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Phylogenetic relationships of the north-eastern Atlantic and Mediterranean blenniids

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The phylogenetic relationships of 27 north-eastern Atlantic and Mediterranean blennioids are analysed based on a total of 1001 bp from a combined fragment of the 12S and 16S mitochondrial rDNA. The most relevant results with implications in current blenniid taxonomy are: (1) *Lipophrys pholis* and *Lipophrys* (= *Paralipophrys*) *trigloides* are included in a well-supported clade that by the rule of precedence must be named *Lipophrys*; (2) the sister species of this clade are not the remaining species of the genus *Lipophrys* but instead a monotypic genus comprising *Coryphoblennius galerita*; (3) the smaller species of *Lipophrys* were recovered in another well-supported and independent clade, which we propose to be recognized as *Microlipophrys*; (4) although some authors included the genera *Salaria* and *Lipophrys* in a single group we have never recovered such a relationship. Instead, *Salaria* is more closely related to the genera *Scartella* and *Parablennius*; (5) the genus *Parablennius*, which was never recovered as a monophyletic clade, is very diverse and may include several distinct lineages; (6) the relative position of *Aidablennius sphynx* casts some doubts on the currently recognized relationships between the different blenniid tribes. Meristic, morphological, behavioural and ecological characters support our results and are also discussed. The possible roles of the tropical West African coast and the Mediterranean in the diversification of blenniids are discussed.

ADDITIONAL KEYWORDS: 12S rDNA – 16S rDNA – Blenniidae – phylogeny.

INTRODUCTION

Blennioids have a world-wide distribution, reaching their highest diversity in tropical and subtropical seas (Nelson, 1994). They are one of the most abundant and important fish groups in the north-eastern Atlantic and Mediterranean rocky shores. Study of the phylogenetic relationships of these fishes may provide an important contribution to our understanding of the history of the north-eastern Atlantic and Mediterranean ichthyofauna.

The monophyly of the suborder Blennioidei and the family Blenniidae is supported by morphological and molecular evidence (Springer, 1993; Nelson, 1994; Stepien *et al*., 1997). However, the taxonomic history of the north-eastern Atlantic and Mediterranean blenniids has been marked by many revisions and species reassignments. Some controversies arose with some authors splitting and others clumping the different taxa. A summary of this complex history is presented in Table 1 and is described in the following paragraphs.

In his synopsis of the Blenniidae, Norman (1943) grouped species of this family into three subfamilies, Ophioblenniinae, Salariinae and Blenniinae. He erected the monospecific genus *Coryphoblennius* with *Coryphoblennius galerita* (Linnaeus, 1758) and placed the remaining north-eastern Atlantic and Mediterranean blenniids in the genus *Blennius*. He also

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Table 1. Schematic representation summarizing the main revisions of the taxonomy of the Atlanto-Mediterranean blenniids, by Norman (1943), Bath (1977) and Zander (1986). For simplification purposes only the species analysed in this paper are shown

Norman, 1943			Bath, 1977		Zander, 1986	
Genera	Sub-genera	Species	Genera	Species	Genera	Species
Coryphoblennius		*C. galerita*	*Coryphoblennius*	*C. galerita*	*Coryphoblennius*	*C. galerita*
Blennius	*Blennius*	*B. ocellaris*	*Blennius*	*B. ocellaris*	*Blennius*	*B. ocellaris*
	Salaria	*B. sphynx*	*Aidablennius*	*A. sphynx*	*Aidablennius*	*A. sphynx*
		B. sanguinolentus	*Pictiblennius*	*Pi. sanguinolentus*	*Parablennius*	*P. sanguinolentus*
				Pi. parvicornis		*P. parvicornis*
				Pi. incognitus		*P. incognitus*
		B. gattorugine		*Pi. zvonimiri*		*P. zvonimiri*
		B. tentacularis	*Parablennius*	*P. gattorugine*		*P. gattorugine*
		B. pilicornis		*P. tentacularis*		*P. tentacularis*
				P. pilicornis		*P. pilicornis*
				P. rouxi		*P. rouxi*
						P. ruber
		B. cristatus	*Scartella*	*S. cristata*	*Scartella*	*S. cristata*
		B. fluviatilis	*Salaria*	*S. fluviatilis*	*Lipophrys*	*L. fluviatilis*
				S. pavo		*L. pavo*
	Lipophrys	*B. trigloides*	*Paralipophrys*	*P. trigloides*		*L. trigloides*
		B. pholis	*Lipophrys*	*L. pholis*		*L. pholis*
		B. canevai		*L. canevai*		*L. canevai*
		B. adriaticus		*L. adriaticus*		*L. adriaticus*
		B. nigriceps		*L. nigriceps*		*L. nigriceps*
		B. dalmatinus		*L. dalmatinus*		*L. dalmatinus*

suggested that *Blennius* should be divided into three subgenera: *Blennius*, *Lipophrys* and *Salaria*.

Springer (1968), Springer & Smith-Vaniz (1972) and Smith-Vaniz (1976) addressed the taxonomic classification within the family Blenniidae mainly at a subfamily and tribe level. They recognized six major lineages within the Blenniidae. Bock & Zander (1986) renamed one of these lineages, with the final result being the recognition of the following six tribes: Salariinae, Blenniini, Parablenniini, Omobranchini, Phenablenniini and Nemophini. Nelson (1994) restated this classification with *Ophioblennius atlanticus* (Valenciennes, 1836) in the tribe Salariini, *Blennius ocellaris* Linnaeus, 1758 in the tribe Blenniini and the remaining north-eastern Atlantic and Mediterranean species in the tribe Parablenniini.

Bath (1977) extensively revised these fishes and suggested that *Lipophrys* should be raised to generic status, but he also erected a new monospecific genus with *Paralipophrys trigloides* (Valenciennes, 1836). He was the first to suggest that *Parablennius* is a polyphyletic genus placing several species currently classified as *Parablennius* in a new genus named *Pictiblennius*.

Zander (1978) argued against the inclusion of *Paralipophrys* and *Salaria* in *Lipophrys* and of *Pictiblennius* in *Parablennius*, which was also defended by Bock & Zander (1986) and Nelson (1994). Subsequently, Bath (1981) himself admitted the inclusion of *Pictiblennius* in *Parablennius*.

Papaconstantinou (1977) reported a close relationship between *C. galerita* and *Lipophrys* (= *Paralipophrys*) *trigloides* based on a comparative study of the skulls of Mediterranean blenniids. Also based on skull morphology, Bock & Zander (1986) noted a close relationship between *Coryphoblennius* and *Lipophrys*.

Bath (1996) suggested that the genera *Lipophrys* [but not *L.* (= *Paralipophrys*) *trigloides*] and *Salaria* are more closely related to each other than to any other genus. He also divided the genus *Parablennius* into five distinct groups (see Discussion).

Richtarski & Patzner (2000) compared the morphology of male reproductive systems in Mediterranean blenniids and reported a close similarity between *Lipophrys* [including *Lipophrys* (= *Paralipophrys*) *trigloides*] and *Blennius*. They also argued that *Salaria pavo* (Risso, 1810) is more closely related to *Parablennius sanguinolentus* (Pallas, 1814) than to *Lipophrys*, a view also shared by Garcia, Alvarez & Thode (1987) based on a study of the karyoevolutional pathways of some blenniids. Garcia *et al*. (1987) also stated that *C. galerita* is closely related to *Lipophrys pholis* (Linnaeus, 1758) and *L.* (= *Paralipophrys*) *trigloides*.

Finally, Nieder & Busse (1992) addressed the systematics of the tribe Parablenniini based on a comparison of band patterns from blood serum electrophoresis in seven blenniid species, and recovered three groups: *Parablennius*, *Lipophrys* and *Scartella*. *C. galerita* was not included in their analysis.

This brief review illustrates the controversy on the phylogeny of this group. One major drawback of the studies reported so far was their lack of a cladistic approach (except for Springer, 1993; Stepien *et al*., 1997), which led to definitions of groups based on mere similarities, i.e. mixtures of primitive and derived characters. Additionally, in many of these studies, the different lineages were highly unequally represented, limiting the validity of the generalizations made.

The first molecular and cladistic approach to the phylogeny of blennioid fishes was performed by Stepien *et al*. (1997). However, they addressed the relationships among families and tribes at a similar level to that of Springer (1993) with morphological data. Furthermore, although Stepien *et al*. (1997) did sample four of the six recognized tribes of the Blenniidae, only two of the Parablenniini genera were included. In addition, the eastern Atlantic and Mediterranean species of this tribe were not included, thus leaving the controversy about the phylogeny of the Atlanto-Mediterranean blenniids unresolved. Based on molecular data from a fragment of the mitochondrial 12S rDNA, they suggested that: (1) the family Blenniidae is monophyletic; (2) the tribe Parablenniini appears to be monophyletic – the tribe Salariini is paraphyletic due to the fact that the genus *Ophioblennius* was recovered with the tribes Nemophini and Omobranchini; and (3) the Salariini, with the exception of *Ophioblennius*, are a sister group of the Parablenniini. Stepien *et al*. (1997) also confirmed some relationships within the suborder Blennioidei, providing evidence that the Tripterygiidae are the sister family of the Blenniidae.

In this study we analysed the phylogeny of the north-eastern Atlantic and Mediterranean Blenniidae. Phylogenetic relationships were inferred, based on partial sequences of the 12S and 16S mitochondrial rDNA combined in a single fragment. These relationships were supported by an explicit cladistic perspective and the results were compared with other independent sources of data currently available in the literature, namely meristic, morphometric, behavioural and ecological data. Relationships within and between species were also used to discuss briefly the biogeography of this group.

METHODS

The species sampled in the present study, the origins of the samples and the GenBank accession numbers are listed in Table 2. A total of 25 blenniid species representing some west African species and all genera described for the north-eastern Atlantic and Mediter-

ranean (with the exception of *Hypleurochilus* and *Spaniblennius*) (Almada *et al*., 2001) were analysed. The collection of specimens analysed in this study was deposited in the Oceanographic Museum of the Arrábida Nature Park (references MOPNA580–604).

In an attempt to detect possible intraspecific variability in these species, samples were collected in locations as distant as possible within the geographical range of each species (see Table 2).

The choice of the outgroup species was made according to the results of Stepien *et al*. (1997). The outgroup species used were *Tripterygion delaisi* Cadenat & Blache, 1970 from the family Tripterygiidae and *Labrisomus nuchipinnis* (Quoy & Gaimard, 1824) from the more distantly related blennioid family Labrisomidae (references MOPNA605 and MOPNA606, respectively).

Total genomic DNA was extracted from muscle tissue or from finrays that were preserved in 96% ethanol using a proteinase K/SDS-based extraction buffer, and purified by phenol/chloroform and ethanol precipitation (Maniatis, Fritsch & Sambrook, 1982).

Primers were designed from the alignment of the complete mitochondrial DNA sequences of ten fish species belonging to six different families (Cyprinidae, Homalopteridae, Salmonidae, Bothidae, Gadidae and Latimeriidae) available in the GenBank database (accession numbers AF023183, AF023188, AF038484, NC001606, M91245, NC001717, NC001960, AB000667, X99772, Z21921). Both 12S and 16S rDNA primers proved to be efficient in amplifying DNA from a wide range of fish families in our laboratory, namely: Atherinidae, Batrachoididae, Blenniidae, Cyprinidae, Gobiesocidae, Gobiidae, Labridae, Labrisomidae, Mugilidae, Sparidae and Tripterygiidae. Primer sequences were:

- 12S rDNA (fragment length 404 bp) 12SFor 5′-AAC TGGGATTAGATACCCCAC-3′ and 12SRev 5′-GGG AGAGTGACGGGCGGTGTG-3′.
- 16S rDNA (fragment length 597 bp) 16SFor 5′-AAG CCTCGCCTGTTTACCAA-3′ and 16SRev 5′-CTGAACTCAGATCACGTAGG-3′.

Amplifications were obtained in a total volume of 20 μL with 1.5 μM $MgCl_2$, 200 μM each dNTP, 0.5 μM of each primer, 0.5 units of *Taq* polymerase (Gibco BRL, Life Technologies Inc., Gaithersburg, MD, USA), approximately 20 ng of genomic DNA and 1× buffer supplied by the manufacturer.

PCR was performed in a Biometra thermblock (Biometra, Trio-Thermblock, Göttingen, Germany) and in a Biorad Gene-Cycler. These amplifications consisted of 4 min at 94 °C, and 30 cycles of 1 min at 94 °C, 1 min at 55 °C and 1 min at 72 °C, and 10 min at 72 °C for the 12S and 16S rDNA. Gel purification of PCR products was performed with GFX PCR DNA and gel band purification kit (Amersham Pharmacia Biotech, UK). Samples were processed by either manual or automatic sequencing: (1) manual sequencing – the purified PCR products were cloned into the pGEM-T easy vector (Promega, Madison, WI, USA), with an alkaline-lysis extraction of the DNA, following the dideoxynucleotide chain termination method (Sanger, Nicklen & Coulson, 1977); (2) automatic sequencing – the purified PCR products were sequenced in a CEQ 2000 XL (Beckman Coulter, USA) with the same primers. Both strands of each specimen were sequenced; these sequences are available in the GenBank database (accession numbers are given in Table 1). Alignments were made using ClustalX 1.81 (Thompson *et al*., 1997) with default settings. Transitional saturation was examined by plotting transitions and transversions against sequence divergence. The transition (Ts)/transversion (Tv) ratio for each fragment was determined by the average of the quotient between transitions and transversions for each pair of species. Character congruence between the two fragments was tested using the incongruence-length difference test (ILD) (Farris *et al*., 1995) available in PAUP 4.0b10 Win (Swofford, 2002).

In order to estimate the relative rate of substitutions for the 12S and 16S rDNA sequences, the percentage divergence for each possible pair of 12S rDNA haplotypes was divided by the percentage divergence for the equivalent pair of the 16S rDNA.

The combined data set of the 12S and the 16S rDNA fragments was analysed with three methods of phylogenetic inference: maximum-parsimony (MP), maximum-likelihood (ML) and minimum-evolution (ME) (Saitou & Nei, 1987). Analysis was performed with PAUP 4.0b10 Win (Swofford, 2002). Bootstrapping (Felsenstein, 1985) was used to determine robustness of the nodes in the trees with 1000 replicates for MP and neighbour-joining (NJ) and 100 replicates for ML. The heuristic search option 'random addition of taxa' and tree bisection reconnection (TBR) were used with the three methods of inference. MP analysis was conducted with the ACCTRAN option. In order to choose the model of evolution that best fitted our data we used the program Modeltest 3.06 (Posada & Crandall, 1998). The ML settings selected corresponded to the GTR+I+G model. NJ analysis was performed with the distance derived from the general time reversible model (GTR).

RESULTS

Alignment, base composition and sequence polymorphism

Inspection of the basic information on the DNA fragments analysed in this study (Table 3) shows that

Table 2. List of species studied (names according to Zander, 1986; Bath, 1990a), geographical origin of the samples and GenBank accession numbers

Family	Tribe	Genus	Species	Origin of samples	GenBank accession numbers
Tripterygiidae	–	*Tripterygion*	*T. delaisi*	Mainland Portugal, Madeira, Azores	AY098809, AY098810, AY098811, AY098812, AY098813, AY098849, AY098850
Labrisomidae	–	*Labrisomus*	*L. nuchipinnis*	Cape Verde	AY098807, AY098808, AY098847, AY098848
Blenniidae	Salariini	*Ophioblennius*	*O. atlanticus*	Azores, Cape Verde	AY098805, AY098806, AY098846
	Blenniini	*Blennius*	*B. ocellaris*	Greece, Azores	AY098746, AY098747, AY098815
	Parablenniini	*Aidablennius*	*A. sphynx*	Croatia, Italy	AY098744, AY098745, AY098814, AF549191, AF549192, AF549193
		Coryphoblennius	*C. galerita*	Lebanon, Croatia, Italy, UK, Mainland Portugal, Madeira, Azores	AY098748, AY098749, AY098750, AY098751, AY098752, AY098753, AY098754, AY098755, AY098816, AY098817, AY098818, AY098819
		Parablennius	*P. gattorugine*	Greece, Italy, Mainland Portugal	AF414715, AY098777, AY098778, AY098835
			P. ruber	Azores	AF414716, AY098779, AY098834
			P. pilicornis	Mainland Portugal	AY098795, AY098796, AY098831
			P. sanguinolentus	Lebanon, Greece, Croatia, Italy, Mainland Portugal	AF414697, AF414698, AF414699, AF414700, AF414701, AF414702, AF414703, AF414704, AF414705, AF428241, AF428242, AY098837
			P. parvicornis	Cape Verde, Canaries, Madeira, Azores	AF414706, AF414707, AF414708, AF414709, AF414710, AF414711, AF414712, AF428238, AF428239, AF428240, AY098830
			P. rouxi	Spain, Italy	AY098781, AY098782, AY098783, AY098832, AY098833
			P. tentacularis	Italy	AY098780, AY098838, AY098839
			P. zvonimiri	Lebanon, Greece, Italy, Spain	AY098790, AY098791, AY098792, AY098793, AY098794, AY098840, AY098841
			P. incognitus	Croatia, Italy, Mainland Portugal, Madeira, Azores	AY098784, AY098785, AY098786, AY098787, AY098788, AY098829
			P. salensis	Cape Verde	AY098789, AY098836
		Salaria	*S. pavo*	Lebanon, Israel, Greece, Italy, Mainland Portugal	AY098798, AY098799, AY098800, AY098801, AY098802, AY098842
			S. fluviatilis	Mainland Portugal	AY098797, AY098843
		Lipophrys	*L. pholis*	Mainland Portugal, UK, Azores	AY098761, AY098762, AY098763, AY098764, AY098765, AY098766, AY098767, AY098825
			L. canevai	Lebanon, Greece, Croatia, Mainland Portugal	AF414713, AY098773, AY098774, AY098775, AY098821
			L. dalmatinus	Greece, Italy	AY098756, AY098757, AY098823
			L. nigriceps	Spain	AF414714, AY098776, AY098824
			L. adriaticus	Croatia	AY098758, AY098820
			L. caboverdensis	Cape Verde	AY098759, AY098760, AY098822
			L. (= *Paralipophrys*) *trigloides*	Greece, Mainland Portugal, Madeira, Azores	AY098768, AY098769, AY098770, AY098771, AY098772, AY098826, AY098827, AY098828
		Scartella	*S. cristata*	Spain	AY098803, AY098845
			S. caboverdiana	Cape Verde	AY098804, AY098844

Table 3. Summary of the basic information on the 12S and 16S rDNA fragments analysed in this study. *Abbreviations*: Ts, transition; Tv, transversion

	Size (bp)	Conserved sites	Phylogenetically informative sites	Ts/Tv ratio	Adenine	Cytosine	Guanine	Thymine
12S rDNA	404	55%	39%	1.63	30%	25%	22%	22%
16S rDNA	597	61%	30%	1.76	29%	25%	23%	23%

both 12S and 16S fragments are somewhat richer in adenine than in other bases, as described for the ribosomal mtDNA of other fish (see Kocher *et al*., 1989; Meyer, 1993). Saturation analysis showed no mutational saturation either in 12S, in 16S rDNA or in the combined 12S + 16S fragments.

The fragment from the 12S rDNA evolved on average 1.26 times faster than the fragment from the 16S rDNA (SD = 0.34; min. = 0; max. = 3.84; $N = 593$). The alignments showed that indels represent 4% (9 indels) of the 12S rDNA and 5% (16 indels) of the 16S rDNA fragments. One of these indels had up to 11 nucleotides, which probably resulted from one insertion in *S. pavo* 16S rDNA. Both 12S and 16S fragments of *S. pavo* presented several insertions that were not observed in any other species analysed in this study, including the closely related *Salaria fluviatilis* (Asso, 1801).

Differences within species were analysed when samples from different geographical areas were available. Intraspecific genetic distances (uncorrected *p*-distance) were rather low for both fragments analysed ($\text{mean}_{12S} = 0.006$ and $\text{mean}_{16S} = 0.017$). The higher genetic distance between haplotypes within a single species was that of *C. galerita* with a maximum of 0.015 divergence for 12S rDNA and 0.043 divergence for 16S rDNA between samples from the Azores and Croatia. Interestingly, these intraspecific genetic distances are higher than the genetic distances found between several closely related blenniid species [e.g. 0.007/0.017 between *Lipophrys canevai* (Vinciguerra, 1880) and *Lipophrys nigriceps* (Vinciguerra, 1883), 0.010/0.019 between *Parablennius rouxi* (Cocco, 1833) and *Parablennius tentacularis* (Brünnich, 1768) and 0.013/0.030 between *Scartella cristata* (Linnaeus, 1758) and *Scartella caboverdiana* Bath, 1990b for 12S rDNA and 16S rDNA, respectively]. Nevertheless, although the sequences within each species were not always identical for all specimens, the different haplotypes of each species were always recovered in the same clade with all methods of phylogenetic inference used.

Phylogenetic analysis

The null hypothesis of congruence between the two data sets (12S rDNA and 16S rDNA) was rejected ($P = 0.01$) by the ILD test (Farris *et al*., 1995). Recently, however, Dolphin *et al*. (2000) showed that even when ILD tests reveal significant differences between two fragments it is frequently preferable to analyse them combined in a single data set. They note that this is especially true when the two fragments evolve at different rates or when one of them is small or noisy or 'lacks the ability to fully resolve trees'. Preliminary analysis of our data showed that, in general, the 12S and 16S rDNA gave the same information, although the small 12S rDNA fragment (404 bp) seems to lack phylogenetic signal for higher rank taxa. In addition, the combination of the two fragments in a single data set of 1001 bp yielded better resolved trees and an overall increase in bootstrap support. Therefore, we present the results of the analysis of this combined data set.

The phylogenetic relationships that resulted from the MP, ML and ME methods are shown in Figure 1. Parsimony analysis yielded 12 equally parsimonious trees with a length of 1451 steps, consistency index of 0.45 and retention index of 0.65.

All inference methods support the following results:

1. The phylogenetic relationships between *A. sphynx*, from the tribe Parablenniini, *B. ocellaris*, from the tribe Blenniini, and *O. atlanticus*, from the tribe Salariini, with the remaining Parablennini analysed in this study suggest that the tribal relationships among the Blenniidae should be further investigated.
2. Species of the genus *Lipophrys* were recovered in two distinct and very well-supported monophyletic clades. One of these clades includes *L. pholis*, *L.* (= *Paralipophrys*) *trigloides* and *C. galerita*. The other includes the small sized *Lipophrys*, whose males present facial masks during the breeding season, *L. canevai*, *L. nigriceps*, *L. caboverdensis*, *Lipophrys adriaticus* (Steindachner & Kolombatovic, 1883) and *Lipophrys dalmatinus* (Steindachner & Kolombatovic, 1883). The genetic distance (uncorrected *p*-distance) between the species of these two clades (mean = 0114, SD = 0.011, $N = 40$, range = 0.087–0.137) is greater than those between *L. pholis* or *L. trigloides* with *C. galerita* (0.047 between *L. pholis* and *L. trigloides*, 0.068 between *L. pholis* and *C. galerita* and 0.075

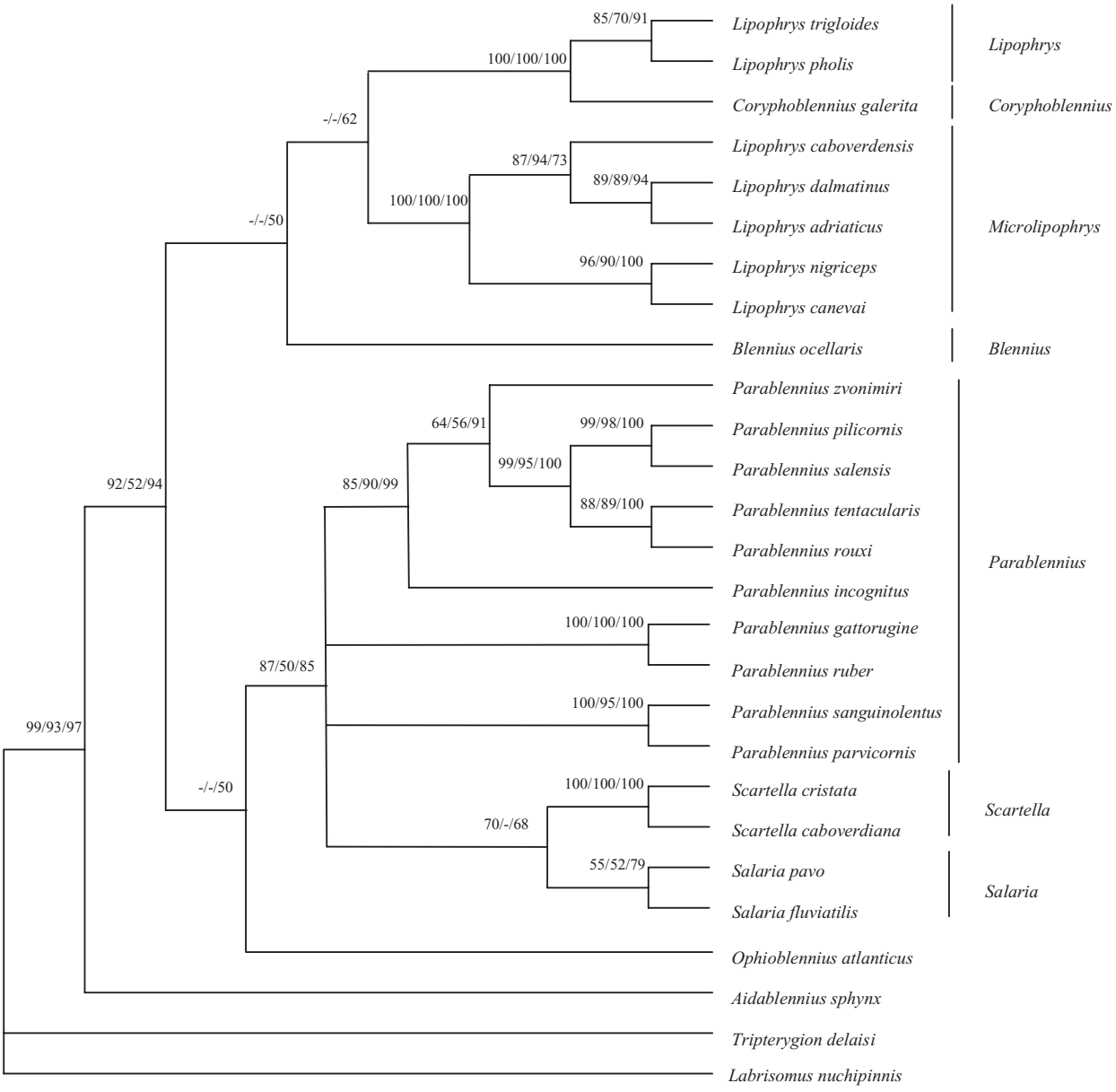

Figure 1. Phylogenetic tree obtained for the combined 12S–16S rDNA fragments sequenced. *Labrisomus nuchipinnis* and *Tripterygion delaisi* were used as outgroups. Bootstrap values for each node are shown as percentages for maximum-parsimony, maximum-likelihood and neighbour-joining, respectively. Parsimony analysis parameters: tree length = 1451; consistency index = 0.45; retention index = 0.65. Only bootstrap values above 50% for maximum-parsimony are shown.

between *L. trigloides* and *C. galerita*), which further emphasizes the paraphyly of *Lipophrys* as currently defined. To test our results against the currently accepted taxonomy we compared our tree with that obtained if we constrain the *Lipophrys* species to form a single group excluding *C. galerita*. The Kishino–Hasegawa test showed that our tree was significantly shorter than the constrained tree, which was 23 steps longer ($P = 0.0016$).

3. Concerning the genus *Salaria*, although we were not able to obtain samples of one of the species, *Salaria basilisca* (Valenciennes, 1836), and this node is not strongly supported, the available evidence does not argue against its monophyly.
4. *Parablennius* is not clearly recovered as a monophyletic clade. Instead, at least three clades emerge from our analysis: (i) *P. sanguinolentus* and *P. parvicornis*; (ii) *P. gattorugine* and *P. ruber*; and (iii) *Parablennius pilicornis* (Cuvier, 1829), *Parablennius salensis* Bath, 1990b; *P. tentacularis*, *P. rouxi*, *Parablennius incognitus* (Bath, 1968) and *Parablennius zvonimiri* (Kolombatovic, 1892).

5. Finally the genera *Parablennius*, *Salaria* and *Scartella* were recovered as a large suprageneric group that had already been mentioned by Norman (1943), although this was not supported by strong bootstrap values.

DISCUSSION

TAXONOMIC ISSUES

Tribes

The eastern Atlantic Blenniidae make up only a small fraction of the taxa within this family. Therefore, it is premature to draw conclusions on subfamilies or tribes based only on the results presented here. However, some of the relationships between the tribes, namely the separation between the Parablenniini and the Blenniini, should be reanalysed because the relative positions of *A. sphynx* and *B. ocellaris* cast some doubts on the phylogenetic relationships traditionally accepted. The value of the characters traditionally used to separate the two tribes, such as the type of suture of the dentaries, may not be decisive. Additionally, the distances between *Blennius* and other taxa, traditionally included in the Parablenniini, are smaller than those among several Parablenniini. This suggests that a return to a tribe more similar to the Blenniini of Norman (1943), Springer (1968) and Bath (1977) with the appropriate corrections and adjustments may be preferable.

Williams (1990) and Stepien *et al*. (1997) already noted that the Salariini and the Parablenniini are sister groups. Stepien *et al*. (1997) argued that the Salariini are not a monophyletic group, owing to the deviant position of *Ophioblennius*. Recently, Bath (2001) showed that the characters used to divide the Salariini and the Parablenniini are invalid because intermediate stages were found in several genera. Our results on *O. atlanticus* support the conclusions of Stepien *et al*. (1997) and Bath (2001) concerning the strong affinity between *Ophioblennius* and the Blenniini/Parablenniini group.

It is possible that the fragments analysed in this preliminary study are not appropriate to recover some old phylogenetic signals. In addition to more DNA data, adequate coverage of the genera included in the family Blenniidae at a global scale is necessary to solve the pending issues on tribal or subfamilial groupings.

Genera

The results presented in this study indicate that *Lipophrys* (*sensu* Bath, 1977) is a paraphyletic genus. *L. pholis* appears closer to *L.* (= *Paralipophrys*) *trigloides* (as already suggested by Bock & Zander, 1986) and to *Coryphoblennius galerita* than to the other small *Lipophrys* species, which form their own independent monophyletic group (see Fig. 1). A close relationship between *L. pholis*, *L. trigloides* and *C. galerita* had already been proposed by other authors based on osteological and karyological data (Papaconstantinou, 1977; Bock & Zander, 1986; Garcia *et al*., 1987). The three species also share the ecological specialization of living in a rocky intertidal zone and are among the blenniids more tolerant to cold waters reaching the west coast of France (*L. trigloides*), the British Isles (*C. galerita*) and the Norwegian coast (*L. pholis*) (Zander, 1986; Bath, 1990a). The evidence presented above, together with the eco-ethological specializations of the small *Lipophrys* species, in our view justifies their placement in a separate genus. The males of these species use holes that tightly fit the body as nests, and present conspicuous black and yellow or black and red facial masks, which are important signals in courtship and territorial displays during the breeding season (Zander, 1975; Wirtz & Bath, 1989). These courtship and agonistic displays are markedly different from those performed by other blennioid species, including *L. polis* and *L. trigloides* (see Abel, 1964, 1980, 1993; Gibson, 1968; Wirtz, 1978, 1980; Almada *et al*., 1983, 1990; Heymer, 1987). Recently, Raventós & Macpherson (2001) provided further evidence for the distinctiveness of these two monophyletic groups. They studied the duration of the planktonic larval stage in a number of littoral fishes and concluded that they tend to be similar within the same genus. The one exception to this trend was the genus *Lipophrys* with *L. trigloides* presenting a planktonic larval duration that is twice as long as found for *L. adriaticus* and *L. canevai*.

A closer look at the morphology of these species also shows that they fall into not only genetically but also morphologically distinct groups. We therefore suggest the following two new generic definitions.

1. In the absence of an available valid name we propose the name *Microlipophrys* to designate the new genus. *Microlipophrys* is here defined by a combination of the following characters: species of the former group Parablenniini (*sensu* Bock & Zander, 1986) that lack supraorbital tentacles and have 12 pectoral rays. Additional but not exclusive characteristics are the presence of glands on the tip of the second dorsal fin rays and the absence of glands on the tip of the anal fin spines in breeding males, and a comparatively small body size (from 4 cm in *M. dalmatinus* to 7 cm in *M. canevai*). The newly defined genus *Microlipophrys* encompasses the species *adriaticus* (Steindachner & Kolombatovic, 1883), *bauchotae* Wirtz & Bath, 1982, *caboverdensis* (Wirtz & Bath, 1989), *canevai* (Vinciguerra,

1880), *dalmatinus* (Steindachner & Kolombatovic, 1883), *nigriceps* (Vinciguerra, 1883) and *velifer* (Norman, 1935). As type species of the new genus we designate *Microlipophrys canevai* (Vinciguerra, 1880), the first described species of the genus. We could not analyse *M. velifer* (Norman, 1935) and *M. bauchotae* Wirtz & Bath, 1982, but we did analyse *M. caboverdensis*, which is very closely related to those two species (Wirtz & Bath, 1989). Thus, we think that our data are sufficiently complete to justify the argument for the monophyly of this group.

2. The genus *Lipophrys* Gill, 1896, which has precedence over *Paralipophrys* (Bath, 1977), is here redefined by a combination of the following characters: species of the former group Parablenniini (*sensu* Bock & Zander, 1986) that lack supraorbital tentacles and have 13 pectoral rays. Additional but not exclusive characteristics are the presence of glands on the tip of the second dorsal fin rays and the absence of glands on the tip of the anal fin spines in breeding males, and a comparatively large body size (up to 14 cm in *L. trigloides* and up to 30 cm in *L. pholis*). The newly defined genus *Lipophrys* encompasses the species *pholis* (Linnaeus, 1758) and *trigloides* (Valenciennes, 1836). The type species of the genus is *Lipophrys pholis* (Linnaeus, 1758), as designated by Gill (1896). The difference in the lateral line system morphology of the two species, stressed by Bath (1977), seems to be an adaptation to the different habitats they colonized, as suggested by Zander (1978).

We are aware that changes in taxonomy should be avoided to preserve stability in biological classification unless there is strong evidence that the previous situation is inadequate. We believe, however, that the erection of *Microlipophrys* clarifies the taxonomy of these fish and helps to define groups that are monophyletic and well characterized both in morphology and in eco-ethology.

An alternative hypothesis to the erection of *Microlipophrys* would be to retain all the species traditionally ascribed to *Lipophrys* in that genus (including *L. trigloides*). Apart from lack of support in the results presented above this option would render the genus *Lipophrys* paraphyletic unless *C. galerita* was also included in this genus. However, the monotypic genus *Coryphoblennius* should not be extinguished by merging it with *Lipophrys* because *C. galerita* presents several important morphological peculiarities. *Coryphoblennius* is the only other genus in the former group Parablenniini (*sensu* Bock & Zander, 1986) lacking supraorbital tentacles and presenting glands on the tip of the soft dorsal fin rays but not on the anal fin spines in breeding males. It is characterized by a large number of autapomorphies such as a fleshy, triangular appendage on the nape, which were described in detail by Bath (1977). In order to retain *Coryphoblennius* as a distinct genus avoiding at the same time the paraphyly of *Lipophrys* it seems preferable to define *Lipophrys* more narrowly and to recognize the distinctiveness of *Microlipophrys* as proposed above.

We found a genetic divergence between samples of *C. galerita* captured in different locations that is similar to the genetic distances found between closely related species. Interestingly, three distinct haplotypes were identified: one in the Azores; one in Madeira, mainland Portugal and Great Britain; and another in Italy, Croatia and Lebanon. These findings seem to be congruent with the geographical variations in body coloration patterns described by Bath (1978). However, a population genetics study with large sample sizes is needed to resolve this issue.

For the time being the genus *Salaria* should be retained although the moderate level of bootstrap support warrants further investigation of this genus. The insertions in the 12S and 16S rDNA sequences of *S. pavo* mentioned in the Methods could be related with the low bootstrap values obtained for this genus. A comparison of our own sequences with the secondary structure models presented by Ortí *et al*. (1996) for similar fragments in piranhas showed that the 11-bp-long insertion found in the 16S fragment of *S. pavo* occurred in a loop region (loop L of the 16S rDNA model presented by these authors). Contrary to Zander (1978, 1980, 1986), these fish are not related at all to the redefined *Lipophrys* or *Microlipophrys* but rather are more closely related to *Scartella* and *Parablennius*. A relationship between *Salaria* and some *Parablennius* (e.g. *P. sanguinolentus*) had already been suggested by other authors, namely Norman (1943), Bath (1977) and Garcia *et al*. (1987).

As already noted by Bath (1996), the genus *Parablennius* proved to be a heterogeneous group in which many fishes that could not be included in other groups were placed. It is a collection of old lineages widely spread in the Indo-Pacific and both sides of the Atlantic, so our study is insufficient to allow a proper resolution of the relationships of this putative genus. Bath (1996) identified several *Parablennius* subgroups based on morphological data. It is interesting to note that all Atlanto-Mediterranean subgroups were also recovered by our analysis with a single modification: group 4 of Bath (*P. pilicornis* and *P. salensis*), although forming a very well-supported monophyletic clade, was included in our analysis in a larger clade that also includes the species of Bath's group 3 (*P. incognitus*, *P. zvonimiri*, *P. rouxi* and *P. tentacularis*).

In our study, the divergence between the clade *P. parvicornis*/*P. sanguinolentus* and the other *Parablennius* is considerable. They are morphologically and

ecologically very specialized, being typically herbivorous fish of very shallow waters, a rare condition in the eastern Atlantic blennies, exploring boulder habitats and even intertidal pools (Gibson, 1968; Goldschmid *et al*., 1980; Santos & Almada, 1988). *P. sanguinolentus* and *P. parvicornis* are morphologically very similar and present one synapomorphy with 13 rays in the pectoral fins, whereas all other *Parablennius* have 14 rays. Although Bath (1977) has proposed placing these two species into a new genus (*Pictiblennius*), he later returned them to the genus *Parablennius* stressing that they form a distinct group within the genus (Bath, 1996). We suggest that this group needs further investigation to clarify its taxonomy. Similar considerations are applicable to the pair *P. ruber*/*P. gattorugine*.

PHYLOGEOGRAPHICAL CONSIDERATIONS

Considering the distribution patterns of the lineages identified in this study the following phylogeographical considerations are worth mentioning.

1. *Scartella* and some lineages of the group that is currently named as *Parablennius* are widely distributed in the Indo-Pacific and the Atlantic. They either evolved prior to the closure of the Tethys sea in the east caused by the contact between the African plate and Arabia about 23 million years ago (Briggs, 1995), and/or moved around the Cape of Good Hope in South Africa when water temperatures were higher. However, if such a route was operative it must have played a minor role in the history of this group. Indeed, the blennioid fauna of South Africa has a rich endemic component, suggesting that interchanges of fish with adjacent areas had substantial limitations for a considerable span of time.

   These two hypotheses will only be adequately tested when a reliable molecular clock is available. The remaining lineages, namely *Aidablennius*, *Blennius*, *Salaria*, *Lipophrys*, *Coryphoblennius* and *Microlipophrys*, are endemic to the eastern Atlantic and the Mediterranean.
2. Some lineages include pairs of species in which one member occurs in the mainland and the other in one or more groups of Atlantic islands. Examples include: (i) *M. caboverdensis* closely related to the West African *M. bauchotae* and *M. velifer* (Wirtz & Bath, 1989); (ii) *S. caboverdiana* closely related to the West African *S. cristata*; (iii) *P. salensis* and the western and eastern Atlantic *P. pilicornis*; (iv) although sometimes captured in the western European shore *P. ruber* is specially common in some north-eastern Atlantic islands, particularly at the Azores (Almeida, 1982; Almeida & Harmelin-Vivien, 1983) where it may have originated according to the model of speciation proposed by Zander (1980) (see also Bath, 1982). Its sister species *P. gattorugine* is widely distributed in the Mediterranean and the north-eastern Atlantic.

   We propose that all these examples may reflect dispersal events by which fishes of mainland origin colonized the Atlantic islands at different times.
3. Finally, another interesting pattern contrasts fishes with distributions centred in the Mediterranean and others with distributions centred in the tropical and subtropical eastern Atlantic. Examples include the *Microlipophrys* from the Mediterranean and adjacent Atlantic waters (*M. dalmatinus*, *M. adriaticus*, *M. canevai* and *M. nigriceps*) and the African *M. caboverdensis*. Another example includes the Mediterranean centred *P. sanguinolentus*, which can be presently found in the Atlantic between Morocco and the Bay of Biscay, and the west African *P. parvicornis* that is also found in Cape Verde, the Canaries, Madeira and the Azores. The distribution of *P. rouxi* and *P. tentacularis* is centred in the Mediterranean and adjacent Atlantic waters. This clade is sister to the clade *P. pilicornis*/*P. salensis*, which are Atlantic warm-water species.

During the Pliocene and Pleistocene many decreases in sea surface temperatures are known to have occurred in the eastern Atlantic (Briggs, 1995). For instance at the last glacial maximum the polar front was located along the Iberian Peninsula (Dias, Rodrigues & Magalhãs, 1997). Even the Cape Verde islands suffered a high sea temperature drop, excluding them from the much reduced tropical Atlantic during this period (CLIMAP Project Members, 1981; Briggs, 1995).

The impact of these decreases in sea temperature on the Mediterranean seems to have been attenuated, especially in the south-west and south-east (Thiede, 1978). Almada *et al*. (2001) proposed that, during cold periods, both the Mediterranean and the west coast of Africa must have served as refugia for warm-water species. At the same time, these two areas would probably have remained separated even during interglacial periods, owing to the intense upwelling occurring along the Mauritanian shore (see Marañón *et al*., 2001). This upwelling could favour speciation of endemic forms due to the cold water barrier separating the west African shore and the Mediterranean together with the north-eastern Atlantic adjacent coast northwards from Morocco. This means that the low number of endemic species now found in the Mediterranean may be misleading. In interglacial periods, such as the current one, several species dispersed out of the Mediterranean, thus becoming non-endemic. A similar pattern of expansion is also expected to occur among tropical species. In the future with a reliable

molecular clock we hope that this hypothesis may be tested rigorously.

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