage, 1883)	-	-	-	JX188985	JX189754	-	-
<i>Enneapterygius minutus</i> (Günther, 1877)	-	JFBM 46377-1224	-	KF678492	KF678594	-	-

Total genomic DNA was extracted from muscle or fin clips using a Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA), according to manufacturer suggested protocol. Polymerase chain reaction (PCR) was used to amplify fragments of two nuDNA exons (ectodermal-neural cortex 1-like protein, Enc1, and the cardiac muscle myosin heavy chain 6 alpha, myh6) and two mtDNA fragments (16S ribosomal RNA, 16S, and the control region, D-loop). PCR reactions contained 1.5 µL template DNA, 2.75 µL water, 6.25 µL GoTaqR Green Master Mix (Promega, Madison, WI), with 1.0 µL of each primer (10µM) (see [12,21,22] for the primer pairs used for the different loci). Exonuclease 1 and shrimp alkaline phosphatase were added to PCR products for enzymatic purification at manufacturer-suggested thermal profiles. Automated Sanger sequencing of purified PCR products was performed using ABI Prism R BigDye Terminator v. 3.1 chemistry (Applied Biosystems, Foster City, CA, USA) at the Biomedical Genomics Center DNA Sequencing and Analysis Facility at the University of Minnesota, USA. Complementary heavy and light strands were aligned into contiguous sequences (contigs) and edited in Geneious v. 6.1.8 (Biomatters Ltd., Auckland, New Zealand). Alignments were visually inspected for potential misalignments and, when appropriate, verified by checking amino acid translations.

All sequences were aligned with the software MEGAX [23], using the ClustalW method [24]. All novel sequences were deposited in GenBank (see Table 1 for their Accession Numbers). The alignment of the novel fragments and those downloaded from GenBank were trimmed to fragments of 801 bp (Enc1) 754 bp (myh6) 517 bp (16S) 308 bp (D-loop), respectively. In addition, publicly available sequences belonging to the study taxa and the outgroups *Diademichthys lineatus* (Sauvage, 1883) (Gobiesocidae) and *Enneapterygius minutus* (Günther, 1877) (Tripterygiidae) were downloaded from GenBank and included in the analyses (see Table 1 for their GenBank Accession Number, AN).

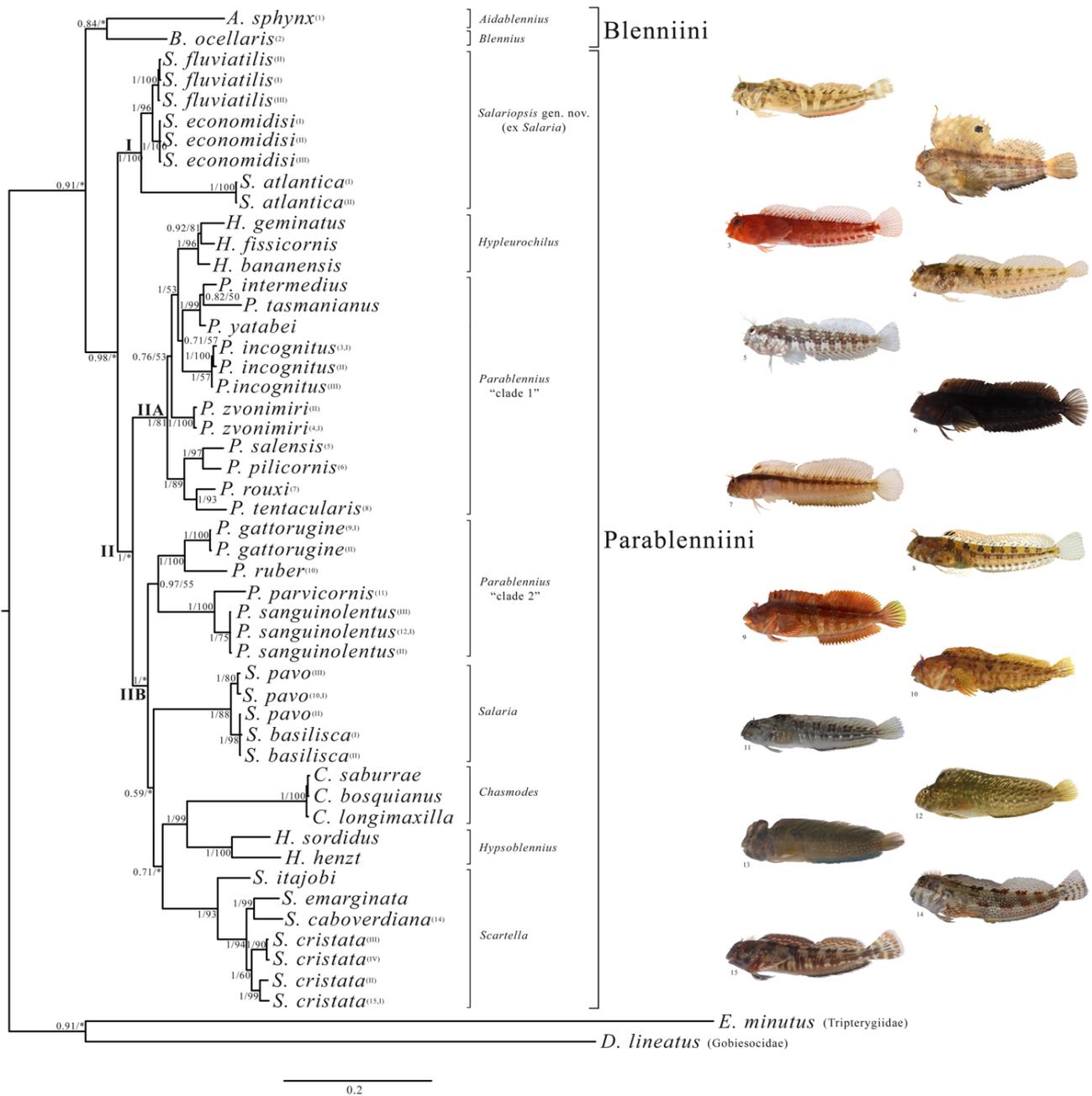


Figure 1. Bayesian phylogram of the studied Blenniidae samples based on the concatenated mtDNA and nuDNA dataset. Node statistical support is reported as nodal posterior probabilities (Bayesian Inference of phylogeny, BI)/bootstrap values (maximum likelihood, ML). Asterisks indicate a bootstrap support value lower than 50. Square brackets group the samples according to the current taxonomy. Arabic numbers in brackets refer to the blennies' images attached next to the phylogram. Roman numbers in brackets refer to specimens listed in Table 1. (I), freshwater *Salariopsis* gen. nov. (ex *Salaria*) clade; (II), marine Parablenniini clade; (IIA), *Hypleurochilus* and *Parablennius* "clade 1" subclades; (IIB), *Parablennius* "clade 2", *Salaria*, *Chasmodes*, *Hypsoblennius* and *Scartella* subclades.

In order to test whether the mitochondrial and nuclear fragments could be combined for joint analyses, the incongruence length difference test (ILD, [25]) as implemented in PAUP* v. 4.0b10 [26] was used. According to Cunningham [27], if $p > 0.01$, pooling the data improves the phylogenetic accuracy, and thus it is admissible to merge the tested datasets into a single matrix. This condition was fulfilled both for the concatenation of all the genetic markers analysed in the frame of this study ($p = 1$). Therefore, the fragments of both the

mtDNA and nuDNA loci were concatenated in a single, partitioned dataset. The best evolutionary model for each locus was selected among models analysed by MrBayes v. 3.2.6 [28] using Bayesian model choice criteria (nst = mixed, rates = gamma). The phylogenetic analyses of the partitioned concatenated dataset, including the fragments of the amplified DNA loci, were conducted using Bayesian Inference (BI) and Maximum Likelihood (ML) framework in the software package MrBayes and PhyML v. 3 [29], respectively. Bootstrap values [30] were calculated with 1000 replicates in the ML trees, whereas the node posterior probability values were reported in the BI tree. In the BI analyses, two independent Markov Chain Monte Carlo analyses were performed with 1 million generations (temp.: 0.2; default priors). Trees and parameter values were sampled every 100 generations, with the result of 10,000 trees for each analysis. Convergence of chains was assessed to ensure proper mixing (Effective Sample Size, ESS, greater than 200 in all the analyses performed). The initial 25% of trees were discarded as “burn-in”.

3. Results

All phylogenetic analyses based on the concatenated DNA dataset were congruent and nodes were well-supported. Most of the genera included in the analyses proved to be monophyletic, with the noteworthy exception of *Salaria* Forsskål, 1775 and *Parablennius* Miranda Ribeiro, 1915, which were paraphyletic.

The *Parablenniini* are separated from *Blenniini* by a cladogenetic event, with an uncorrected *p*-distance between the two tribes of 15.5% (Figure 1). There are two well-supported major subclades within *Parablenniini*: a clade that includes the investigated freshwater *Salaria* species (see Figure 1, clade “I”), and a clade that includes the remaining analysed ingroup taxa (see Figure 1, clade “II”). Within clade “II”, the genus *Parablennius* is split into two different subclades; one subclade includes *Parablennius intermedius*, *P. tasmanianus*, *P. yatabei*, *P. incognitus*, *P. zvonimiri*, *P. salensis*, *P. pilicornis*, *P. rouxi*, *P. tentacularis* and the genus *Hyppleurochilus* Gill, 1861 (subclade “IIA”, see Figure 1); the second subclade includes the rest of the analysed *Parablennius* species (i.e., *P. gattorugine*, *P. ruber*, *P. parvicornis*, and *P. sanguinolentus*) along with representatives of the genera *Chasmodes* Valenciennes, 1836, *Hypsoblennius* Gill, 1861, *Scartella* Jordan, 1886 and *Salaria* (subclade “IIB”, see Figure 1). The uncorrected *p*-distance between the two subclades (i.e., “IIA” and “IIB”) is 13.4%.

4. Discussion

The phylogenetic trees obtained in the present study highlight some important inconsistencies in the current taxonomy of *Parablenniini*: (i) the genera *Parablennius* and *Salaria* are paraphyletic; (ii) some alleged *Parablennius* species cluster with the genus *Hyppleurochilus*; (iii) a scarce-to-absent genetic differentiation was observed between the three species belonging to the genus *Chasmodes*. Our study strongly supports prior findings which suggested a sharp differentiation between the marine and freshwater species currently ascribed to the genus *Salaria* (e.g., [12,17]). To date, three species are formally described within the freshwater clade of *Salaria*: the widespread *S. fluviatilis*; *S. economidisi*, endemic to Lake Trichonis (Greece), and *S. atlantica*, endemic to Morocco. Moreover, a further undescribed taxon of putative species rank occurs in the Middle East (see [31,32]).

This group of freshwater blennies is deeply divergent from its alleged marine congeneric taxa, by an extent much greater than that reported by Doadrio et al. [33], Hundt et al. [12] and Vecchioni et al. [17], thus stressing the inappropriateness of their current generic assignment. Even though some studies (e.g., [34]) found some clear osteological differences between *S. pavo* and *S. fluviatilis*, to date, the absence of morphological synapomorphies is a recurrent issue [35]). Based on these results, the taxonomical status of the freshwater species currently ascribed to the genus *Salaria* must be revised. Considering that the type taxon of the genus *Salaria* is *S. basilisca* (Valenciennes, 1836) (see also [36]), the species of the marine clade belong to *Salaria* s.s.. Conversely, no genus-level epithet is available for the divergent freshwater clade currently ascribed to “*Salaria*”. We propose the

new genus *Salariopsis*, which includes the species *Salariopsis fluviatilis*, *S. economidisi* and *S. atlantica*.

5. Systematics

Family: Blenniidae Rafinesque, 1910

Genus: *Salariopsis* new genus (Zoobank link LSID: <http://zoobank.org/urn:lsod:zoobank.org:pub:1884E670-F6E7-48F8-AF7F-19380579DB8>)

Type species of the genus: *Salariopsis fluviatilis* (Asso, 1801)

Synonyms: none

Etymology: By adding the suffix—opsis, from the ancient Greek ὄψις (view, appearance), to the epithet “*Salaria*”, we want to highlight its apparent, but misleading, morphological similarity to the blenniid genus *Salaria* Forsskål, 1775

Morphological diagnosis: Fishes of the genus *Salariopsis* and *Salaria* have many overlapping meristic counts. However, *Salariopsis* possess fewer soft dorsal and anal fin elements than *Salaria*. In fact, *Salariopsis* has 16–17 dorsal and 16–19 anal fin rays, whereas *Salaria* has 22–25 and 23–28 fin rays, respectively (see Table 2).

Table 2. Meristic data compiled from literature for comparison of fin element counts. Superscripts indicate source: ^a Bath [8], ^b Kottelat [37], ^c Doadrio et al. [33], and ^d Tiralongo [20]. Presence of two spines in the pelvic fins of *Salaria atlantica* could not be confirmed. Tiralongo [20] added two new observations from *S. basilisca*: a specimen with 28 anal fin rays and another with 2 pelvic fin rays.

Species	Dorsal Fin	Anal Fin	Pectoral Fin	Pelvic Fin
<i>Salariopsis fluviatilis</i>	XII–XIII, 16–17 ^d	II, 16–19 ^c	12–14 ^d	I, 3 ^a
<i>Salariopsis economidisi</i>	XII–XIII, 16–17 ^b	II, 16–19 ^c	13–14 ^b	I, 3 ^d
<i>Salariopsis atlantica</i>	XII–XIII, 16–17 ^c	II, 16–17 ^c	10–11 ^c	II, 2–4 ^c
<i>Salaria pavo</i>	XI–XIII, 22–25 ^b	II, 23–26 ^c	14 ^a	I, 3 ^a
<i>Salaria basilisca</i>	XI–XIII, 23–27 ^a	II, 25–28 ^{c,d}	14 ^a	I, 2–3 ^{a,d}

The novel data used in this study provided results in accordance with previous molecular studies of the Almadablennius clade (e.g., [11,12,16,17,35]): the genus *Parablennius* s.l. proved to be paraphyletic, supporting the likely presence of at least two distinct and distantly related genera currently joined together within this name. Furthermore, the genus *Hyppleurochilus* s.l. was nested within *Parablennius* (Figure 1, subclade “IIA”). Considering that these two genera share similar morphological features [35] and that the phylogenetic relationships are not in accordance with the current systematics, the taxonomic status of these two genera should be reassessed.

The remaining results largely agree with previous phylogenetic studies and taxonomy, while also providing direction for future studies of speciation and phylogeography. For example, a clade containing *Chasmodes*, *Scartella*, and *Hypsoblennius* was recovered, similar to previous studies (e.g., [16]).

The genus *Chasmodes* includes three species, *Chasmodes saburrae*, *C. bosquianus* and *C. longimaxilla*. Recently, Javonillo and Harold [38] highlighted the existence of a scarce interspecific divergence among the species of this genus, and their sister group relationship with a clade including the genera *Scartella*, *Hypsoblennius* and *Hyppleurochilus* based on 12S mitochondrial DNA sequences. Our results are partially in contrast to those reported by Javonillo and Harold [38]. In fact, even if we detected a very low interspecific divergence (mean uncorrected *p*-distance about 0.22%) among the *Chasmodes* species and a sister group relationship between *Chasmodes* and the genera *Scartella* and *Hypsoblennius*, we did not observe the same phylogenetic relationship with the *Hyppleurochilus* taxa (see Figure 1). This is probably due to our richer sampling effort, which includes more species than those investigated by Javonillo and Harold [38]. The scarce differentiation detected between the *Chasmodes* species is possibly related to their recent origin linked to sea-level fluctuations,

as proposed by Javonillo and Harold [38]. However, bearing in mind that these species have a different ecology, phenotypic plasticity, i.e., an adaptive response to different local habitats and ecology, might be playing a major role in driving the diversification of the three *Chasmodes* lineages and might be accountable for their morphological variations.

Finally, our phylogenetic analyses confirm the monophyly of the genus *Scartella*, as already proposed by other authors [12,35,39], finding a sister clade relationship of this genus with the clade that includes *Chasmodes* spp. and *Hypsoblennius* spp. Within the *Scartella* clade, an uncorrected *p*-distance of 5.23% separating the Mediterranean versus the Atlantic specimens of *S. cristata* (see Table 1) suggests the possible presence of well-characterised parapatric lineages within this species, whose taxonomical rank should be the object of dedicated research.

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Data Availability Statement: The data presented in this study are available in GenBank (see Table 1 for their Accession Numbers).

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