

# Second world record for *Barathronus roulei* Nielsen, 2019 (Ophidiiformes, Bythitidae), from the Porcupine Bank (Northeast Atlantic)

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## Funding information

European Maritime, Fisheries and Aquaculture Fund, Grant/Award Number: PORCUEM-20233FMP001

## Abstract

*Barathronus* is a genus of blind cusk eels comprising 11 valid species. In this paper, we report the second specimen ever documented of *Barathronus roulei* (Bythitidae) obtained from the Porcupine Bank by R.V. *Vizconde de Eza* using a bottom trawl at a depth of 1349 m. Morphological description and illustrations, including a radiograph, are provided. In addition, three new sequences corresponding to three different genes, cytochrome c oxidase subunit I (COI)-DNA barcoding, 16S ribosomal RNA (16S), and recombination activating protein 1 (RAG1), have been added to the molecular repositories, representing the first sequences for the species.

## KEYWORDS

blind cusk eels, COI, deep-sea fish, DNA barcoding, integrative taxonomy, morphology, Northeast Atlantic

The order Ophidiiformes encompasses a vast range of mostly marine fish species that thrive in a variety of environments, from the deep sea to shallow reef habitats (Møller et al., 2016). The order currently comprises 121 genera and 571 species, which are grouped into two viviparous families (Bythitidae and Dinematichthyidae) and two oviparous

families (Carapidae and Ophidiidae) (Fricke et al., 2024; Møller et al., 2016). Viviparous brotula species belonging to the Bythitidae family inhabit both pelagic and benthopelagic regions within the bathyal and abyssal zones, spanning depths of approximately 500–5500 m (Nielsen et al., 2019). The family Bythitidae currently

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comprises 36 genera and 129 recognized species (Fricke et al., 2024). Knowledge of the family is not yet complete, and new species are still being discovered (de Melo et al., 2022; Nielsen et al., 2019).

*Barathronus* Goode & Bean, 1886 was previously included in the family Aphyonidae Jordan & Evermann 1898, but Møller et al. (2016) placed Aphyonidae as a derived clade of the family Bythitidae Gill, 1861. This genus is characterized by having joined vertical fins; loose and transparent scaleless skin; mouth almost vertical, with fangs on vomer, dentaries, and occasionally on palatines; 20–35 long gill rakers on the first branchial arch; 62–84 dorsal-fin rays, 46–73 anal-fin rays, 21–27 pectoral-fin rays, and one pelvic-fin ray; 31–38 precaudal vertebrae and 67–89 total vertebrae; adult specimens with hourglass-shaped vertebral centra and mature males with penis length up to 15%  $L_S$  (Nielsen, 2019). The genus *Barathronus* has been subject to several revisions (Cohen & Nielsen, 1978; Nielsen, 1969; Nielsen et al., 1999). In the latest revision, Nielsen (2019) established 11 valid species, 6 of which are found in the Atlantic Ocean: *Barathronus bicolor* Goode & Bean, 1886, *Barathronus linsi* Nielsen, Mincarone & Di Dario, 2015, *Barathronus pacificus* Nielsen & Eagle, 1974, *Barathronus multidentis* Nielsen, 1984, *Barathronus parafaiti* Vaillant, 1888, and *Barathronus roulei* Nielsen, 2019.

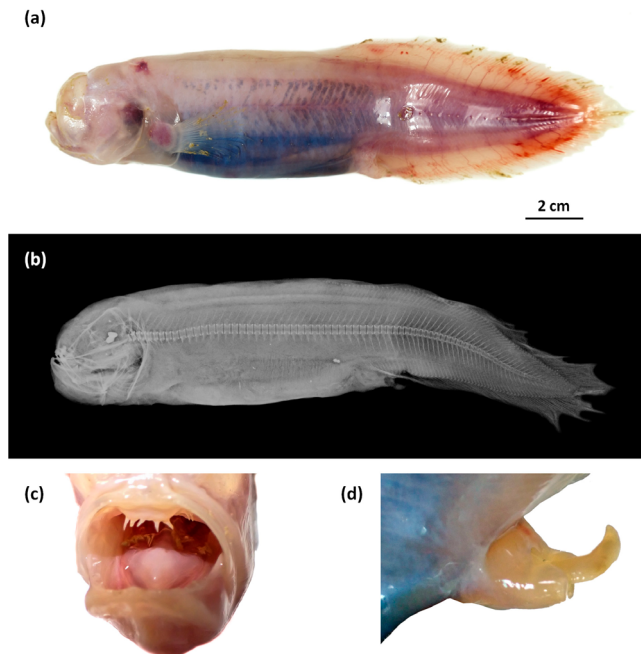
Deep-sea ecosystems constitute the largest biome within the global biosphere, yet our understanding of their biodiversity remains limited (Danovaro et al., 2010). Porcupine Bank is a seamount located in the northeastern Atlantic, approximately 200 km off the west coast of Ireland. Ongoing research efforts are focused on exploring the fish biodiversity of this region and its neighboring area (Bañón et al., 2020, 2021, 2022). Only a few bythitids have been reported in the area, such as *Bellotia apoda* Giglioli, 1883 (Fernández-Zapico et al., 2013), *Cataetix alleni* (Byrne, 1906), and *Cataetix laticeps* Koefoed, 1927 (Merrett et al., 1991).

Although traditional fish species identification has relied heavily on external morphological characters, there is a growing trend to use molecular taxonomy for this purpose (Bañón et al., 2022). A few molecular markers have been previously used in *Barathronus* species for taxonomic or phylogenetic purposes: cytochrome c oxidase subunit I (COI) (Chang et al., 2017; Evseenko et al., 2018; Nielsen et al., 2019), 16S ribosomal RNA (16S) and NADH (Nicotinamide Adenine Dinucleotide + Hydrogen) dehydrogenase subunit 4 (ND4) (Evseenko et al., 2018; Møller et al., 2016; Near et al., 2013), and recombination activating protein 1 (RAG1) (Evseenko et al., 2018; Near et al., 2013).

This research aims to document the first occurrence of *B. roulei* on the Porcupine Bank, the second known worldwide, based on morphometric and meristic data. In addition, the first molecular sequences of three different genes were obtained and deposited in international databases.

On October 10, 2023, a single male specimen of *B. roulei*, measuring 182.3 mm  $L_T$  and weighing 46 g (Figure 1), was collected using bottom trawl gear on Porcupine Bank, 51.5819 N, 12.4842 W, at a depth of 1349 m (Figure 2). Temperature was 6.544°C and salinity was 35.276.

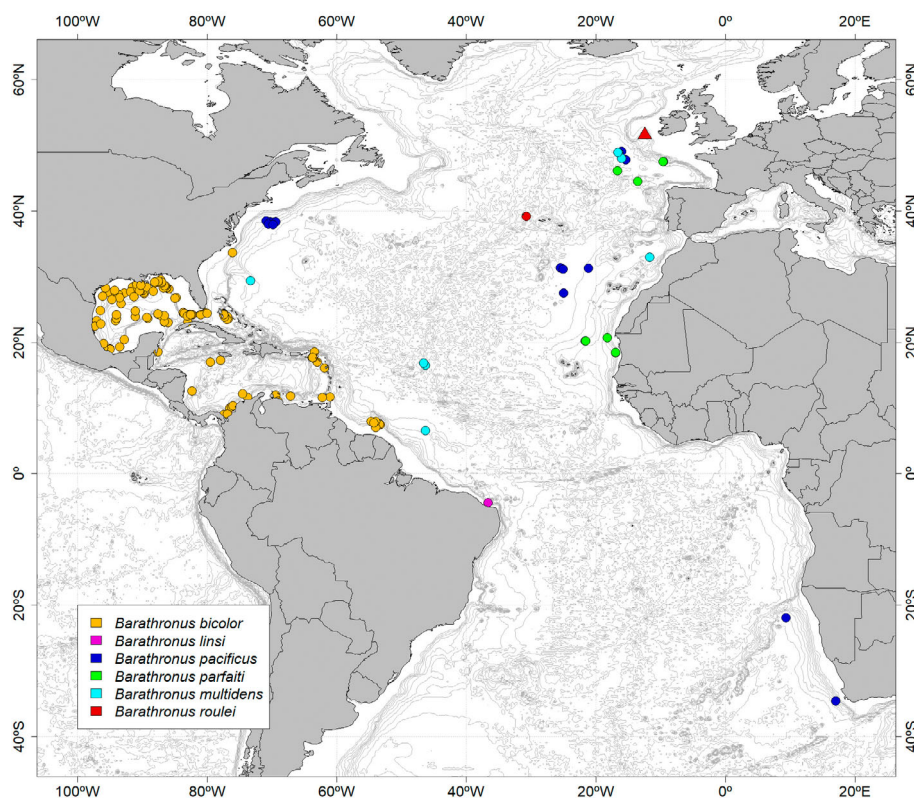
Sampling was conducted onboard the R.V. *Vizconde de Eza*. The specimen was taken using the Baca-GAV 39/52 bottom trawl with a mesh-size of 20 mm in the codend during the 2023 Spanish Bottom Trawl Survey on the Porcupine Bank (SP-PORC-Q3). The survey area



**FIGURE 1** Specimen of *Barathronus roulei* Museo Luis Iglesias de Ciencias Naturais in Santiago de Compostela (MHN USC)\_25223, 182.3 mm  $L_T$ , caught in Porcupine Bank (Northeast Atlantic). (a) Lateral view of fresh specimen; (b) radiograph of the specimen; (c) vomer teeth; (d) penis.

extended from 12° W to 15° W longitude and from 51° N to 54° N latitude. After a small sample was taken for molecular analysis, the specimen was fixed in 10% formalin and then preserved in 70% ethanol and subsequently deposited in the fish collection of the Museo Luis Iglesias de Ciencias Naturais in Santiago de Compostela (MHN USC) under the reference number MHN USC\_25223. Ichthyological terminology and counts follow Nielsen (2019). Vertebrate counts were obtained from radiographs taken using mammography computed radiography equipment (Fujifilm Capsula XL II, Japan), with exposure factors of 44 kVp × 35 mAs.

The body of the specimen is elongated and posteriorly depressed, tapering to the caudal fin (Figure 1a,b), maximum body depth of 3.3 times in  $L_S$ . The head is globular and truncated anteriorly, moderately long at 5.5 times in  $L_S$  with a blunt snout. The eyes are deep-set and indistinct, forming a subcircular whitish ring with a diameter of 4.6 times in the head. The mouth is wide and almost vertical with prominent lower jaw. The maxilla extends to the vertical center of the eye. The teeth are present on the vomer, palatine, and jaws; the premaxillary teeth are villiform in several rows anteriorly, decreasing to one posteriorly; the dentaries have small teeth anteriorly and four to five large recurved fangs; the vomer has five large fangs (Figure 1c); and there are two smaller palatine fangs on the left side. The ventral fins are jugular, with an elongated and filamentous ray that does not reach the anus. There are no bulbs developed at the base of the penis (Figure 1d). The body and fins are pinkish with lighter fins, and discontinuous lateral blue markings along myomere transect on the anterior part of the body between the origins of pectoral and anal fins. The peritoneum is dark blue, divided into three bluish stripes by pale



**FIGURE 2** Location of *Barathronus roulei* on Porcupine Bank (red triangle). Also shown is the location of records of Atlantic *Barathronus* species (GBIF.org, 2023; OBIS, 2023).

medial infracranial muscles, with the opercle and nape being reddish. The most important meristic and morphometric characters are listed in Table 1.

To attempt molecular identification, three different gene markers were PCR-amplified and sequenced based on previously described procedures (Barros-García et al., 2022). DNA extraction was performed on 25 mg of muscle tissue using the EZNA Tissue DNA Kit from Omega Bio-tek. The standard 5' barcoding region of the *COI* gene was then amplified by PCR using the designated universal primer cocktail for fish DNA barcoding *COI-3* (Ivanova et al., 2007). The mitochondrial 16S ribosomal RNA gene and the partial nuclear *RAG1* gene were also amplified. Primers for 16S were 16Sar-5' and 16Sbr-3' (Palumbi, 1996). A nested-PCR approach was used to amplify the *RAG1* fragment: 2 µL of the product of the first round PCR was used as template for the second PCR. Primers for nested PCR and sequencing were those described in Roa-Varón and Ortí (2009).

The sequencing reactions were migrated in a SeqStudio Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) at the Centro de Apoyo Científico y Tecnológico a la Investigación of the Universidade de Vigo (CACTI) facilities. The quality of sequences was verified, and both direct and reverse runs were assembled into a consensus sequence. The *COI* sequence, along with other specimen data, has been deposited in the BOLD database ([www.boldsystems.org](http://www.boldsystems.org)) within the project titled "Fishes of the Porcupine Bank" (code PORCU) under process ID PORCU038-24. Additionally, the sequence is available in GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) under accession number PP313084. Sequences of the 16S and *RAG1* genes have also been deposited in GenBank under accession numbers PP256263 and PP259110, respectively.

Available *COI* barcodes of *Barathronus* were retrieved from the GenBank and BOLD repositories and used to construct a molecular cladogram. For the *COI* marker analysis, nucleotide differences between sequences were calculated using the p-distance method (Nei & Kumar, 2000), and a Neighbor-Joining diagram for taxonomic identification purposes was constructed, employing the MEGA v11 software suite (Tamura et al., 2021).

The three molecular sequences obtained—*COI*, 16S, and *RAG1*—were compared with reference libraries for positive identification, but no matches were found. Figure 3 illustrates the attempted molecular identification of the *B. roulei* specimen comparing its *COI* barcode with those of the same genus in the BOLD and GenBank databases using the Neighbor-Joining method. Of the six sequences available, four clustered as *Barathronus maculatus* and two clustered as *B. pacificus*, with intraspecific distance values varying between 0% and 0.18%. The putative barcode of the *B. roulei* specimen forms an independent branch with minimum distances of 8.76% with *B. maculatus* and 9.06% with *B. pacificus*, highlighting species distinctiveness and a well-defined barcoding gap within the genus. It is worth noting that sequences initially reported as *B. maculatus* were later identified as *Barathronus algrahami* (see below).

Previously, only one specimen of *B. roulei* was known, collected off the Azores in 1896 (MOM P01-0003665) at a depth of 1846 m. Often referred to as *B. parfaiti* (Roule, 1916, 1919), it was later described as a valid new species *B. roulei* (Nielsen, 2019). Therefore, only the holotype is available for comparisons. The morphometric and meristic data of our specimen generally agree with the holotype, with differences within the natural variability in the species (Nielsen personal communication). However, the colouration is different: The

**TABLE 1** Morphometric and meristic data of *Barathronus roulei* and comparison with the holotype data of 100 mm  $L_s$  reported by Nielsen (2019).

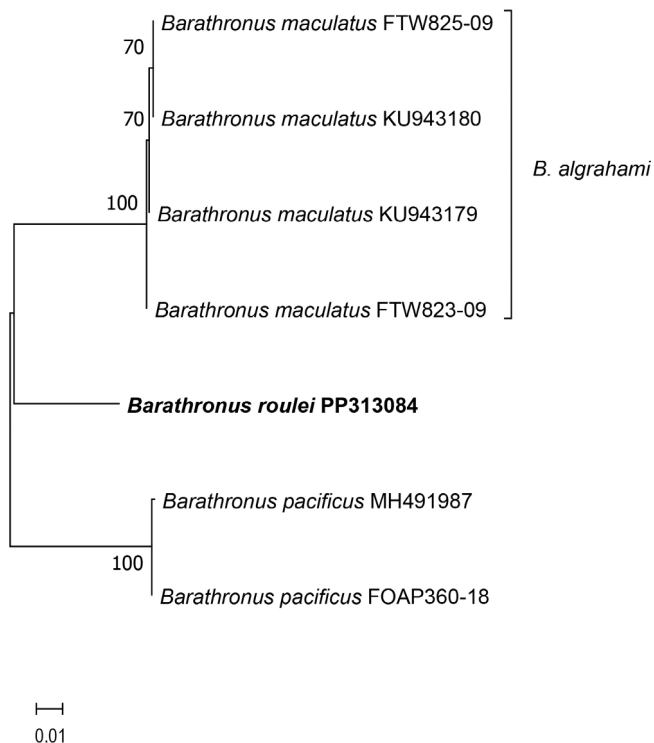
Morphometric and meristic data	MHN USC_25223		MOM P01-0003665 holotype
	Length (mm)	Standard length %	Standard length %
Total length	182.3		
Standard length	170.4		100
Head length	31.1	18.3	21.5
Preorbital length	9	5.3	-
Postorbital length	19.2	11.3	-
Eye diameter	6.5	3.8	-
Interorbital width	3.5	2.1	-
Pre-dorsal-fin length	86.4	50.7	51.0
Dorsal-fin base	87	51.1	-
Upper jaw length	16.7	9.8	11.0
Pre-anal-fin length	106	62.2	59
Anal-fin base	64.4	37.8	-
Penis length	12	7.0	-
Pre-pectoral-fin length	42	24.6	-
Pectoral-fin length	27.8	16.3	-
Pre-pelvic-fin length	31.2	18.3	-
Pelvic-fin length	20.2	11.9	-
Body depth anterior anal-fin rays	28.4	16.7	-
Body depth (maximum)	51.3	30.1	-
Distance from base of pelvic fin to anal-fin origin	86.2	50.6	44
Meristic			
Dorsal-fin rays	73		71
Anal-fin rays	62		66
Pectoral-fin rays	23		24
Pelvic-fin rays	1		1
Caudal-fin rays	10		10
Gill rakers	6 + 20		6 + 20
Precaudal vertebrae	32		34
Total vertebrae	79		82
Origin of anal fin above vertebra number	29		29
Origin of anal fin below dorsal-fin ray number	13		12

Abbreviation: MHN USC, Museo Luis Iglesias de Ciencias Naturais in Santiago de Compostela.

bluish fins with ochraceous bases described in the holotype (Roule, 1919) are not evident in our specimen, and the bluish colouration on the body and peritoneum is not described in the holotype.

Only six COI sequences are available in public repositories for two *Barathronus* species, *B. pacificus* Nielsen & Eagle, 1974 and *B. maculatus* Shcherbachev, 1976. Two GenBank sequences (KU943179 and KU943180), originally named *B. maculatus* by Chang et al. (2017), were reassigned to *B. algrahami* Nielsen, Pogonoski & Appleyard, 2019 by Nielsen et al. (2019), though GenBank did not update the species status. The other two sequences in BOLD systems (FTW823-09 and FTW825-09) group with the former, suggesting misidentification.

This report documents the first occurrence of *B. roulei* on the Porcupine Bank, its second known occurrence worldwide, and extends our understanding of its distribution. Successful PCR amplification and sequencing of three gene markers (COI, 16S, and RAG1) provided essential genetic data, revealing the genetic distinctiveness of *B. roulei*. Morphometric and meristic comparisons with the holotype confirmed its natural variability despite differences in colouration. Our results highlight the importance of combining molecular and morphological data for accurate documentation of marine biodiversity and suggest a potentially wider distribution for *B. roulei*, highlighting the need for further deep-sea exploration and research on the elusive genus *Barathronus*.



**FIGURE 3** Neighbor-Joining diagram of available cytochrome c oxidase subunit I (*COI*) gene sequences of *Barathronus* congeners. The percentage of replicate trees in which associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The bar represents the number of base differences per site after computing the nucleotide distances among the sequences as p-distances. There were a total of seven sequences and 651 positions in the final dataset.

### AUTHOR CONTRIBUTIONS

Rafael Bañón and Francisco Baldó collected the specimen and developed the research idea. Alejandro de Carlos and Ángel Sebastián Comesaña conducted molecular analyses. José Daniel Barreiro Vázquez makes the X-ray photograph. All authors were involved in writing and editing the paper.

### ACKNOWLEDGMENTS

The authors would like to thank the staff involved in the 2023 Porcupine research survey carried out by the Spanish Institute of Oceanography (IEO, CSIC) on board the R.V. *Vizconde de Eza* (Ministry of Agriculture, Fisheries and Food, Spain). This survey, included in the PORCUDEM project, was partly funded by the EU through the European Maritime, Fisheries, and Aquaculture Fund as part of the Spanish National Programme for the Collection, Management, and Use of Data in the Fisheries Sector and Support for Scientific Advice in relation to the Common Fisheries Policy.

### FUNDING INFORMATION

European Maritime, Fisheries, and Aquaculture Fund (grant/award number: PORCUDEM-20233FMP001).

### CONFLICT OF INTEREST STATEMENT

The authors declare that they are not aware of any competing interests.

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**How to cite this article:** Bañón, R., de Carlos, A., Comesaña, Ángel Sebastián, Barreiro Vázquez, J. D., & Baldó, F. (2024). Second world record for *Barathronus roulei* Nielsen, 2019 (Ophidiiformes, Bythitidae), from the Porcupine Bank (Northeast Atlantic). *Journal of Fish Biology*, 105(4), 1348–1353. <https://doi.org/10.1111/jfb.15878>