



SHORT COMMUNICATION

First record of the deep-water shark *Etmopterus spinax* (Chondrichthyes: Etmopteridae) from the southern Baltic Sea (Pomeranian Bay)

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Received 29 September 2017; accepted 1 February 2018

Available online 14 February 2018

KEYWORDS

Etmopterus spinax;
Anisakis simplex;
Pomeranian Bay;
Southern Baltic Sea;
Inflow from the North
Sea

Summary *Etmopterus spinax* is a deep-sea shark species that inhabits the northeast Atlantic and the western Mediterranean Sea. Skagerrak and Kattegat are reported to be part of the distribution of the species, but it has never been noted in the southern Baltic. Lacking any commercial value and commonly discarded in trawl and longline fisheries, *E. spinax* has been poorly studied. We reported on the first record of one specimen of *E. spinax* caught in the Pomeranian Bay on October 13, 2016 at a depth of 10 m. It was a female measuring 42.7 cm in total length. The morphological examination of the specimen was supported with COI barcode analysis, whereas species assignment to the population of origin was conducted based on a control region (CR) sequence of mtDNA. COI and CR sequence searches against GeneBank confirmed its identity as *E. spinax* and revealed that the specimen shared identical haplotypes with fish from populations in the Azores, Rockall Trough, and west of Ireland in the northeast Atlantic. The stomach contents, parasitic fauna, and hepatosomatic index of the individual were also examined. Only one L3 larval *Anisakis simplex* nematode specimen was collected from the stomach lumen of the shark. The specimen could have arrived in the Pomeranian Bay along with an inflow

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from the North Sea. In December 2014, a strong Major Baltic Inflow brought large amounts of water into the Baltic Sea, followed by some inflows of moderate intensity.

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The velvet belly lanternshark, *Etmopterus spinax* (L., 1758), is a deep-sea bioluminescent squaloid shark (Claes and Mallefet, 2009) found predominantly in the northeast Atlantic, the western Mediterranean Sea and in deep waters off southern Africa (south of Senegal) at depths of 70–2000 m and most abundantly at 200–500 m (Compagno et al., 2005). According to McEachran and Branstetter (1984), it occurs rarely in the North Sea and is absent from the Baltic; however, Bergstad et al. (2003) report that this species is a characteristic member of the Skagerrak deep-water fish assemblage at depths of 300–700 m, while Compagno (1984) report Skagerrak and Kattegat to be part of the distribution of the species. *E. spinax* is a non-commercial species that is caught only as by-catch in bottom trawls fishing for *Nephrops norvegicus* (L. 1758) and *Pandalus borealis* Krøyer, 1838 in the Skagerrak and Kattegat by Swedish fishermen. All specimens are probably discarded, which limits the data that is available. It has never been recorded in logbooks. However, in Swedish waters, bottom-trawls are required to have a selective grid that should help to reduce by-catch of *E. spinax* (Coelho et al., 2009).

Data from the Mediterranean Sea and the eastern central and south Atlantic indicate that the species is caught relatively commonly in scientific trawl surveys, and since there is no evidence that the population has declined there, its IUCN Red List status is least concern (LC) (Coelho et al., 2009). However, the over-exploitation of shelf fish stocks has triggered many fisheries to exploit marine resources at ever greater ocean depths (Morato et al., 2006), and this species is considered vulnerable to over-fishing because of its late maturity (Coelho and Erzini, 2008). Furthermore, the reproductive cycle in *E. spinax* has been suggested to last from two to three years before viviparous parturition, which suggests this species has low fecundity. Occurring frequently as by-catch in deep-water fisheries landing crustaceans and teleosts, the species is exposed to relatively high levels of mortality (Porcu et al., 2014). Therefore, lacking any commercial value and commonly discarded in trawl and longline fisheries, *E. spinax* has been poorly studied (Coelho and Erzini, 2008).

According to Klimpel and Palm (2002), parasitological studies on deep-water chondrichthyans from the northeastern Atlantic are scarce. To date, no parasitic investigations of this species have been done in the Baltic Sea area.

The aim of the study was to present the first record of *E. spinax* in the Pomeranian Bay, which is the first time it has been noted in the southern Baltic, as well as to assign its population of origin through genetic studies. Additionally, the stomach contents, parasitic fauna, and hepatosomatic index were examined.

One specimen of the deep-water shark *E. spinax* was caught in the Pomeranian Bay (western Baltic; 54°04'80"N

14°44'00"E) on October 13, 2016, at a depth of 10 m with a cod gillnet (110 mm mesh size).

The Pomeranian Bay (Arkona Basin) is a large, shallow basin off the Polish and German coasts, no more than 30 m deep. It is a highly dynamic environment and is one of the most important ecological areas in the southwestern Baltic Sea. Salinity does not deviate from that prevailing in southern Baltic surface waters (7 psu) (Czugała and Woźniczka, 2010).

Sixteen diagnostic metric characters were studied, according to McEachran and Branstetter (1984) and Compagno et al. (2005), to the nearest mm. Weight was measured to the nearest 0.1 g.

Material for the genetic study consisted of muscle tissue fragments collected during morphological examinations of *E. spinax*. DNA was isolated using the peqGOLD Tissue DNA Mini Kit (PeqLab, Germany) following the manufacturer's instructions. Qualitative and quantitative assessment of the isolates was conducted by separation on 1.5% agarose gel and spectrophotometric assays using NanoDrop 2000 (Thermo Scientific). In order to confirm the morphological identification of the specimen, a PCR assay was performed based on the mitochondrial DNA (mtDNA) marker cytochrome oxidase subunit 1 (COI). COI sequences were amplified based on FishF2_t1 and FishR2_t1 primers according to the method described by Ivanova et al. (2007). Additionally, Elasmocr15642 and Elasmocr16638 primers were used to amplify the control region (CR) of mtDNA, and these were used to assign the specimen to populations identified in a paper published by Stonero et al. (2003). Both PCR reactions were prepared based on GoTaq[®] G2 Flexi DNA Polymerase (Promega) and subjected to a heating-cooling treatment using a thermal cycler GeneAmp PCR system 9700 thermocycler (Applied Biosystems). The results of amplification were assessed by separating the samples on 1.5% agarose gel. The COI and CR PCR products were bidirectionally sequenced using Genomed (Warsaw, Poland) and assembled with Geneious 8.1.6 (Kearse et al., 2012). Then the GeneBank database was searched BALST for matches.

The stomach content was also examined, and the hepatosomatic index (HSI) (%), which is the ratio between liver weight and fish weight, was calculated as follows:

$$HSI = 100 \times \frac{LW}{W},$$

where LW – liver weight (g) and W – fish wet weight (g).

The parasitological examination focused on the skin, vitreous humor, eye lens, mouth, buccal and nasal cavities, gills, gastrointestinal tract, kidney, peritoneum, and muscles. The nematode was isolated and transferred to glycerin

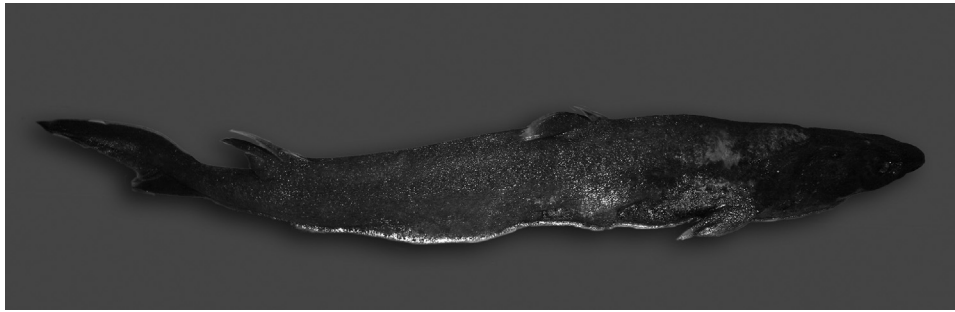


Figure 1 The specimen studied from the Pomeranian Bay.

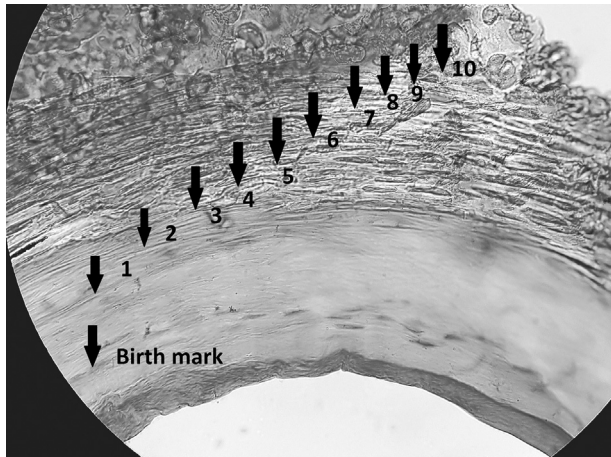


Figure 2 Age validation of *Etmopterus spinax* female collected in the Pomeranian Bay.

to increase transparency. Vertebrae for age determination were removed from the specimen examined from the cervical regions. First, a thin section through the center of the centrum was prepared, then it was stained with hematoxylin and eosin, embedded in a hard epoxy and mounted onto a microscope slide (Campana, 2014). The age was determined under the microscope Motic BA 210 (at 100 \times magnification).

The *E. spinax* specimen examined was a female 42.7 cm in total length TL (Fig. 1) and 311.6 g in weight (gutted weight was 229.5 g). The fish specimen was ten years old (Fig. 2).

Head length (HL) was 21.17% of TL. The snout of the individual examined was long (47.49% of HL). The nostrils were located about midway from the snout tip to the eye (slightly closer to the eye – 54.35%). The eye diameter was long (25.43% of HL), but it was not longer than the distance from the snout tip to the eye (28.34%). The mouth width (43.55% of HL) was slightly less than snout length (47.49% of HL). The spiracle was above and behind the eye (Fig. 3). The gill openings were similar in size to the spiracle (7.06% of HL each), and they were less than one-third of eye length. The interdorsal space was short (21.47% of TL) and was much shorter than the pectoral-pelvic fin distance (34.15%). The bases of dorsal fins D_2 and D_1 were 8.16% and 5.19% of TL, while the heights of D_2 and D_1 were 7.71% and 5.65% of TL, respectively. Similarly, the spine in D_2 was higher than that in D_1 (6.40% and 4.27% of TL, respectively). The distance from the pelvic insertions to the ventral caudal origin was slightly

longer than that from the tip of the snout to the first gill openings (20.96% and 17.55%, respectively). The morphological characters of the specimen described in this work correspond to data reported in McEachran and Branstetter (1984) and Compagno et al. (2005), with the exception of the eye diameter dimension, which in the current study was not longer than the distance from the snout tip to the eye.

BLAST analysis of the COI barcode confirmed the identity of the collected specimen as *E. spinax*, and the barcode for this species was previously submitted to GenBank (i.e., KJ128486.1). Specimen assignment to the population of origin based on the 913 bp sequence of CR revealed that, according to work published by Gubili et al. (2016), the *E. spinax* specimen collected in the Baltic Sea shared identical sequences (haplotypes) with fish collected in the north Atlantic, from the Azores (KX494600.1, KX494606.1, KX494607.1, KX494608.1), the Rockall Trough (KX494695.1, KX494680.1), and west of Ireland (KX494644.1, KX494637.1, KX494634.1).

Velvet belly lanternsharks from these populations had significantly negative F_u 's F_s values, but only that from Ireland also exhibited a unimodal distribution, suggesting population expansion for the CR. It is interesting that the haplotype identified in the specimen examined was not described in regions that are geographically adjacent (i.e., Norway or the North Sea), but in Atlantic basins. One of the reasons for this could be the small sample size of Norwegian sample collection or the lower level of haplotype diversity of the North Sea samples. The results presented by Gubili et al. (2016) demonstrating high levels of connectivity and gene-flow in this species across the northeast Atlantic suggest similar events among populations from the eastern North Sea and the Baltic Sea.

Previous genetic work has produced little evidence of population structure in members of deep-water shark groups with gene-flow occurring at all but the largest oceanic distances assessed, which supports the generally held paradigm of high connectivity and low population structure in marine species (Ward et al., 1994). Recently, the study by Gubili et al. (2016) demonstrated high levels of gene-flow in *E. spinax* across the northeast Atlantic, adding to a relatively limited literature examining the population genetics and phylo-geography of potentially vulnerable deep-sea sharks. Furthermore, the significant population sub-division observed between Atlantic and Mediterranean lanternsharks highlights the potentially significant role of bathymetry as a barrier to connectivity, with important implications for fisheries management.



Figure 3 Head of the specimen studied, with the spiracle above the eye.

The diet of *E. spinax* has been analyzed as follows: crustaceans 74.8%, fishes 16.9%, cephalopods 6.9%, polychaetes 0.9%, and others 0.5% (Cecchi et al., 2004). In this work, the stomach of the specimen studied was empty, and only one nematode *Anisakis simplex* (Rudolphi, 1809) L3 larva from the stomach lumen was identified. The larvae of this nematode occur in marine fish (mainly in herring, mackerel, and cod-like families), in which they migrate from the stomach to the body cavity and locate in the intestine, as well as on the peritoneum and under the liver and gonad capsules. The larva found in *E. spinax* specimen was dead, hence its presence in the empty stomach.

The larvae of *A. simplex* in this host was first identified by Klimpel and Palm (2002) in specimens from the Norwegian Deep. In addition to internal parasites such as the nematode larvae of two species (*A. simplex*) and *Hysterothylacium aduncum* (Rudolphi, 1802) and three species of tapeworms, they found two monogenean species that are ectoparasites. *A. simplex* and *H. aduncum* are listed in the Baltic Proper (in the Gulf of Finland, the Gulf of Gdańsk, and the Pomeranian Bay; Unger et al., 2014). It is worth mentioning that nematodes exhibit great flexibility with regard to environmental changes. No ectoparasites were found in the specimen examined; however, since they are more sensitive to environmental changes, they could have died while their host was in the low-saline waters of the Baltic Proper. It is not known how long the specimen was in Baltic Sea waters, or whether the nematode infection occurred in the Baltic Sea, because there was no prey in the stomach. The infection could have occurred in the North Sea by swallowing the larva along with its first intermediate host, such as marine planktonic copepods (euphausiids), or it could have occurred in the Baltic Sea via teleosts as intermediate or paratenic hosts (Klimpel and Palm, 2002).

The HSI presented in this work is in agreement with Porcu et al. (2014). According to their work, the liver of *E. spinax* is relatively small at 11.5% and 10.3% of total body weight in females and males, respectively (11.07% in the present study). HSI values are higher in mature females, but it decreases during pregnancy, and a slight increase is observed in spent females.

The specimen of *E. spinax* studied in this work was found in the Pomeranian Bay trapped in a cod gillnet at a depth of

10 m. The velvet belly lanternshark, however, is usually found on, near, or well above the substrate from 70 to 2000 m, although mostly between depths of 200 and 500 m (Compagno, 1984). In the northeast Atlantic, *E. spinax* has been caught at depths of 400–1000 m (Merrett et al., 1991). It is known to segregate by size into different depths with large, mature females found at the greatest depths (Gibson et al., 2006). In general, smaller (<30 cm) individuals tend to occur at depths of less than 500 m, while mature individuals are found at moderate depths (500–600 m) (Coelho et al., 2009). This may suggest that these mature sharks are being affected more by commercial deep-water fisheries than other life stages of *E. spinax* that are found in shallower waters. The female *E. spinax* examined in this study can be considered large, because specimens of this species rarely exceed 45 cm (Compagno, 1984). The age determined of specimen examined (ten years) is in agreement with Gennari and Scacco (2007) from the Tyrrhenian Sea (ten years old females were of 37.5–42.0 cm TL) and in Coelho and Erzini (2008) from the waters off Portuguese coast (ten years old females were of 41.1 cm TL).

The specimen collected could have arrived in the Pomeranian Bay along with an inflow from the North Sea. In December 2014, a strong Major Baltic Inflow (MBI), classified as a rare, very strong event, brought large amounts of saline water into the Baltic Sea (Naumann et al., 2016). In November 2015, a series of twelve low-pressure cells crossed the Baltic Sea. During the winter of 2015–2016, again two MBIs of moderate intensity followed the previous one. In this warm winter, only one phase of cold continental weather conditions triggered an outflow at the beginning of January. Afterward, warm maritime weather continued again with storms at the end of January and the beginning of February. In September 2016, a baroclinic inflow was observed at the Darss Sill (Naumann et al., 2016).

A better understanding of the biology, ecology, population structure, and migratory activity of deep-water chondrichthyans is needed to make reliable predictions on the long-term effects of fishing and to ensure that these species are sustainably managed in the future (Cunha et al., 2012).

Acknowledgments

Authors thank Prof. Małgorzata Sobczak for technical support in the microscope slide preparation of the *E. spinax* vertebrae.

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