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Northernmost record and new biological data of the Phaeton dragonet *Synchiropus phaeton* (Callionymiformes: Callionymidae) in the eastern Atlantic

by

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Résumé. – Signalement le plus septentrional et nouvelles données biologiques du Dragonnet phaéton *Synchiropus phaeton* (Callionymiformes: Callionymidae) dans l'Atlantique Est.

Une femelle reproductrice active du Dragonnet phaéton *Synchiropus phaeton* (Günther, 1861) de 22 cm de long, a été capturée avec un chalut de fond dans la zone portugaise de l'océan Atlantique. L'identification taxonomique est confirmée par des paramètres morphométriques et méristiques ainsi que du Barcode. Ce signalement est le plus septentrional dans l'Atlantique pour l'espèce et il documente une nouvelle longueur maximale pour l'espèce. L'examen histologique du tissu reproducteur chez *S. phaeton* révèle que cette espèce est un reproducteur par lots avec une organisation de développement ovarien asynchrone.

Key words. – Callionymidae – *Synchiropus phaeton* – NE Atlantic – Portugal waters – Maximum length – Barcoding – Reproduction.

Dragonets of the family Callionymidae are a group of benthic fishes that occur in the upper 900 m of all temperate, subtropical and tropical oceans (Fricke, 2016a). The family Callionymidae consists of small, bottom-associated fishes usually living on sand and mud beds. It includes 193 valid species organized in 11 genera (Farias *et al*., 2016). The Phaeton dragonet *Synchiropus phaeton* (Günther, 1861) lives on sandy or muddy bottoms at a depth between 99 m and 650 m. It is distributed from the Azores and Portugal south to Gabon and the Mediterranean Sea (Fricke, 2016b).

This study aims i) to record the northernmost and greater specimen of *Synchiropus phaeton* in Atlantic waters, and ii) to provide original information on basic reproductive features of the species.

MATERIAL AND METHODS

A specimen of *Synchiropus phaeton* was captured with bottom trawl in Portugal in 2017. The specimen was first preserved frozen. Then it was fixed in 10% formalin, and finally transferred to 70% ethanol and deposited in the fish collection of the Museum Luis Iglesias de Ciencias Naturais of Santiago de Compostela (Galicia, Spain) with the reference number MHNUSC 25105. The main morphometric and meristic characters were taken following Fricke (1981, 2016b).

After morphometric measurements, the gonads were removed and immediately fixed in 10% formalin buffered with $NaH_2PO_4*H_2O$ (0.046 M, molar concentration) and NaH2PO4*H2O (0.029 M). Serial portions from the gonad (5 in total) were extracted, dehydrated, embedded in paraffin, sectioned at 3 µm and stained with haematoxylin-eosin for histological examination. The specimen was sexed and classified within its correspondent maturity phase using histological criteria (Brown-Peterson *et al*., 2011). The follicles (oocytes and surrounding follicular layer) were classified into stages of development using histological criteria (Murua and Saborido-Rey, 2003).

A sample of muscle from the *S. phaeton* specimen was employed for DNA purification and sequencing of the standard 5' barcoding region of the mitochondrial COI gene, following procedures previously described (Bañón *et al*., 2016). PCR amplification was carried out with Thermo Scientific Phire Green Hot Start II PCR Master Mix and the primer set C_FishF1t1-C_FishR1t1 (Ivanova *et al*., 2007). A 658 nucleotides-long sequence was submitted to the GenBank repository and given the accession number MG210569. In order to explore the taxonomic status of the sample, its sequence was aligned with other phylogenetically-related barcodes using the MEGA6 software (Tamura *et al*., 2013) and the Neighbor-Joining method (Saitou and Nei, 1987) was employed to construct a tree diagram. The divergence among sequences was calculated in the units of the number of base differences per site (p-distances). Confidence limits of this analysis were tested using a bootstrap procedure (Felsenstein, 1985).

RESULTS

One specimen of *Synchiropus phaeton* of 220 mm TL (Fig. 1) was recorded on 24 September 2017, near Peniche (Portugal), at 39°18'N, 9°55'W with bottom trawl at 254 m depth. The body is elongate and slightly depressed; snout shorter than eye diameter, 1.3 times in eye; eye large, 3.4 times in head; interorbital narrow, 8 times in eye diameter; gill opening sublateral; preopercular spine with 2 dorsal points backward and no antrorse spine at base; soft dorsal rays branched, the last divided through its base. Body

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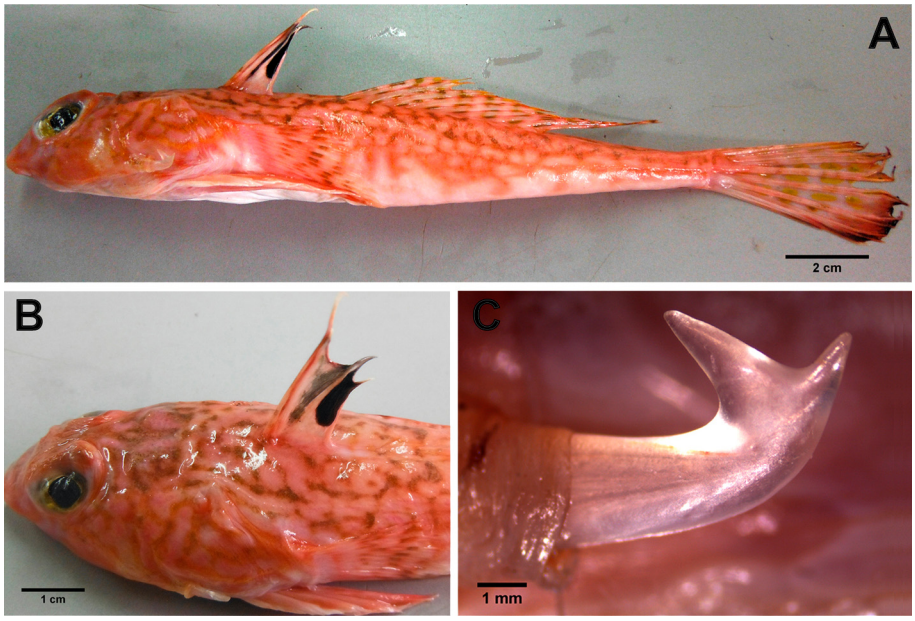

Figure 1. – Specimen of *Synchiropus phaeton*. **A**: View of the entire specimen of 220 mm TL; **B**: Detail of head and first dorsal fin; **C**: Detail of the preopercular spine showing 2 dorsal points.

Table I. – Measurements and counts of specimen of *Synchiropus phaeton*.

	L (mm)	%SL
Total length	220	
Standard length	165	
Head length	54	44.6
Preorbital length	12	9.9
Postorbital length	26	21.5
Orbital length	16	13.2
Bony Interorbital	2	1.7
Predorsal (1) length	48	39.7
Predorsal (2) length	74	61.2
Dorsal (1) base	13	10.7
Dorsal (2) base	56	46.3
Preanal length	85	70.2
Anal base	49	40.5
Pectoral-fin length	35	28.9
Pelvic-fin length	43	35.5
Prepectoral length	56	46.3
Prepelvic length	36	29.8
Body height	25	20.7
Body wide	34	28.1
Length of 1st spine of first dorsal fin	27	22.3
Length of last ray of second dorsal fin	37	30.6
Caudal peduncle length	35	28.9
Caudal peduncle depth	8	6.6
Caudal fin length	56	46.3
Dorsal (1) fin rays	IV	
Dorsal (2) fin rays	viii,1	
Anal fin rays	vii,1	
Pectoral fin rays	i,20,i	
Pelvic fin rays	I,5	
Caudal fin rays	(ii),i,7,ii,(ii)	

orange-pinkish with irregular darker markings; first dorsal fin with a black blotch between third and four spine; second dorsal fin pale with irregular greenish spots; anal fin with a distal black streak; caudal fin distally black. The main morphometric and meristic characters are showed in the table I.

Regarding the molecular identification, the resulting Neighbor-Joining tree (Fig. 2) clustered the DNA barcode of the *S. **phaeton*** specimen caught in Galician waters with two publicly available sequences from the same species, JQ774749 and JQ774751, which correspond to specimens from the southern coast of Portugal (Costa *et al*., 2012). The mean nucleotide divergence in this group was 0.22%. When comparisons were made with other *Synchiropus*, this value rose to 5.80% with *S. agassizii* (Goode & Bean, 1888) and up to 17.72% with *S. masudai* (Nakabo, 1987) representing the minimal and maximal values among the species included in the alignment. These figures are in agreement with the values described within species and genus in fishes (Ward *et al*., 2009).

Although the freezing process, prior formalin fixation, prevented a detailed analysis of the ovarian cell structures, basic classification of oocyte development was feasible (Fig. 3). The stages assigned were primary growth, secondary growth (cortical alveoli and vitellogenic oocytes) and maturation (germinal vesicle migration to hydration). It was not possible to identify accurately some ovarian structures, such as atretic oocytes. However, postovulatory follicles (POF), as a signal of spawning activity, were clearly identified.

DISCUSSION

Morphological data of *Synchiropus phaeton* is consistent with the description, measurements and counts reported by other authors (Fricke, 1981, 2016b). The molecular identification supports the reliability of the morphological one.

The histological examination of the gonads revealed that *S. phaeton* exhibits an asynchronous ovarian development organization, *i.e*. oocytes of all stages of development are present without a dominant population (Murua and Saborido-Rey, 2003). The specimen examined was sexually mature and in an actively spawning phase, with evidence of recent spawning activity (signs of ovula-

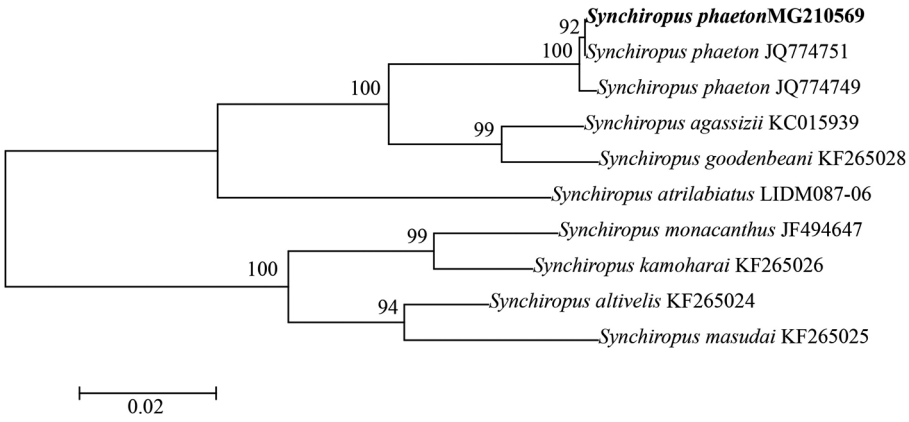

Figure 2. – Neighbor-Joining analysis of related callionymid species showing the specimen captured in Galician waters is bolded.

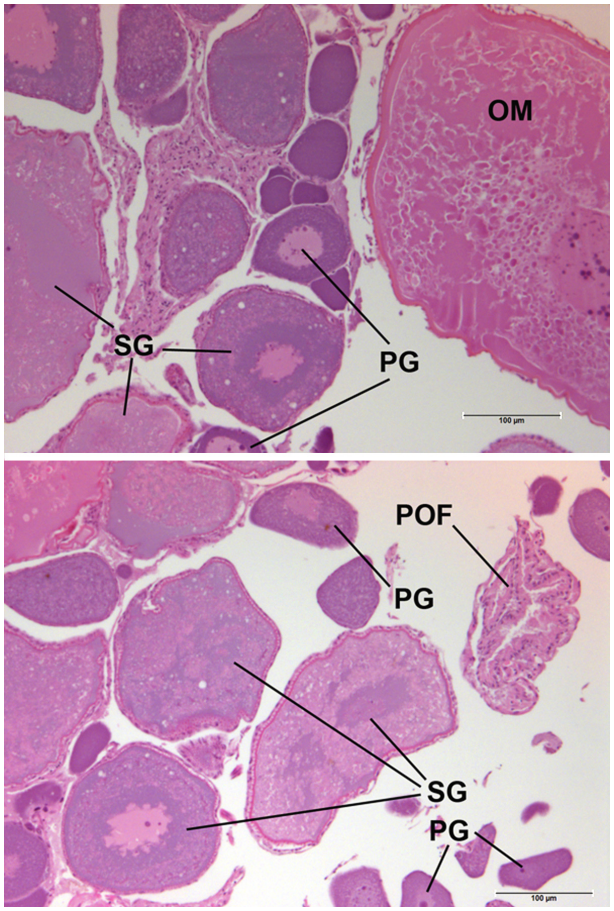

Figure 3. – Histological slides of *Synchiropus phaeton* ovary with evidence of past spawning (POF: postovulatory follicles) and next spawning (OM: oocyte maturation). PG: primary growth oocytes; SG: secondary growth oocytes (includes cortical alveoli and vitellogenic oocytes).

tion and the presence of recent postovulatory follicles). To date, no single study has depicted the spawning season of *S. phaeton* in the study area.

The histological examination of the ovaries showed the presence of postovulatory follicles and several modes in the secondary growth follicle pool, which are clear evidences of batch spawning type (Murua and Motos, 2006). This agrees with findings in other Callionymidae species, for example *Callionymus maculatus* and *C. lyra*, releasing small batches of eggs over a long spawning period (Gibson and Ezzi, 1979; King *et al*., 1994).

The maximum length of organisms is an important parameter related to other major biological processes such as the length at first maturity and asymptotic body length, and many times is the only life history trait known for a given species (Juan-Jordá *et al*., 2016). Regarding *S. phaeton*, males reach to 18 cm (usually 10-14 cm), whereas females reach to 12 cm (usually 6-10 cm) (Fricke, 1986, 2016b). Our specimen, a female of 22 cm TL, has notably increased the maximum size known for this species. However, in many callionymid species, males attain greater size than females (Harrington, 1997). Therefore, it would be logical to speculate that males longer than 22cm exist but remain unfound.

Very little information has been so far provided about this species in the Atlantic European waters. Lopes da Cunha and Antunes (2012) revised the distribution and abundance of callionymid species in the mainland Portuguese waters, but only data on *Callionymus* species were reported, which may be related to the lack of records of *S. phaeton*. The northward distribution of *S. phaeton* in the Atlantic is known to reach the Azores and Portugal but generally without exact limit reported. So far, the northernmost one was reported by Palmer (1971) in Sezimbra Bay, Portugal, ca. 38°26'N. Our record, at 39º18'N, extends the distribution range of the species slightly northwards in the Eastern Atlantic. A detailed knowledge of fish spatial distribution is required to identify shifts in their distribution range, notably linked to global warming and the tropicalization process. More and more tropical fish species have been reported north to their known natural distribution range in the eastern Atlantic (Kaimuddin *et al*., 2016) and off the western Iberian coast (Bañón and Sande, 2008; Carneiro *et al*., 2014). Although callionymid species are rarely mentioned in these types of reports, species such as *C. reticulatus* have been recorded in the northernmost areas of the Eastern Atlantic (Fröiland and Greve, 1976; Neudecker and Damm, 2004) and in the Mediterranean (Fricke and Ordines, 2017), suggesting a recent northern expansion. Given its tropical distribution, *S. phaeton* seems to be a good candidate to spread further northwards in the present global warming scenario.

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