

***Gaidropsarus* (Gadidae, Teleostei) of the North Atlantic Ocean: a brief phylogenetic review**

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The phylogenetic relationships among the North Atlantic *Gaidropsarus* and between the three Gaidropsarinae genera *Gaidropsarus*, *Ciliata* and *Enchelyopus* are reviewed with the hitherto most comprehensive taxonomic sampling of this group. Phylogenetic results (maximum parsimony, maximum likelihood and Bayesian inference) based on nuclear (*rhodopsin*) and concatenated mitochondrial (12S, 16S and *cytb*) markers clearly support this subfamily. For the north-eastern Atlantic species of *Gaidropsarus*, two previously unreported clades were strongly supported, clarifying the relationships within the genus, and revealing fewer distinct taxa in the north Atlantic *Gaidropsarus* than previously stipulated. The data challenge the specific status of *Gaidropsarus mediterraneus* and *Gaidropsarus guttatus* and raise doubts concerning the distinctiveness of other species. A taxonomic revision of the genus is suggested.

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Key words: *Gaidropsarinae*; mitochondrial and nuclear markers; multilocus inference.

INTRODUCTION

The gadoids (cod, hake and allies) include some of the most important commercial fish species inhabiting mostly cool oceanic waters around the world and occupying habitats from the deep-sea to coastal waters (Nelson, 2006). Despite many taxonomic efforts, the phylogeny of these fishes has been subjected to many recent changes and proposals, especially at the family and subfamily levels (Svetovidov, 1948; Cohen, 1984; Teletchea *et al.*, 2006; Roa-Varón & Ortí, 2009). For example, the genera *Gaidropsarus* Rafinesque 1810, *Ciliata* Couch 1832 and *Enchelyopus* Bloch & Schneider 1801, although viewed as forming a well-defined clade, have been alternatively placed in the families Phycidae (Whitehead *et al.*, 1986), Lotidae (Froese & Pauly, 2013) and Gadidae (Teletchea *et al.*, 2006; Roa-Varón & Ortí, 2009). More recently, authors resurrected the subfamily Gaidropsarinae (Gadidae) (Howes, 1991) to accommodate these three genera (Teletchea *et al.*, 2006; Roa-Varón & Ortí, 2009).

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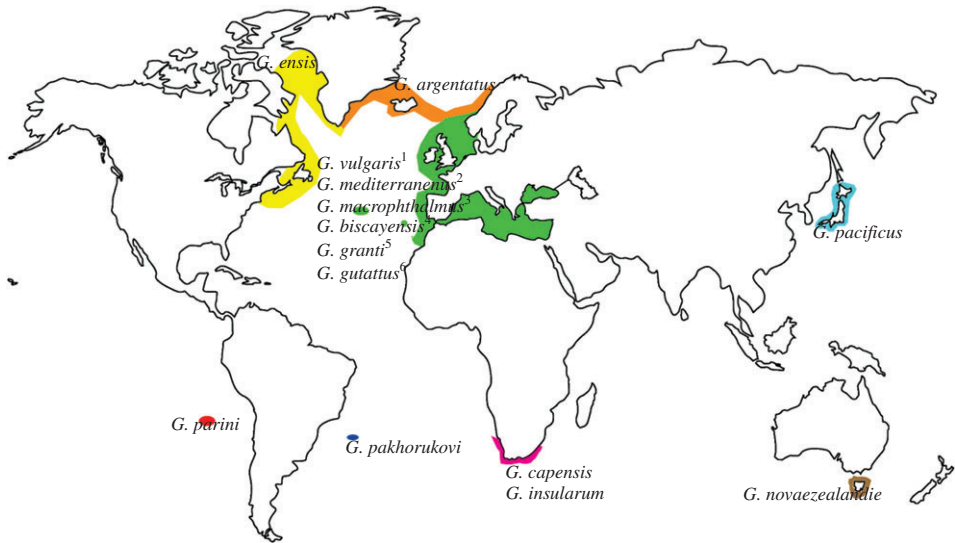


FIG. 1. Distributions of species of *Gaidropsarus*. ¹The central Norwegian coast and the Faroe Islands southwards through the North Sea and around the British Isles to the region around the Strait of Gibraltar; also reported from Iceland and the western and northern coasts of the Mediterranean Sea. ²Southern Norway, around the western shores of the British Isles to the south-west and south coasts of Europe, into the Black Sea, and on the northwest African coast. ³Bay of Biscay and northwards along the west coast of the British Isles to the Faroe Islands. ⁴Western Mediterranean Sea and northern part of the central Mediterranean; also from Spain and Portugal to Morocco and Madeira. ⁵Canary Islands and the Azores; also from the central and eastern Mediterranean Sea, and Galicia, Spain. ⁶Canary Islands, Madeira and the Azores; also from Galicia, Spain.

This study focuses on the phylogenetic relationships between these three *Gaidropsarus* genera (*Gaidropsarus*, *Ciliata* and *Enchelyopus*) and the phylogenetic relationships between the North Atlantic *Gaidropsarus*. This genus includes 14 recognized species: seven in the north-eastern Atlantic Ocean, one in the north-western Atlantic Ocean [*Gaidropsarus ensis* (Reinhardt 1837)], one in the south-western Atlantic Ocean, two in the south-eastern Atlantic and south-western Indian Oceans, one in the north-western Pacific Ocean, one in the south-western Pacific Ocean and one in the south-eastern Pacific Ocean (Fig. 1 and Table I). Most of these species occur in the Atlantic Ocean (11 of 14) with the peak of diversity in its north-eastern margin (seven of 14). The genus expresses a remarkable ecological diversity, from shallow waters to considerable depths and from warm-temperate to Arctic waters (Froese & Pauly, 2013).

Hitherto only five species of *Gaidropsarus* were genetically characterized: *col* and *cytb* were amplified for *G. ensis* and *Gaidropsarus vulgaris* (Cloquet 1824) (Teletchea *et al.*, 2006); *12s*, *16s* and *rag1* for *G. ensis* and *Gaidropsarus argentatus* (Reinhardt 1837) (Roa-Varón & Ortí, 2009); *cytb* for *G. argentatus* (Bakke & Johansen, 2005); *col* for *Gaidropsarus biscayensis* (Collett 1890) (Costa *et al.*, 2012) and *cytb* and *rhodopsin* for *Gaidropsarus mediterraneus* (L. 1758) and *G. biscayensis* (FishTrace, 2013).

This study aimed to infer the phylogenetic relationships among all the species of *Gaidropsarus* inhabiting the North Atlantic Ocean, complementing previous sequences

TABLE I. Species of *Gaidropsarus* and their distribution

Species	Distribution
<i>Gaidropsarus ensis</i>	North-western Atlantic Ocean
<i>Gaidropsarus argentatus</i>	North-eastern Atlantic Ocean
<i>Gaidropsarus macrophthalmus</i>	
<i>Gaidropsarus biscayensis</i>	
<i>Gaidropsarus vulgaris</i>	
<i>Gaidropsarus granti</i>	
<i>Gaidropsarus mediterraneus</i>	
<i>Gaidropsarus guttatus</i>	
<i>Gaidropsarus pakhorukovi</i>	South-western Atlantic Ocean
<i>Gaidropsarus capensis</i>	South-eastern Atlantic and South-western Indian Oceans
<i>Gaidropsarus insularum</i>	
<i>Gaidropsarus pacificus</i>	North-western Pacific Ocean
<i>Gaidropsarus novaezealandiae</i>	South-western Pacific Ocean
<i>Gaidropsarus parini</i>	South-eastern Pacific Ocean

Baffin Bay (Greenland) extending from the east coast of North America (Canada to off Cape Hatteras, U.S.A.) (bathydemersal)
 Off the south-eastern coast of Greenland to the Norwegian coasts north from Bergen (bathydemersal)
 Faroe-Shetland Channel south to the Bay of Biscay along the lower continental shelf
 Western Mediterranean, also in the adjacent Atlantic from Spain and Portugal to Morocco and Madeira
 Central Norwegian coast and the Faroe Islands southwards through the North Sea and British Isles to the Strait of Gibraltar; also into the Mediterranean Sea
 Canary Islands and the Azores; it is also known from Galicia (Spain) and the Mediterranean Sea
 Southern Norway to the north-west African coast, Mediterranean and Black Sea
 Canary Islands, Madeira and the Azores, and also found in Galicia (Spain)
 Rio Grande Rise
 From Cape Town to East London in South Africa
 South Africa, Tristan da Cunha and Gough Island (Atlantic Ocean); St Paul and Amsterdam Islands (Indian Ocean)
 Japan
 Tasmania and New Zealand
 South-eastern Pacific Ocean. Possibly, also in South Atlantic Ocean

with mitochondrial sequences of additional species and analysis of a nuclear marker. This comprehensive study of the North Atlantic members of this relatively unknown genus contributes to the understanding of the Gadidae, a family of high economic importance.

MATERIALS AND METHODS

SAMPLING

Specimens were sampled from members of the Gadidae, including species belonging to the subfamilies Gadinae, Gaidropsarinae, Lotinae and Phycinae. Fin or muscle tissue was excised and preserved in 96% ethanol. Tissue vouchers (or entire specimens when available) were deposited at ISPA Instituto Universitário das Ciências Psicológicas, Sociais e da Vida (Portugal). The dataset was completed with sequences retrieved from GenBank (Table II). Eight species of *Gaidropsarus*, all from the North Atlantic Ocean, were sampled: *G. vulgaris*, *G. mediterraneus*, *G. biscayensis*, *Gaidropsarus guttatus* (Collett 1890), *Gaidropsarus macrophthalmus* (Günther 1867), *Gaidropsarus granti* (Regan 1903), *G. ensis* and *G. argentatus*. Some closely related taxa were also sampled in order to provide taxonomic context: subfamily Gaidropsarinae [*Ciliata mustela* (L. 1758) and *Enchelyopus cimbrius* (L. 1766)], subfamily Gadinae [*Trisopterus minutus* (L. 1758), *Trisopterus luscus* (L. 1758), *Gadiculus argenteus* Guichenot 1850, *Gadus morhua* L. 1758 and *Gadus ogac* Richardson 1836], subfamily Phycinae [*Phycis phycis* (L. 1766) and *Phycis blennoides* (Brünnich 1768)], subfamily Lotinae [*Lota lota* (L. 1758), *Molva macrophthalma* (Rafinesque 1810), *Molva molva* (L. 1758), *Molva dipterygia* (Pennant 1784), *Molva elongata* (Otto 1821) and *Brosme brosme* (Ascanius 1772)] and family Merlucciidae [*Merluccius merluccius* (L. 1758)] (Table II).

DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

Total genomic DNA was isolated from tissue samples using REDExtract-N-Amp Kit (Sigma Aldrich; www.sigmaaldrich.com) following the manufacturer's instructions. Fragments of the mitochondrial *12s* rRNA, *16s* rRNA, cytochrome *b* gene (*cytb*) and the *rhodopsin* nuclear gene were amplified with the following pairs of primers: *12s*: *12sfor* and *12srev* (Almada *et al.*, 2005); *16s*: *16sfor* and *16srev* (Almada *et al.*, 2005); *cytb*: *fish-cytb-f* and *truc-cytb-r* (Sevilla *et al.*, 2007) and *rhodopsin* gene: *rod-f2b* and *rod-r4n* (Sevilla *et al.*, 2007). Polymerase chain reaction (PCR) amplifications were performed in a 20 µl total-reaction volume with 10 µl of REDExtract-N-ampl PCR mix (Sigma Aldrich), 0.8 µl of each primer (10 µM), 4.4 µl of Sigma-water and 4 µl of template DNA. Details on the primers and amplification procedures are in Appendix I. Purification and sequencing were performed by Stabvida (Oeiras; www.stabvida.net) using the same primers. Obtained sequences, their geographical location and voucher label were deposited in GenBank (accession numbers KC980927–KC981068) (Table II).

PHYLOGENETIC ANALYSES

Sequences were edited with CodonCode Aligner 4.0.1. (CodonCode Corporation; www.codoncode.com) and aligned using Clustal X2 (Larkin *et al.*, 2007). MEGA 5.1 (Tamura *et al.*, 2011) was used to compute estimates of net evolutionary divergence between species, applying a likelihood distance and using parameters selected under the Akaike information criterion (AIC) (Nei & Kumar, 2000).

The appropriate model of sequence evolution for *rhodopsin* and concatenated mitochondrial datasets was determined using jModeltest (Guindon & Gascuel, 2003; Posada, 2008), under the AIC. Phylogenetic relationships were inferred with three methods using *M. merluccius* as an outgroup. Maximum parsimony (MP) trees were estimated using PAUP* (Swofford, 2003) and robustness was tested with 1000 bootstrap replicates (Felsenstein, 1985). Maximum likelihood

TABLE II. Species analysed, labels and GenBank accession numbers

Family	Subfamily	Species	Label	GenBank accession numbers				
				<i>12s</i>	<i>16s</i>	<i>cytb</i>		
Gadidae	Gaidropsarinae	<i>Gaidropsarus mediterraneus</i>	GAME	KC980968-74	KC980950-54	KC981020-24; EF427563-64*	KC981032-37; EF439116-17*	
		<i>Gaidropsarus guttatus</i>	GAGU	KC980963-67	KC980947-49	KC981016-19	KC981038-42	
		<i>Gaidropsarus grantii</i>	GAGR	KC980975	KC980946	KC981025	KC981050	
		<i>Gaidropsarus vulgaris</i>	GAVU	KC980976-77	KC980944-45	KC981026-27; DQ174050-51*	KC981048-49	
		<i>Gaidropsarus macrophthalmus</i>	GAMA	KC980978-79	KC980942-43	KC981028-29	KC981045-47	
		<i>Gaidropsarus biscayensis</i>	GABI	KC980980	KC980940-41	EF427562*	KC981043-44; EF439115*	
		<i>Gaidropsarus ensis</i>	GAEN	FJ215034*	FJ215143*	FJ215142*	DQ174048-49*	
		<i>Gaidropsarus argentatus</i>	GAAR	FJ215033*	FJ215142*	KC980955-56;	AJ517500*	
		<i>Ciliata mustela</i>	CIMU	KC980981-82; FJ215012*	KC980981-82; FJ215121*	FJ215121*	KC981015; DQ174039*	
		<i>Enchelyopus cimbrius</i>	ENCI	KC980983-84; FJ215025*	KC980957-59; FJ215134*	KC980957-59; FJ215134*	EU492211-12*; EU224005-06*; EU469624*; AJ517498*	
		Gadinae	<i>Gadiculus argenteus</i>	GADAR	KC980985-86	KC980933-36	KC981007-09; DQ174043*; EU224053*; AJ517492*	KC981056-59; EU224200-01*
				GAMO	KC980992; NC002081*	NC002081*; AM489716*	DQ174045-46*; DQ356941*; AF081684*	EF439113-14*
				GAOC	DQ356941*	DQ356941*; FJ215140*		-

TABLE II. Continued

Family	Subfamily	Species	Label	I2s	GenBank accession numbers		
					I6s	cytb	rhodopsin
Merlucciidae	Merlucciinae	<i>Trisopterus minutus</i>	TRMI	KC980990-91	KC980930-32; FJ215196*	KC981001-03; EU492138-39*; EU224043-44 KC981004-06; JF309486; JF309484; EU224041; EU492067	KC981063-65
		<i>Trisopterus luscus</i>	TRLU	KC980987-89	KC980927-29	KC981060-62	
Phycinae	Phycinae	<i>Phycis blennoides</i>	PHBL	KC980996-97; AY368283; AY845393	KC980960	KC981054-55; EU036474-75; EU224072-73; EF439218	KC981066-68
			PHPH LOLO	- NC004379; AJ002633; AP004412	- NC004379; AP004412	EU036476-77 EU036576-77 EU036578-79 -	
		<i>Phycis phycis</i> <i>Lota lota</i>	PHPH LOLO	- NC004379; AJ002633; AP004412	- NC004379; AP004412	EU036476-77 EU036576-77 EU036578-79 -	
			MOMO	-	FJ215173	EU224021-22 KC981010-12; AY960858; DQ174070	
Merlucciidae	Merlucciinae	<i>Molva molva</i>	MOMA	KC980998-1000; AY845396	KC980937-39	KC981066-68	
			MOMA	-	-	EF439141-42	
		<i>Molva macrophthalma</i>	MOMA	-	-	EF439141-42	
		<i>Molva dipterygia</i> <i>Molva elongata</i> <i>Brosme brosme</i> <i>Merluccius merluccius</i>	MODI MOEL BRBR MEME	- - EU048342 KC980993-95; FR751402	- AY850364 - KC980961-62; FR751402	EF439139-40 EF439139-40 EU492245-46 KC981051-53; EF439400-01	

*Sequences drawn from GenBank.

(ML) phylogenetic analyses were employed using RAxML 7.4.2 (Stamatakis *et al.*, 2012) on Cipres (Miller *et al.*, 2010), with 100 rapid bootstrap inferences, using the GTRGAMMA model. Bayesian inference (BI), as implemented in MrBayes 3.2.1 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003), was computed for the *rhodopsin* and for the concatenated mitochondrial dataset, using the corresponding substitution models selected by jModeltest and with the *cytb* and *rhodopsin* fragments partitioned by codon position. Four independent runs of five million generations of Markov chain Monte-Carlo (MCMC) simulations were used to determine posterior probabilities. Topologies were sampled every 1000 generations, and a majority rule consensus tree was estimated after discarding the first 5000 sampled generations.

The Bayesian MCMC method implemented in *BEAST 1.7.2. (Heled & Drummond, 2010; Drummond *et al.*, 2012) was also used to infer the genealogical relationships between the species of Gaidropsarinae. The analysis was performed on a dataset combining the mitochondrial concatenation and the *rhodopsin* fragment, implementing independent gene-specific substitution models, and assuming a Yule speciation process tree prior. Three independent tree searches of 50×10^6 generations each were run, retaining one in every 1000 samples from the posterior distribution of the model parameter log files and tree files. Convergence of the runs was confirmed, using Tracer 1.5 (Rambaut *et al.*, 2013), by effective sample sizes (ESS) of parameter estimates over 200.

RESULTS

MOLECULAR DATA

The *12s* dataset comprised 370 bp (96 parsimony informative sites), the *16s* fragment yielded a dataset of 564 bp (98 parsimony informative sites) and the *cytb* dataset comprised 1060 bp (461 parsimony informative sites). The concatenated dataset for the three mitochondrial fragments comprised a total of 1994 bp (655 parsimony informative sites), with the best fit evolutionary model being the generalized time-reversible (GTR) + gamma distribution (Tavaré, 1986). For the *rhodopsin* dataset, the sequenced fragment was 760 bp long (no heterozygous positions were found), comprising 232 parsimony informative characteristics and an optimal molecular evolutionary model of Tamura 3-parameter + invariable sites + gamma distribution (Tamura, 1992).

PHYLOGENETIC RELATIONSHIPS

The estimated genetic distances revealed lack of evolutionary divergence between *G. mediterraneus* and *G. guttatus* for both mitochondrial and nuclear markers (Appendix II). The divergence between *G. macrophthalmus* and *G. biscayensis* was also zero for *rhodopsin*.

Phylogenetic relationships were congruent among the different methods and fragments analysed (Figs 2 and 3), with the *rhodopsin* being least informative. The species tree topology obtained with the *BEAST software reached similar results (Fig. 4). Two strongly supported clades were found within *Gaidropsarus* (clade C; Figs 2 and 3): a first group comprising *G. guttatus* and *G. mediterraneus* (clade F), and a second one with the remaining species studied (clade E; Fig. 2). Within the first clade, monophyly of neither *G. guttatus* nor *G. mediterraneus* was recovered, but together they formed a clade. This relationship was recovered for every method and fragment analysed and is in accordance with the evolutionary divergence results (Appendix II). Within the second clade (E), a group with *G. ensis* and *G. argentatus* (clade H; Fig. 2) appeared as sister to the remaining four species (*G. vulgaris*, *G. biscayensis*, *G. macrophthalmus*

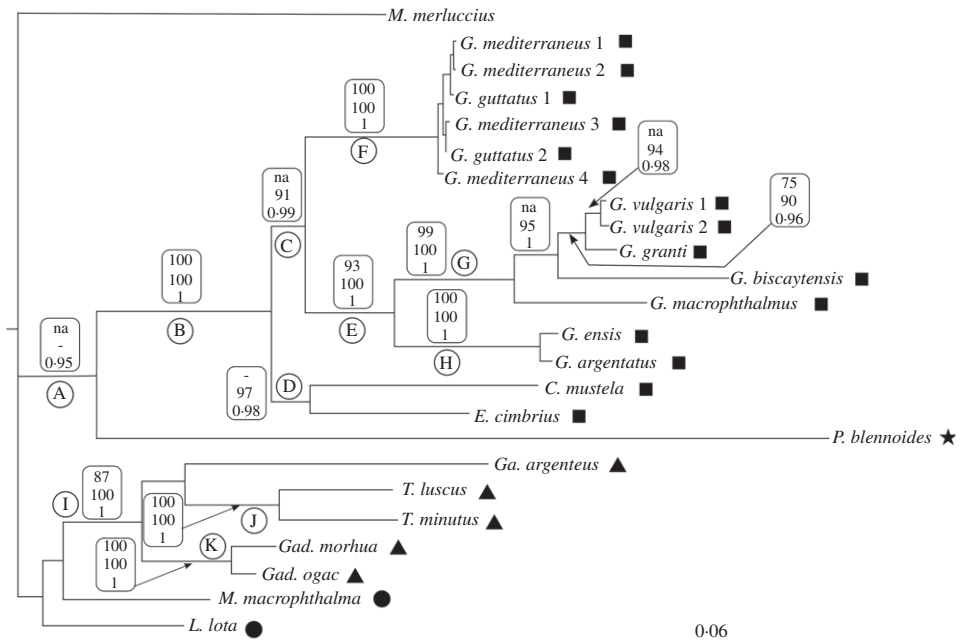


FIG. 2. Phylogenetic relationships of *Gaidropsarus* based on Bayesian analyses of the concatenated mitochondrial dataset (*12s*, *16s* and *cytb*). Support values for each node correspond to bootstrap values [maximum parsimony (MP) and maximum likelihood (ML)] and posterior probabilities [Bayesian inference (BI)]. Circled capital letters correspond to main nodes. Subfamilies of the Gadidae: Gadinae (▲), Gaidropsarinae (■), Lotinae (●) and Phycinae (★). *M.*, *Merluccius*; *G.*, *Gaidropsarus*; *C.*, *Ciliata*; *E.*, *Enchelyopus*; *T.*, *Trisopterus*; *Ga.*, *Gadiculus*; *Gad.*, *Gadus*; *P.*, *Phycis*; *L.*, *Lota*; *M.*, *Molva*.

and *G. granti*) (clade G; Figs 2 and 3). The Gaidropsarinae subfamily (clade B) was recovered, including *Gaidropsarus* and a clade comprising *C. mustela* and *E. cimbrius* (clade D; Fig. 2). Clade I comprises the species of the subfamily Gadinae considered in this study, including a *Gadus* clade (clade K; Fig. 2), a *Trisopterus* clade (clade J) and *G. argenteus*. The phylogenetic relationships between this subfamily, Lotinae and Phycinae remain unclear. Although clade A (Gaidropsarinae + Phycinae) has high mitochondrial support (Fig. 2), the same does not hold for *rhodopsin* (Fig. 3). This marker indicated monophyly of the family Gadidae, comprising subfamilies Gaidropsarinae, Gadinae, Phycinae and Lotinae, with high supporting values, but was unable to resolve relationships among these subfamilies (Fig. 3).

DISCUSSION

This work was based on a mitochondrial concatenated dataset and a nuclear marker, *rhodopsin*. The North Atlantic *Gaidropsarus* always emerges as monophyletic and, in the majority of the analysis, as sister to a clade that includes *Ciliata* and *Enchelyopus*. These results are in accordance with the findings of Roa-Varón & Ortí (2009).

Concerning *Gaidropsarus*, two strongly supported clades were found: a first group comprising *G. guttatus* and *G. mediterraneus*, and a second one with the remaining

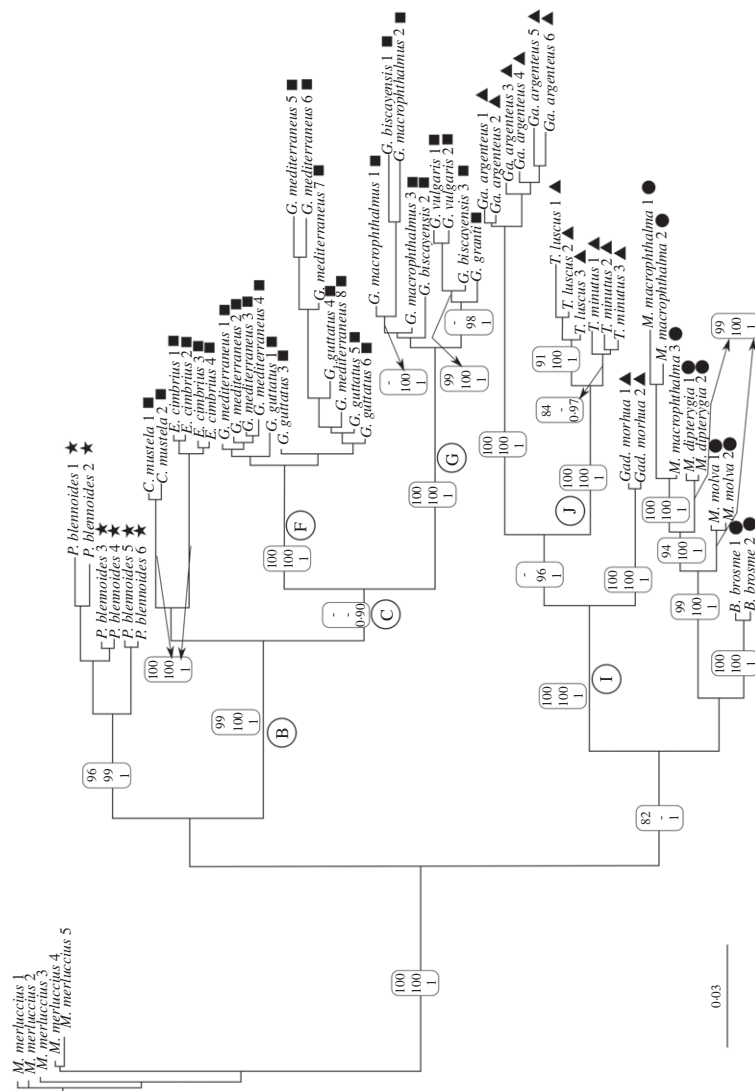


FIG. 3. Phylogenetic relationships of *Gaidropsarus* based on Bayesian analyses of the *rhodopsin* dataset. Support values for each node correspond to bootstrap values [maximum parsimony (MP) and maximum likelihood (ML)] and posterior probabilities [Bayesian inference (BI)]. Circled capital letters correspond to main nodes. Subfamilies of the Gadidae: (▲), Gaidropsarinae (■), Lotinae (●) and Phycinae (★). *M.*, *Merluccius*; *G.*, *Gaidropsarus*; *C.*, *Ciliata*; *E.*, *Enchelyopus*; *T.*, *Trisopterus*; *Ga.*, *Gadiculus*; *Gad.*, *Gadus*; *P.*, *Phycis*; *L.*, *Lota*; *M.*, *Molva*; *B.*, *Brosmie*.

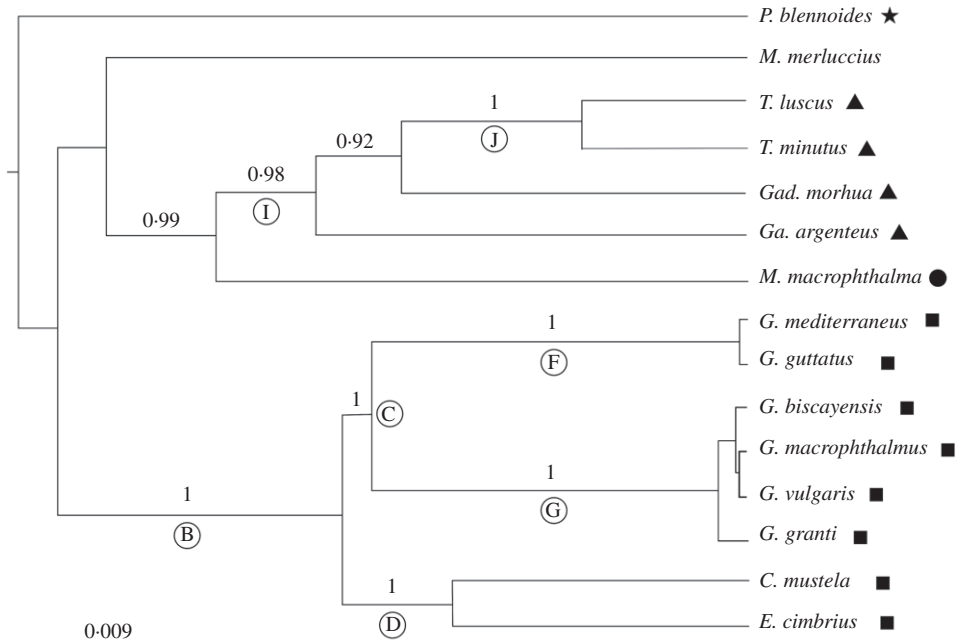


FIG. 4. Phylogenetic relationships of *Gaidropsarus* based on the species tree obtained with the *BEAST for the mitochondrial concatenation and *rhodopsin* datasets. Support values for each node correspond to posterior probabilities. Subfamilies of the Gadidae: Gadinae (▲), Gaidropsarinae (■), Lotinae (●) and Phycinae (★). *M.*, *Merluccius*; *G.*, *Gaidropsarus*; *C.*, *Ciliata*; *E.*, *Enchelyopus*; *T.*, *Trisopterus*; *Ga.*, *Gadiculus*; *Gad.*, *Gadus*; *P.*, *Phycis*; *M.*, *Molva*.

species studied (*G. ensis*, *G. argentatus*, *G. vulgaris*, *G. biscayensis*, *G. macrophthalmus* and *G. granti*). This subdivision was not reported in previous analyses, most likely as a consequence of poor taxonomic sampling. Previously, only *G. ensis*, *G. argentatus*, *G. vulgaris*, *G. mediterraneus* and *G. biscayensis* were genetically analysed, but never in the same study. *Gaidropsarus guttatus* was never included in previous studies, which explains the absence of the two clades reported here.

This work found less species richness and a lower number of distinct taxa among *Gaidropsarus* from the North Atlantic Ocean. The molecular data do not validate the species status of *G. mediterraneus* or *G. guttatus* (Appendix II and Figs 2 and 3). In fact, the diagnostic characteristics (both morphological and meristic) between these two species mostly overlap, only their distribution ranges are distinct (Svetovidov, 1986). *Gaidropsarus guttatus* was, until recently, considered to be exclusively Macaronesian. Recently, it has also been described for the Atlantic coast of Spain (Galicia) (Bañon *et al.*, 2010) and, thus, its distribution overlaps with *G. mediterraneus*. Doubts are also raised concerning the species status of some of the taxa within the second clade recovered for this genus (*G. vulgaris*, *G. biscayensis*, *G. macrophthalmus* and *G. granti*) (Fig. 3). Therefore, the taxonomy of the genus should be revised.

Rhodopsin has been found to be under selection (Yokoyama & Takenaka, 2004; Ebert & Rose, 2009). For example, in Gobiidae, Larmuseau *et al.* (2010) found discordant patterns between the phylogeny of neutral genes (*12s* and *16s*) and *rhodopsin*,

recommending against the use of this marker for phylogeographic reconstruction of aquatic taxa. In the present work, however, results from MP and inferences based on neutral models of molecular evolution (ML and BI) were largely coherent, and similar to results from the mitochondrial concatenated dataset. In fact, as noticed by Roje (2014), comparisons of topology congruence of the trees inferred by the individual markers are good indicators of non-neutral *v.* neutral markers. The phylogenetic relationships among species were inferred from the genealogies of both genetic markers and by inferring the most probable species tree given both genealogies.

The genus *Gaidropsarus* includes several commercially important species of wide distribution. This study is the first phylogenetic approach of *Gaidropsarus* that comprises all the North Atlantic species, including species that were genetically sampled for the first time. Future works should include all the remaining species of this genus and, if possible, several specimens per species, ideally from different locations, across their distribution ranges.

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References

- Almada, F., Almada, V. C., Guillemaud, T. & Wirtz, P. (2005). Phylogenetic relationships of the north-eastern Atlantic and Mediterranean blenniids. *Biological Journal of the Linnean Society* **86**, 283–295.
- Bakke, I. & Johansen, S. D. (2005). Molecular phylogenetics of Gadidae and related Gadiiformes based on mitochondrial DNA sequences. *Marine Biotechnology* **7**, 61–69. doi: 10.1007/s10126-004-3131-0
- Bañon, R., Villegas-Ríos, D., Serrano, A., Mucientes, G. & Arronte, J. C. (2010). Marine fishes from Galicia (NW Spain): an updated checklist. *Zootaxa* **2667**, 1–27.
- Cohen, D. M. (1984). Gadiformes: overview. In *Ontogeny and Systematics of Fishes Based on an International Symposium Dedicated to the Memory of Elbert Halvor Ahlstrom* (Moser, H. G., Richards, W. J., Cohen, D. M., Fahay, M. P., Kendall, A. W. Jr. & Richardson, S. L., eds), pp. 259–265. Lawrence, KS: American Society of Ichthyologists and Herpetologists.
- Costa, F. O., Landi, M., Martins, R., Costa, M. H., Costa, M. E., Carneiro, M., Alves, M. J., Steinke, D. & Carvalho, G. R. (2012). A ranking system for reference libraries of DNA barcodes: application to marine fish species from Portugal. *PLoS One* **7**, e35858. doi: 10.1371/journal.pone.0035858
- Drummond, A. J., Suchard, M. A., Xie, D. & Rambaut, A. (2012). Bayesian Phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29**, 1969–1973. doi: 10.1093/molbev/mss075

- Ebert, D. & Rose, A. (2009). Rhodopsin population genetics and local adaptation: variable dim light vision in sand gobies is illuminated. *Molecular Ecology* **18**, 4140–4142.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Guindon, S. & Gascuel, O. (2003). A simple, fast and accurate method to estimate large phylogenies by maximum likelihood. *Systematic Biology* **52**, 696–704. doi: 10.1080/10635150390235520
- Heled, J. & Drummond, A. J. (2010). Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution* **27**, 570–580. doi: 10.1093/molbev/msp274
- Howes, G. J. (1991). Biogeography of gadoid fishes. *Journal of Biogeography* **18**, 595–622.
- Huelsenbeck, J. P. & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* **17**, 754–755. doi: 10.1093/bioinformatics/17.8.754
- Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J. & Higgins, D. G. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics* **23**, 2947–2948. doi: 10.1093/bioinformatics/btm404
- Larmuseau, M. H. D., Huysse, T., Vancampenhout, K., Van Houdt, J. K. J. & Volckaert, P. A. M. (2010). High molecular diversity in the rhodopsin gene in closely related goby fishes: a role for visual pigments in adaptive speciation? *Molecular Phylogenetics and Evolution* **55**, 689–698. doi: 10.1016/j.ympev.2009.10.007
- Nei, M. & Kumar, S. (2000). *Molecular Evolution and Phylogenetics*. New York, NY: Oxford University Press.
- Nelson, J. S. (2006). *Fishes of the World*, 4th edn. Hoboken, NJ: John Wiley & Sons.
- Posada, D. (2008). jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* **25**, 1253–1256. doi: 10.1093/molbev/msn083
- Roa-Varón, A. & Ortí, G. (2009). Phylogenetic relationships among families of Gadiformes (Teleostei, Paracanthopterygii) based on nuclear and mitochondrial data. *Molecular Phylogenetics and Evolution* **52**, 688–704. doi: 10.1016/j.ympev.2009.03.020
- Roje, D. M. (2014). Evaluating the effects of non-neutral molecular markers on phylogeny inference. *PLoS One* **9**, e87428. doi: 10.1371/journal.pone.0087428
- Ronquist, F. & Huelsenbeck, J. P. (2003). MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574. doi: 10.1093/bioinformatics/btg180
- Sevilla, R. G., Diez, A., Norén, M., Mouchel, O., Jérôme, M., Verrez-Bagnis, V., van Pelt, H., Favre-Krey, L., Krey, G., The Fish Trace Consortium & Bautista, J. M. (2007). Primers and polymerase chain reaction conditions for DNA barcoding teleost fish based on the mitochondrial cytochrome b and nuclear rhodopsin genes. *Molecular Ecology Notes* **7**, 730–734. doi: 10.1111/j.1471-8286.2007.01863.x
- Stamatakis, A., Aberer, A. J., Goll, C., Smith, S. A., Berger, S. A. & Izquierdo-Carrasco, F. (2012). RAXML-Light: a tool for computing TeraByte phylogenies. *Bioinformatics* **28**, 2064–2066. doi: 10.1093/bioinformatics/bts309
- Svetovidov, A. N. (1948). *Gadiformes. Fauna of USSR, Fishes* **9** (4). Zoological Institute of the Academy of Sciences of the USSR (in Russian). English translation (1962) by W. J. Walters and V. W. Walters. Jerusalem: Israel Program for Scientific Translations.
- Svetovidov, A. N. (1986). Gadidae. In *Fishes of the North-Eastern Atlantic and the Mediterranean*, Vol. 2 (Whitehead P. J. P., Bauchot M. L., Hureau J. C., Nilssen J. & Tortonese E., eds), pp. 521–1014. Paris: UNESCO.
- Swofford, D. L. (2003). *PAUP* Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4b.10*. Sunderland, MA: Sinauer Associates Inc.
- Tamura, K. (1992). Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G + C-content biases. *Molecular Biology and Evolution* **9**, 678–687.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using likelihood, distance, and parsimony methods. *Molecular Biology and Evolution* **28**, 2731–2739. doi: 10.1093/molbev/msr121
- Tavaré, S. (1986). Some probabilistic and statistical problems in the analysis of DNA sequences. *Lectures on Mathematics in the Life Sciences (American Mathematical Society)* **17**, 57–86.

- Teletchea, F., Laudet, V. & Hänni, C. (2006). Phylogeny of the Gadidae (sensu Svetovidov, 1948) based on their morphology and two mitochondrial genes. *Molecular Phylogenetics and Evolution* **38**, 189–199. doi: 10.1016/j.ympev.2005.09.001
- Whitehead, P. J. P., Bauchot, M. L., Hureau, J. C., Nielsen, J. & Tortonese, E. (Eds) (1986). *Fishes of the Northeastern Atlantic and Mediterranean*. Paris: UNESCO.
- Yokoyama, S. & Takenaka, N. (2004). The molecular basis of adaptive evolution of squirrelfish rhodopsins. *Molecular Biology and Evolution* **21**, 2071–2078.

Electronic References

- FishTrace (2013). *Genetic Catalogue, Biological Reference Collections and Online Database of European Marine Fishes*. Available at www.fishtrace.org (last accessed 6 July 2013).
- Froese, R., Pauly, D. (2013). *FishBase*. Available at www.fishbase.org (last accessed 10 November 2013).
- Miller, M. A., Pfeiffer, W., Schwartz, T. (2010). Creating the CIPRES Science Gateway for inference of large phylogenetic trees. *Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA*, pp. 1–8. Available at http://www.phylo.org/index.php/portal/cite_us/
- Rambaut, A., Suchard, M. A., Xie, D., Drummond, A. J. (2013). *Tracer v1.5*. Available at <http://beast.bio.ed.ac.uk/Tracer/>

Appendix I: Primers used and polymerase chain reaction (PCR) cycling conditions for amplification and sequencing

Primer Name	Sequence	Reference	PCR conditions
<i>12s</i>			
<i>12sfor</i>	AACTGGGATTAGATACCCAC	Almada <i>et al.</i> (2005)	4 min at 94° C, 30 cycles (1 min at 94° C, 1 min at 55° C, 1 min at 72° C), 10 min at 72° C
<i>12srev</i>	GGGAGAGTGACGGGGGTGTG		
<i>16s</i>			
<i>16sfor</i>	AAGCCTCGCCTGTTTACCAA	Almada <i>et al.</i> (2005)	4 min at 94° C, 30 cycles (1 min at 94° C, 1 min at 55° C, 1 min at 72° C), 10 min at 72° C
<i>16srev</i>	CTGAACTCAGATCACGTAGG		
<i>cytb</i>			
<i>fish-cytb-f</i>	ACCACCGTTGTTTATTCAACTACAAGAAC	Sevilla <i>et al.</i> (2007)	3 min at 94° C, 35 cycles (30 s at 94° C, 35 s at 55° C, 2 min at 72° C), 10 min at 72° C
<i>truc-cytb-r</i>	CCGACTTCCGGATTACAAGACCG		
<i>Rhodopsin</i>			
<i>rod-f2b</i>	GTCTGCAAGCCCCATCAGCAACTTCCG	Sevilla <i>et al.</i> (2007)	3 min at 94° C, 30 cycles (30 s at 94° C, 35 s at 55° C, 2 min at 72° C), 10 min at 72° C
<i>rod-r4n</i>	GGAACTGCTTGTTTCATGCAGATGTAGAT		

Appendix II: Estimates of net evolutionary divergences within Gaidropsarinae

Rhodopsin													
Mitochondrial			GAGU	GAVU	GABI	GAMA	GAGR	GAEN	GAAR	CIMU			
GAGU	-0.001												
GAVU	0.147	0.143											
GABI	0.148	0.144	0.078										
GAMA	0.140	0.137	0.086	0.108									
GAGR	0.109	0.105	0.014	0.025	0.060								
GAEN	0.147	0.138	0.140	0.176	0.146	0.113							
GAAR	0.090	0.088	0.087	0.089	0.101	0.089	0.009						
CIMU	0.132	0.126	0.163	0.175	0.158	0.127	0.167	0.112					
ENCI	0.136	0.131	0.162	0.189	0.159	0.134	0.174	0.116	0.137				

GAGU, *Gaidropsarus guttatus*; GAME, *Gaidropsarus mediterraneus*; GAAR, *Gaidropsarus argentatus*; GAEN, *Gaidropsarus ensis*; GAMA, *Gaidropsarus macrophthalmus*; GAGR, *Gaidropsarus granti*; GAVU, *Gaidropsarus vulgaris*; GABI, *Gaidropsarus biscayensis*; CIMU, *Cillata mustela*; ENCI, *Encheiropus cimbricus*.