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Huffmanela hamo sp. n. (Nematoda: Trichosomoididae: Huffmanelinae) from the dagger-tooth pike conger Muraenesox cinereus off Japan

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Abstract: Huffmanela hamo sp. n. is described from eggs only, which were found in black spots in the somatic musculature of a dagger-tooth pike conger, Muraenesox cinereus (Forsskål), caught off Japan. The eggs are $66-77~\mu m$ (mean $72~\mu m$) in length and $33-38~\mu m$ (mean $35~\mu m$) in width. The surface of the eggs is smooth and bears neither envelope nor filaments. The species is distinguished from other members of the genus by the dimensions of its eggs and the characteristics of their surface. This is the first species of Huffmanela Moravec, 1987 to be described from an anguilliform fish, and the twentieth nominal species in the genus. Similar black spots with eggs were reported four times in ten years from this fish caught off Japan; although eggs could not be examined, it is likely that the same species was involved in all cases.

Keywords: new species, egg morphology, Pacific Ocean

Huffmanela Moravec, 1987 comprises 19 valid species (Moravec 2001, Justine 2007, 2011, Ruiz and Bullard 2013, Ruiz et al. 2013). These tissue-dwelling nematodes lay numerous eggs that generally form black spots in the flesh or other tissues of fishes. After being laid, eggs finish their maturation, become dark, and are often noticed only when the adults are dead and unavailable for study (Moravec 2001). For these reasons, most species have been described from eggs only, and generally eggs are considered syntypes.

This paper describes, from eggs only, a new species from the dagger-tooth pike conger, *Muraenesox cinereus* Forsskål, caught off Japan. This is the first *Huffmanela* species to be reported from an anguilliform fish and from a member of the Elopomorpha.

MATERIALS AND METHODS

One of us (T.I.) received information about black spots in the flesh of dagger-tooth pike conger in four occasions: (1) July 2004, place unknown, probably western Japan; (2) July 2006, Wakayama Prefecture, Japan; (3) August or September 2009, Yamaguchi Prefecture, Japan; (4) August 2013 (specimens received 5 September), probably western Japan. More precise information about localities could not be obtained. In each case the flesh with the black spot was photographed and eggs were photographed under a microscope. In all four cases, the eggs were similar to that described in this paper, but in the three first

cases, the sample could not be kept. Observations and photographs presented in this paper concern only the fourth sample. Photographs suggest that a trichosomoidid nematode, probably the same *Huffmanela* species, was found in all four cases, but in the absence of detailed observation and measurement of eggs that cannot be ascertained.

The tissue was fixed in ethanol. Fragments of fixed tissue were placed on a microscope slide in a drop of lactophenol and covered with a coverslip, and the slide was heated under a flame for a few seconds. Drawings were made with an Olympus BH2 microscope equipped with DIC optics. Measurements were taken on pencil drawings and are given as the range, with the mean \pm standard deviation in parentheses, in micrometres. Pencil drawings were redrawn using Adobe Illustrator.

RESULTS

The piece of flesh sent for inspection was somatic musculature. It showed a few black spots, 1–2 mm in size (Fig. 1A). Examination of black spots showed that they were clusters of dark eggs (Fig. 1B,C).

Huffmanela hamo sp. n.

Figs. 1, 2

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Description of eggs

Advanced eggs (Figs. 1D, 2A-D). Advanced eggs dark-brown; less advanced eggs brown. Eggs embryonat-

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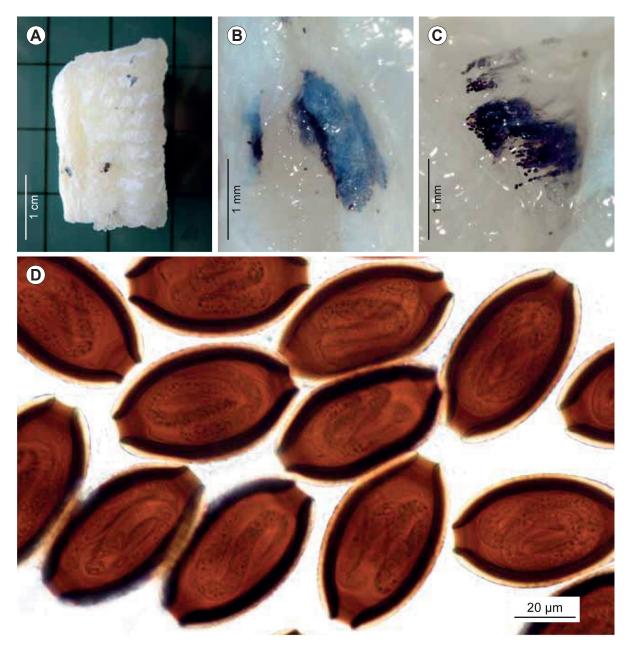


Fig. 1. Huffmanela hamo sp. n. in somatic musculature of the dagger-tooth pike conger Muraenesox cinereus from off Japan. A – piece of flesh of dagger-tooth pike conger with black spots; B, C – black spots, higher magnification (B – deep in flesh, C – on surface); D – eggs (brown eggs with larva visible in each egg).

ed, elongate, with slightly protruding plugs. Size of advanced eggs including polar plugs 66–77 (72 ± 2 , n = 52) × 33–38 (35 ± 1 , n = 52), excluding polar plugs 65–78 (69 ± 2 , n = 72) × 33–38 (35 ± 1 , n = 72) (eggs with or without polar plugs measured on different specimens); thickness of eggshell 5–6. Surface of eggs: smooth, with barely visible ornamentation; no envelope seen; no filament. Larva visible in brown eggs but not in darker eggs in which shell is too opaque.

Aberrant eggs (Fig. 2E–L). Clear eggs, empty (no larva, no embryo) often grouped in small numbers (4–5) amongst normal brown eggs. These eggs were clear and

transparent. Size of clear eggs including polar plugs 70–79 (75 \pm 3.5, n = 9) \times 34–39 (36 \pm 2, n = 9), excluding polar plugs 62–80 (70 \pm 5, n = 11) \times 33–37 (35 \pm 1, n = 11) (eggs with or without polar plugs measured on different specimens); thickness of eggshell 7–10; size of polar plug variable. Deformed clear eggs (Fig. 2I–K) of same characteristics, eggshell wrinkled. An aberrant clear egg without plug found (Fig. 2L). Observation of surface of clear eggs easier than for dark opaque eggs; as for brown eggs, surface smooth, only faint ornamentation visible.

Male and female: Unknown.

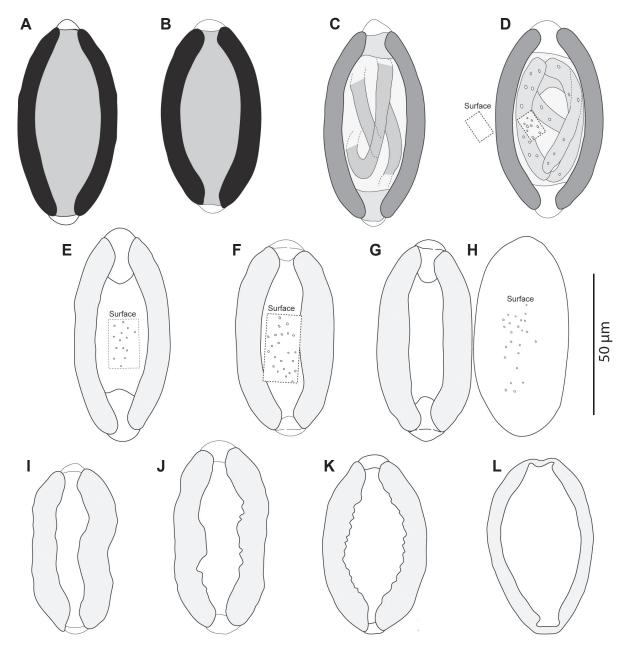


Fig. 2. Huffmanela hamo sp. n. from Muraenesox cinereus. A, B – dark eggs, larva non visible; C, D – brown eggs with larva; E–H – clear eggs; I–K – deformed clear eggs; L – aberrant clear egg without plugs. All drawings are optical sections; in addition, surface of eggs (as seen with DIC optics with focus on upper surface) is drawn and indicated in D–F, H.

Type host: *Muraenesox cinereus* (Forsskål) (Muraenesocidae: Anguilliformes).

Type locality: North West Pacific Ocean, probably off western Japan (more precise information could not be obtained).

Site of infection: Eggs deposited in the somatic musculature.

Collecting date: August 2013.

Prevalence and intensity: Hundreds of eggs in infected fish. Four cases of flesh with black spots reported in 10 years; photographs suggest that the same species was involved but that cannot be ascertained in the absence of specimens.

Deposition of type specimens: Syntypes have been deposited in the Meguro Parasitology Museum, To-kyo, Japan, MPM 20937; the Muséum National d'Histoire Naturelle, Paris, France, MNHN JNA73; and the Institute of Parasitology, Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic, N – 1048.

Etymology: The specific name *hamo* means the Japanese name of the fish. Invariable.

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Remarks. Moravec (2001) provided a key to the eight nominal species of *Huffmanela* known at that time, based on size and superficial envelope. Justine (2007) provided a table of characteristics of the eggs of 21 known species, including all 16 nominal species and five species described without a valid name. Ruiz et al. (2013) provided a table including the 18 nominal species known. We do not provide another new table here and use these tables for comparisons. With the recently described *H. markgracei* Ruiz et Bullard, 2013, there are, before the description of the present species, 19 nominal species.

The diagnosis of Huffmanela eggs is based on measurements and characteristics of their surface. We use only advanced (brown or dark-brown) eggs for this comparison and exclude clear eggs. We list here the six species for which the egg length variations overlap that of H. hamo sp. n., i.e. mean 72 μm \times 35 μm; range 66–77 μm \times 33–38 µm. These are *H. balista* Justine, 2007 (70 µm \times 35 µm; 63–78 µm \times 32–41 µm); *H. japonica* Moravec, Koudela, Ogawa et Nagasawa, 1998 (58–69 μm × 26–30 μ m); H. longa Justine, 2007 (66 μ m × 27 μ m; 58–72 μ m \times 23–32 µm); *H. ossicola* Justine, 2004 (79 µm \times 36 µm; 72–88 μ m \times 32–40 μ m); H. plectropomi Justine, 2011 $(69 \mu m \times 32 \mu m; 64-76 \mu m \times 29-35 \mu m);$ and H. schouteni Moravec et Campbell, 1991 (69–75 μm × 27–30 μm). H. hamo can thus be differentiated from all known species by the measurements of its eggs and the characteristics of their surface:

Huffmanela balista has an envelope, often inconspicuous (vs no envelope in H. hamo), and the surface has inconspicuous longitudinal ridges (vs smooth). The species was described from the external mucosa of the swimbladder of Abalistes stellatus (Anonymous) (Balistidae) off New Caledonia (Justine 2007).

Huffmanela japonica has a thin smooth envelope (vs no envelope), and eggs are smaller and markedly narrower (26–30 vs 33–38). They were described from the muscles of *Upeneus japonicus* Houttuyn (Mullidae) in the Inland Sea of Japan (Moravec et al. 1998).

Huffmanela longa has long filaments at extremities of eggs (vs no filament), narrower eggs (27 μm vs 35 μm). The eggs were found in the mesentery, mucosa of the abdominal cavity and outer swimbladder wall of Gymnocranius oblongus Borsa, Béarez et Chen (type-host) and G. grandoculis (Valenciennes) (Lethrinidae) off New Caledonia (Justine 2007, Justine et al. 2010).

Huffmanela ossicola has numerous filaments on the eggs surface surrounded by thin envelope (vs no envelope, no filament) and larger eggs. The species was described from the bones of *Bodianus loxozonus* (Snyder), B. perditio (Quoy et Gaimard) and B. busellatus Gomon (Labridae) off New Caledonia (Justine 2004).

Huffmanela plectropomi is typified by the possession of a conspicuous layer of filaments around egg (vs no filament) and narrower eggs (32 μm vs 35 μm). The eggs are from the mesentery of *Plectropomus leopardus* (Lacepède) (Serranidae) off New Caledonia (Justine 2011).

The eggs of *Huffmanela schouteni* have a thin envelope with protuberances (*vs* no envelope) and are narrower (27–30 μm *vs* 33–38 μm). This nematode was described from the serosa of the intestine, abdominal cavity and swimbladder of *Hirundichthys affinis* (Günther), *Cheilopogon cyanopterus* (Valenciennes) and *C. heterurus* (Rafinesque) (Exocoetidae) off Curaçao, Caribbean Sea, and off Italy, Ligurian Sea (Moravec and Campbell 1991, Moravec and Garibaldi 2003).

Off Japan, only three species of *Huffmanela* were found: *H. shikokuensis* Moravec Moravec, Koudela, Ogawa et Nagasawa, 1998, *H. japonica* and *H. hamo* sp. n. The species can be distinguished from each other by measurements of their eggs: *H. japonica* has smaller eggs, $58-69 \ \mu m \times 26-30 \ \mu m$ than *H. hamo*, $(66-77 \ \mu m \times 33-38 \ \mu m)$; whereas *H. shikokuensis* from *Stephanolepis cirrhifer* (Temminck et Shlegel) (Monacanthidae) has much larger eggs, $78-90 \ \mu m \times 36-45 \ \mu m$ (Moravec et al. 1998). The three species were found in the musculature, but *H. japonica* is from a mullid, *H. shikokuensis* from a monacanthid and *H. hamo* from a muraenesocid fish, respectively.

DISCUSSION

In this paper, we described clear eggs in addition to normal dark eggs. Descriptions of *Huffmanela* species are usually restricted to dark eggs, but a few studies have also reported 'aberrant eggs' (in *H. plectropomi* – see Justine 2011) and 'putatively non-viable eggs' (in *H. markgracei* – see Ruiz and Bullard 2013). In *H. markgracei*, non-viable eggs were smaller than normal eggs, but in *H. plectropomi* aberrant eggs were larger, as were clear eggs in the present species. This, of course, urges caution for the measurements of eggs, which are an important diagnostic feature; comparison between species should be done only with embryonated eggs.

Huffmanela species were found (Justine 2004, Ruiz and Bullard 2013) in a single family of sharks (Carcharhinidae) and various teleost families, which belong to eight orders: Perciformes with eight families (Centrarchidae, Labridae, Lethrinidae, Lutjanidae, Mullidae, Nemipteridae, Serranidae and Xiphiidae), Tetraodontiformes with three families (Balistidae, Monacanthidae and Tetraodontidae), and with a family each, Atheriniformes (Atherinopsidae), Beloniformes (Exocoetidae), Gadiformes (Gadidae), Pleuronectiformes (Cynoglossidae), Ophidiformes (Ophidiidae) and Scorpaeniformes (Sebastidae). Huffmanela hamo, with its host belonging to the family Muraenesocidae, is thus the first species from a fish of

the order Anguilliformes and from the superorder Elopomorpha.

The dagger-tooth pike conger, or 'hamo', is a traditional food in Japanese cuisine. It is a major commercial species, with annual catches reaching about 350 000 tonnes in recent years; countries reporting the largest landings are China and Taiwan (FAO 2013). Black spots in the flesh might of course affect the commercial quality of the fish. Eggs of *Huffmanela* were reported four times in dagger-tooth pike congers in ten years in Japan to one of us (T.I.). Photographs (not shown) indicate that the eggs

were similar in all cases, but eggs could not be measured and examined in the three first cases. It is likely that all four infections were conspecific because they all were observed in the same tissue (somatic musculature), in the same host species and collected in the same general geographic area.

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