

RESEARCH NOTE

First record of humpback anglerfish (*Melanocetus johnsonii*) (*Melanocetidae*) in Antarctic waters

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Abstract

The first record of the humpback anglerfish (*Melanocetus johnsonii*) in Antarctic waters (Ross Sea) is documented. Species identification is confirmed by the structure of the escal bulb and analysis of the mitochondrial cytochrome oxidase subunit I (COI) gene. This record extends the known range of *M. johnsonii* by more than 1000 nm to the south. The taxonomic status of *Melanocetus* species in Antarctic waters is discussed.

According to modern concepts (Pietsch & Orr 2007; Pietsch 2009; Mia et al. 2010), the family of black sea-devils (*Melanocetidae*) is represented by the single genus *Melanocetus* with six valid species: *M. eustalis*, *M. johnsonii*, *M. murrayi*, *M. niger*, *M. polyactis* and *M. rossi*. Two species—*M. johnsonii* and *M. murrayi*—are widely distributed in the Atlantic, Pacific and Indian oceans. Three other species—*M. eustalis*, *M. niger* and *M. polyactis*—are known only in the eastern tropical Pacific. It is believed that Antarctic waters of the Ross Sea are inhabited by the sixth representative of the genus—*M. rossi* (Pietsch 1990; Miller 1993; Balushkin & Fedorov 2002; Hanchet et al. 2013)—that was described from a single specimen in poor condition (Pietsch 2009). It had a standard length (SL) of 118 mm and had been caught by otter trawl at a depth of 390 m (Balushkin & Fedorov 1981). Morphological features suggest that *M. rossi* is very close to *M. johnsonii* and *M. murrayi* (Balushkin & Fedorov 2002). However, Pietsch (2009) suggests that *M. rossi* appears to be very similar to *M. johnsonii* and may well prove to be a synonym of this species.

This communication documents the first valid record of *Melanocetus johnsonii* Günther, 1864 in the Ross Sea and discusses the taxonomic status of *Melanocetus* species in Antarctic waters.

Materials and methods

A single specimen of the anglerfish genus *Melanocetus* with a total length (TL) of 157 mm (SL 133 mm) and body weight of 290 g (Fig. 1) was found in the stomach of an Antarctic toothfish (*Dissostichus mawsoni*) with a TL of 138 cm and a body weight of 28 kg, caught on 6 January 2014 by bottom longline (set no. 25) from the fishing vessel *Ugulan* at 1243–1355 m depth in the area with coordinates 75° 13.9'–75° 14.4' S and 175° 05.2'–175° 20.4' W (Fig. 2).

For technical reasons, there was no opportunity to save and bring the anglerfish to the laboratory. It was measured and photographed on board. A pectoral fin clip was cut for subsequent genetic analysis and the illicium was cut off for further morphological study (both fin clip and illicium were preserved in 96% ethanol at a ratio of 1:5). All measurements of illicium and escal bulb were taken according to previously published methods (Bertelsen 1951; Pietsch & Van Duzer 1980; Anderson & Leslie 2001; Pietsch 2009).

The preserved pectoral fin and illicium are registered and deposited in the Russian National Collection of Genetic Reference Materials in the Russian Federal Research



Fig. 1 Anglerfish of the *Melanocetus* genus from the stomach of an Antarctic toothfish captured in the Ross Sea, on 6 January 2014, from the FV *Ugulan*.

Institute of Fisheries and Oceanography (Moscow) under a number MEL 0313.06.01. All molecular genetic studies—DNA extraction, polymerase chain reaction (PCR), PCR product purification and nucleotide sequencing—were performed using standard molecular genetic techniques (Ivanova et al. 2007). For the sequencing reaction, 0.3 pmol of the PCR product and 3.2 pmol of the appropriate primer were taken. Each PCR product was sequenced from both forward (F) and reverse (R) primers. DNA sequencing was performed on ABI Prism 3130xl device (Life Technologies, Carlsbad, CA, USA) according to the manufacturer’s protocol. A set of universal primers was used for mitochondrial cytochrome oxidase subunit I (COI) gene amplification (Ivanova et al. 2007). Data processing was performed, and a molecular genetic tree was constructed by using Geneious 6.0.5 software based on the neighbour joining method, Kimura 2-parameter model and 1000 bootstrap

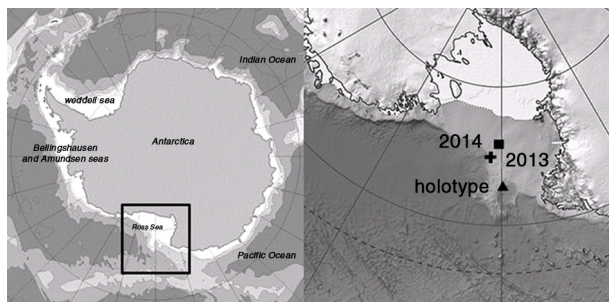


Fig. 2 The capture locations in the Ross Sea of the holotype of *Melanocetus rossi* (Balushkin & Fedorov 1981), the two specimens of *M. rossi* from the stomachs of Antarctic toothfish collected in 2013 (Hanchet et al. 2013) and our specimen from the stomach of an Antarctic toothfish collected in 2014.

replicates (Kimura 1980). Analysis of genetic distances was conducted using the maximum composite likelihood model (Tamura et al. 2004) that involved nine nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 649 positions in the final data set. Evolutionary analyses were conducted in the MEGA6 software package (Tamura et al. 2013). Data on COI sequences of congeneric species were taken from the open BOLD database (<http://www.boldsystems.org>). *Ceratias holboelli* (Ceratiidae) was selected as an out-group (Pietsch & Orr 2007; Mia et al. 2010).

Results and discussion

Morphometry

The study of the illicium of the *Melanocetus* specimen (Fig. 3) and comparison with those of other *Melanocetus* species verifies that the former belongs to the species *M. johnsonii*, whose esca is characterized by the existence of crests and a black pigmented area on the top (Fig. 4). Among other species of the genus, the esca of *M. rossi* bears a medial crest, but its top is not pigmented (Fig. 5). Pietsch (2009) pointed out that the esca of *M. johnsonii* bears anterior and posterior crests (see Fig. 4). However,



Fig. 3 Two lateral views of the esca of an anglerfish of the genus *Melanocetus* from the stomach of an Antarctic toothfish collected in 2014 in the Ross Sea.

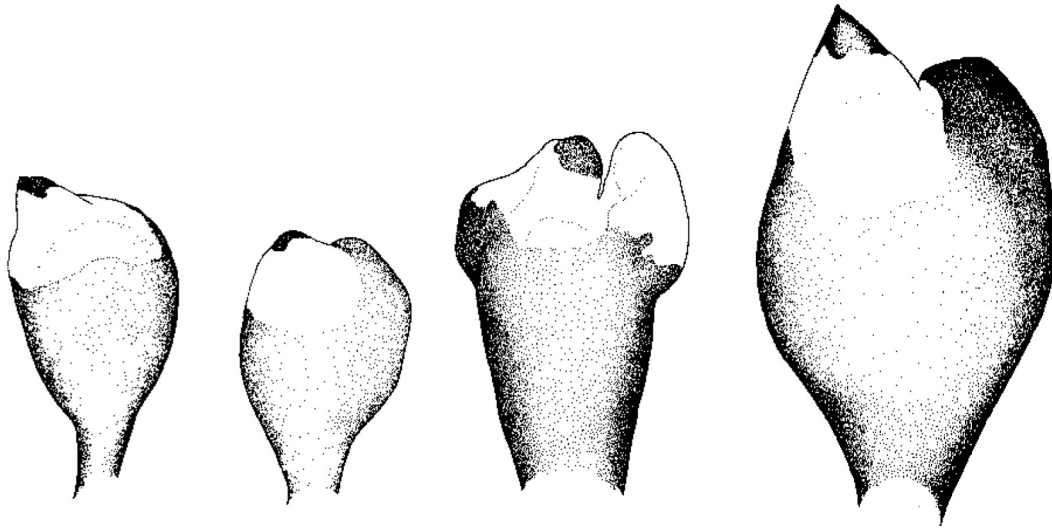


Fig. 4 Variations of esca form in *Melanocetus johnsonii* (after Pietsch & Van Duzer 1980).

there is a single crest in our specimen located in the central part of the esca top with the black pigmented area. Escas of *M. polyactis* and *M. niger* (Fig. 6) are also characterized by a black pigmented area on the top but there are no crests on the escae (Pietsch 2009).

Relative sizes of illicium and esca are considered the main taxonomic features of anglerfishes (Bertelsen 1951;

Pietsch & Van Duzer 1980; Pietsch 2009). The relative length of the illicium in our specimen (25.8% SL) is slightly smaller as compared with literature data (Table 1) for *M. johnsonii* (26.0–60.8%) and *M. rossi* (29.2%). The width of the esca bulb (2.7% SL) in our specimen falls outside of the range of known values for the species mentioned above: 4.3–9.2% and 3.6–3.7% SL, respectively. According to the relative sizes of the illicium and esca, our specimen is closest to *M. murrayi*, from which (apart from the shape of the vomer) it is differentiated by the existence of a crest and a black pigmented area of the top of the esca. Some of the difference of the esca size in our specimen is probably related to the preservation of the illicium in ethanol. The data provided here on the width of the esca suggest greater variability of this feature in *M. johnsonii* as compared to previously known information.



Fig. 5 Esca of *Melanocetus rossi* (after Balushkin & Fedorov 1981).

Genetic analysis

To reveal the species identity using genetic markers, the analysis of the COI gene was performed. Results of genetic analysis definitively confirm that our specimen (GenBank accession number is KM593294) is *Melanocetus johnsonii*. On the molecular genetic tree (Fig. 7), it forms a separate cluster (bootstrap within the cluster varies 62.7–99.9%) with other *M. johnsonii* specimens that differentiates well from congeneric species *M. murrayi* (bootstrap 100%). Genetic distance (Table 2) between our specimen and *M. johnsonii* (mean value for four specimens) is extremely low (unit of the number of base substitutions per site is 0.0015), which confirms its affiliation to *Melanocetus johnsonii*.

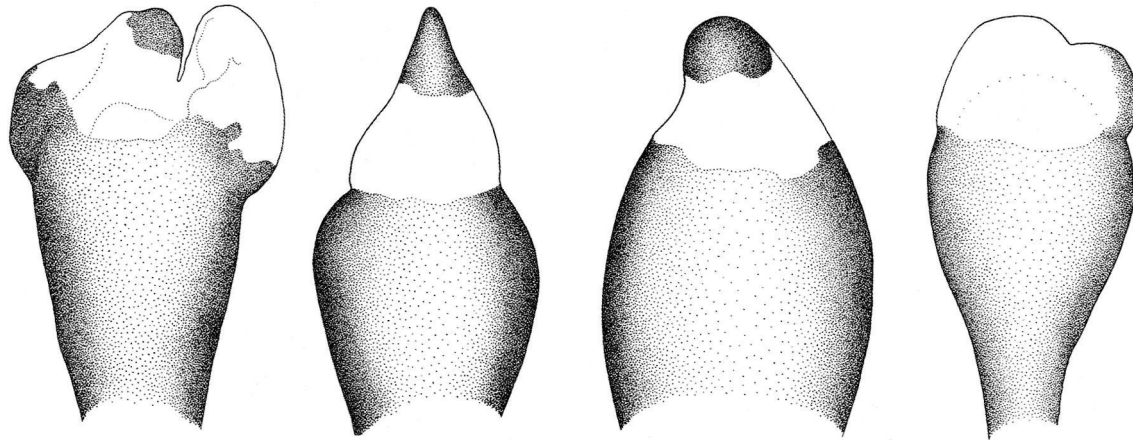


Fig. 6 Variations of esca form in different *Melanocetus* species, from left to right: *M. johnsonii*, *M. polyactis*, *M. niger* and *M. murrayi*. (From Pietsch, Theodore W., *Oceanic anglerfishes: extraordinary diversity in the deep sea*. © 2009 by the Regents of the University of California. Published by the University of California Press.)

Taxonomic and zoogeographic notes

As for the taxonomic status of *M. rossi*, we suggest that it would be premature to synonymize this species with *M. johnsonii* due to the need to examine additional materials (seven preserved specimens and tissue samples of four specimens from the Ross Sea) that are available at the Museum of New Zealand (A. Stewart, pers. comm.). Among all morphological features that differentiate *M. rossi* from *M. johnsonii* the colouration of the esca (lack of black pigment on the top) remains the most important since relative sizes of illicium and esca in *M. rossi* lie within the range of *M. johnsonii*, taking into account the measurements of the specimen reported here (see Table 1).

Table 1 Some distinctive features of *Melanocetus* species.

Species	Illicium length (% standard length)	Esca width (% standard length)	Range/ capture area
<i>M. eustalus</i>	30.6 ^g	11.3 ^g	Eastern tropical Pacific ^g
<i>M. johnsonii</i>	26–55 ^a	4.3–9.2 ^a	All oceans between 66°N and 52°S ^g
	32.4–60.8 ^{b,g}	4.3–8.6 ^{b,g}	
Our specimen	25.8	2.7	Ross Sea
<i>M. murrayi</i>	23–38 ^a	1.4–3.8 ^a	All oceans between 64°N and 43°S ^g
	23.1–37.2 ^{b,g}	1.9–5.1 ^{b,g}	
<i>M. niger</i>	35–47 ^a	4.2–6.4 ^a	Eastern tropical Pacific ^g
	29.8–38.8 ^{b,g}	3.8–5.0 ^{b,g}	
<i>M. polyactis</i>	46–58 ^a	5.2–5.7 ^a	Eastern tropical Pacific ^g
	34.6–56.0 ^{b,g}	5.2–8.5 ^{b,g}	
<i>M. rossi</i>	29.2 ^{c,d,f,g}	3.6 ^{c,f}	Ross Sea ^{c,d,e,f,g,h}
		3.7 ^{d,g}	

^aBertelsen 1951. ^bPietsch & Van Duzer 1980. ^cBalushkin & Fedorov 1981. ^dPietsch 1990. ^eMiller 1993. ^fBalushkin & Fedorov 2002. ^gPietsch 2009. ^hHanchet et al. 2013.

Co-occurrence of two congeneric species (*M. johnsonii* and *M. rossi*) in the same area is quite feasible since there are records of four different *Melanocetus* species in the eastern tropical Pacific (Pietsch & Van Duzer 1980), and *M. johnsonii* and *M. murrayi* are commonly taken in the same trawls in the Gulf of Mexico (T. Sutton, pers. comm.).

On the subject of distribution, it should be noted that 52°S was considered as the southern limit of *M. johnsonii* range to date (Pietsch 2009). Our record significantly extends the species range to the south—23° latitude, or more than 1000 nautical miles—assuming that the toothfish from which our specimen was extracted did not cover this distance after consuming the anglerfish and before being captured. The *M. johnsonii* specimen was in almost fresh condition and did not show signs of advanced digestion.

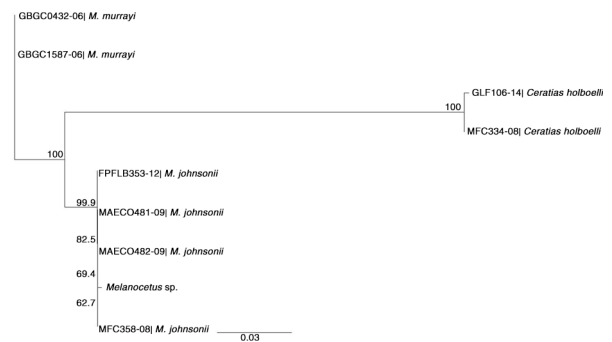


Fig. 7 Molecular genetic tree of selected anglerfish species based on the neighbour joining method, Kimura 2-parameter model and 1000 bootstrap replicates for mitochondrial cytochrome oxidase subunit I (COI) genes. Numbers beside each branch indicate bootstrap values above 50%. The scale bar is according to the Kimura 2-parameter model. Codes besides species names represent the GenBank accession numbers. Our specimen is indicated as *Melanocetus* sp.

Table 2 Estimates of net evolutionary divergence between groups of sequences for investigated anglerfish species based on mitochondrial cytochrome oxidase subunit I (COI) gene (units of the number of base substitutions per site from estimation of net average between groups of sequences are shown; our specimen is indicated as *Melanocetus* sp.).

Species	<i>Melanocetus johnsonii</i>	<i>Melanocetus murrayi</i>	<i>Ceratias holboelli</i>
<i>Melanocetus johnsonii</i>			
<i>Melanocetus murrayi</i>	0.0363		
<i>Ceratias holboelli</i>	2.1196	2.1195	
<i>Melanocetus</i> sp.	0.0015	0.0382	2.1215
Overall mean	0.8351		

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