



***Huffmanella* spp. (Nematoda, Trichosomoididae) parasites in coral reef fishes off New Caledonia, with descriptions of *H. balista* n. sp. and *H. longa* n. sp.**

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Abstract

Two new species of *Huffmanella* Moravec, 1987 are described from coral reef fishes caught off New Caledonia, South Pacific. *H. balista* n. sp. is described from the adults and eggs found in the swimbladder wall of a single *Abalistes stellatus* (Balistidae). The male, 9.9 mm long, has characteristic spermatids and spermatozoa. The female has a characteristic cuticularised anterior oesophagus. The eggs ($70 \times 34 \mu\text{m}$) spontaneously hatch and release larvae, 260 μm long. *H. longa* n. sp. is described from a single female and from eggs collected in the mesentery, swimbladder and body walls of three *Gymnocranius grandoculis* (Lethrinidae). The female, although immature, is the longest (20.7 mm) of all species of the genus. The eggs ($66 \times 27 \mu\text{m}$) have superficial filaments, and are differentiated from those of *H. filamentosa* Justine, 2004 from the same host, by their greater measurements. Eggs of *Huffmanella mexicana* Moravec et Fajer-Avila, 2000, are redescribed for comparison. The host of *Huffmanella* sp. of Justine, 2004, is identified as *Pentapodus aureofasciatus*. New observations on *H. ossicola* Justine, 2004 are reported, including new hosts (*Bodianus busellatus* and *B. perditio*), comparative measurements of eggs in various hosts and organs, description of larvae, 228 μm in length, and X-ray observations of the skeleton of an infected fish.

Key-words: Nematoda, Trichosomoididae, Huffmanelinae, *Huffmanella balista* n. sp., *Huffmanella longa* n. sp., *Huffmanella branchialis* Justine, 2004, *Huffmanella ossicola* Justine, 2004, *Huffmanella mexicana* Moravec et Fajer-Avila, 2000, New Caledonia, fish, *Abalistes stellatus*, *Gymnocranius grandoculis*, *Bodianus perditio*

Résumé

Deux nouvelles espèces d'*Huffmanella* Moravec, 1987 sont décrites de poissons des récifs coralliens de Nouvelle-Calédonie, Pacifique Sud. *H. balista* n. sp. est décrit à partir d'adultes et d'œufs trouvés dans la paroi de la vessie gazeuse d'un unique *Abalistes stellatus* (Balistidae). Le mâle, long de 9.9 mm, a des spermatides et spermatozoïdes caractéristiques. La femelle a un oesophage cuticularisé caractéristique. Les œufs ($70 \times 34 \mu\text{m}$) éclosent spontanément et libèrent des larves longues de 260 μm . *H. longa* n. sp. est décrit d'une unique femelle et d'œufs récoltés dans le mésentère et la paroi de la vessie gazeuse et du corps chez trois *Gymnocranius grandoculis* (Lethrinidae). La femelle, quoique immature, est la plus longue (20.7 mm) de toutes les espèces du genre. Les œufs ($66 \times 27 \mu\text{m}$) ont des filaments superficiels, et sont différenciés de ceux d'*H. filamentosa* Justine, 2004 du même hôte, par leurs plus grandes dimensions. Les œufs d'*Huffmanella mexicana* Moravec et Fajer-Avila, 2000 sont redécrits pour comparaison. L'hôte d'*Huffmanella* sp. de Justine, 2004 est identifié comme *Pentapodus aureofasciatus*. Des nouvelles observations sur *H. ossicola* Justine, 2004 sont rapportées, y compris des nouveaux hôtes (*Bodianus busellatus* et *B. perditio*), des mesures comparatives des œufs dans différents hôtes et organes, la description de la larve, longue de 228 μm , et des observations aux rayons X du squelette d'un poisson infecté.

Introduction

Nematodes of the genus *Huffmanella* Moravec, 1987 are extremely thin tissue-dwelling fish parasites. Prominent accumulations of their dark brown egg are often noticed by fishmongers, because they reduce the commercial value of fish, and are sent to scientists. Unfortunately, eggs finish their maturation after being laid (Moravec, 2001) and the presence of dark brown eggs often means that the adults are dead and decomposed and thus unavailable for study. For this reason, most species have been described from eggs only.

Although a small genus with only eight species known at the beginning of this decade (Moravec 2001), *Huffmanella* has been recently the subject of more intensive study (Conboy & Speare 2002; Cox *et al.* 2004; Moravec *et al.* 2005; Justine 2004, 2005; MacLean *et al.* 2006; Carballo & Navone 2007). Thus, 14 nominal species (one from freshwater and 13 from marine fish) are now known, of which only four have been described from adults (Table 1).

TABLE 1. Egg size and characteristics, host, tissue and locality of *Huffmanella* spp. Species in alphabetical order, with new species at the end. New information in bold.

Species	Eggs size, shell, envelope	Adult	Host	Tissue	Locality
<i>H. banningi</i> Moravec, 1987	99–108 × 42–45, shell 3, envelope with minute canals, thus apparently spinose	unknown	<i>Cynoglossus browni</i> (Cynoglossidae)	muscles	Atlantic Ocean, Senegal and Congo
<i>H. branchialis</i> Justine, 2004	45–50 × 23–26, shell 2–3, thin envelope forming spindle-shaped balloon around egg	unknown	<i>Nemipterus furcosus</i> (Nemipteridae)	mucosa of gills	Pacific Ocean, New Caledonia
<i>H. canadensis</i> Moravec, Conboy & Speare, 2005	48–63 × 24–27, transverse ridges Conboy & Speare (2002): 50–55 × 21–27	described	<i>Sebastes</i> spp. (Sebastidae)	skin, fins	Pacific Ocean, British Columbia, Canada
<i>H. carcharini</i> (MacCallum, 1925) Moravec, 1987	90–105 × 42–54, shell 6–9, envelope visible at pole regions	unknown	<i>Carcharhinus melanopterus</i> , <i>C. plumbeus</i> (Carcharhinidae)	skins and mucosa of connective tissue of gill arches	Atlantic Ocean, USA
<i>H. filamentosa</i> Justine, 2004	48–53 × 25–30, shell 3, no envelope, long thin filaments at eggs extremities	unknown	<i>Gymnocranius grandoculis</i> (Lethrinidae)	mucosa of gills	Pacific Ocean, New Caledonia
<i>H. huffmani</i> Moravec, 1987	60–63 × 33–39, shell 4–6, envelope with minute spines	described	<i>Lepomis</i> spp., <i>Ambloplites rupestris</i> , <i>Micropterus salmoides</i> (Centrarchidae)	inner swim-bladder wall	Freshwater fish, San Marcos River, Texas, USA
<i>H. japonica</i> Moravec et al., 1998	58–69 × 26–30, shell 4–5, covered by thin smooth envelope	unknown	<i>Upeneus japonicus</i> (Mullidae)	muscles	Inland Sea of Japan
<i>H. lata</i> Justine, 2005	77–88 × 52–63, shell 6–8, spinose	unknown	<i>Carcharhinus amblyrhynchos</i> (Carcharhinidae)	skin	Pacific Ocean, New Caledonia
<i>H. mexicana</i> Moravec & Fajera-Avila, 2000	63–69 × 30–33, shell 4–5, thin envelope apparently absent at plugs 57.3±2.7 (48–63) × 29.7±2.2 (27–37) (This paper)	unknown	<i>Sphoeroides annulatus</i> (Tetraodontidae)	inner swim-bladder wall	Pacific Ocean, Mexico
<i>H. moraveci</i> Carballo & Navone, 2007	50–57 × 23–31, shell 3–5, thin envelope	described	<i>Odontesthes smitti</i> , <i>O. nigricans</i> (Atherinopsidae)	skin of fins, epithelium of operculum, mucosa of gills	Atlantic Ocean, Argentina

to be continued.

TABLE 1. (continued)

Species	Eggs size, shell, envelope	Adult	Host	Tissue	Locality
<i>H. ossicola</i> Justine, 2004	72–88 × 32–40, shell 8–10, numerous filaments on eggs surrounded by thin envelope	unknown	<i>Bodianus loxozonus</i> <i>B. perditio</i> <i>B. busellatus</i> (new record) (Labridae)	Within all bones, including gill arch bones	Pacific Ocean, New Caledonia
<i>H. paronai</i> Moravec & Garibaldi, 2000	48–51 × 21–24 (Moravec & Garibaldi, 2002) 43–50 × 19–23 (Justine, 2004), shell 3, thin smooth envelope	unknown	<i>Xiphias gladius</i> (Xiphiidae)	epidermis	Ligurian Sea, Italy
<i>H. schouteni</i> Moravec & Campbell, 1991	69–75 × 27–30, shell 3–5, thin envelope with protuberances	unknown	<i>Hirundichthys affinis</i> , <i>Cheilopogon cyanopterus</i> , <i>C. heterurus</i> (Exocoetidae) (Moravec & Garibaldi, 2004)	Serosa of intestine, abdominal cavity, swim-bladder	Caribbean Sea, Curaçao, and Ligurian Sea, Italy
<i>H. shikokuensis</i> Moravec et al., 1998	78–90 × 36–45, shell 3, thin envelope absent at plugs	unknown	<i>Stephanolepis cirrhifer</i> (Monacanthidae)	muscles	Inland Sea of Japan
<i>H. sp.</i> of Gállego, Riera & Portús, 1993	67–79 × 34–43	unknown	unknown (pseudoparasitism of man)	unknown	Mediterranean Sea, Spain
<i>H. sp.</i> of (Grabda & Ślósarczyk, 1981) Moravec, 2001	114 × 60, delicate transparent envelope	unknown	probably <i>Genypterus blacodes</i> (Ophidiidae)	muscles	Pacific Ocean, New Zealand, South Island
<i>H. sp.</i> of MacLean et al., 2006	73–86 × 39–47 (fresh) 70–79 × 34–41 (formalin)	unknown	<i>Carcharhinus plumbeus</i> (Carcharhinidae)	skin	Aquarium, originally from Atlantic Ocean, North Carolina, USA
<i>H. sp.</i> of Mendes et al., 2005	measurements not given, but “not coincident with the descriptions of other <i>Huffmanella</i> of the skin and muscle”	unknown	<i>Trisopterus luscus</i> (Gadidae)	muscles	Atlantic Ocean, Portugal
<i>H. sp.</i> of Justine, 2004	39–47 × 22–27, shell 3, no filament, no envelope seen	unknown	<i>Pentapodus aureofasciatus</i> (Nemipteridae) (New identification)	mucosa of gills	Pacific Ocean, New Caledonia
<i>H. balista</i> n. sp.	70 (63–78) × 34 (32–41) shell 5–6, no filament	described	<i>Abalistes stellatus</i> (Balistidae)	outer swim-bladder wall	Pacific Ocean, New Caledonia
<i>H. longa</i> n. sp.	63 (58–69) × 29 (26–34), shell 2–4, no envelope, long thin filaments at eggs extremities	female described	<i>Gymnocranius grandoculis</i> (Lethrinidae)	mesentery, mucosa of abdominal cavity, outer swim-bladder wall	Pacific Ocean, New Caledonia

In this paper, two additional species are described, including observations on adults. Additional observations are provided on *H. ossicola* Justine, 2004, previously described from New Caledonia, with the records of new hosts and a comparison of egg measurements in various hosts. Various experiments on egg hatching and a study of the influence of egg preparation on measurements are also included for several species.

Material and methods

Fish specimens were caught with hand-lines on board the R/V 'Coris' off Nouméa, New Caledonia. Live fish were kept in a container with seawater and immediately brought back to the laboratory. All fish were measured, weighed and photographed. A unique number (JNC) was assigned to each fish. The parasitological material was then assigned a corresponding JNC number linked to the respective fish host. Measurements of hosts are indicated as fork length, FL (in mm), and weight, W (in grams).

Material from *Abalistes stellatus*. Pieces of the swimbladder mucosa were observed under a binocular microscope and the tissue was lacerated with thin pincers to collect adults and eggs. Slides of eggs were prepared immediately (designated as 'fresh eggs'). Thorough observation allowed collection of several adults; these were immediately fixed in hot ethanol. About half of the swimbladder mucosa was then fixed in boiling 70% ethanol (and kept in the same fixative) or in hot 4% formalin (and kept in the same fixative); three weeks after collection, slides were prepared from both lots of materials (designated as 'ethanol-preserved eggs' and 'formalin-preserved eggs'). The other half was put in Petri dishes with saline (seawater diluted at $\frac{1}{4}$, i.e. ca. 0.9% NaCl) in the refrigerator (about 5°C) to observe hatching of eggs. After 14h, the dishes were observed under a binocular microscope. Living larvae seen were collected. A single additional adult was collected. The tissues were then fixed in hot ethanol. Three weeks later, slides were prepared from this material (designated as 'ethanol-preserved 24h eggs').

Material from *Gymnocranius grandoculis*. In fish JNC1726, slides were prepared from small parts of the mesentery, and the remainder was preserved in 70% ethanol. In the fishes JNC2166 and JNC2174, the dorsal wall of the abdominal cavity, especially the part surrounding the spinal column, was removed in pieces with pincers. The external fibrous mucosa of the swimbladder was easily separated from the rest of the organ; the tissue underneath and the internal side of the swimbladder showed no eggs. Pieces of the swimbladder or body wall mucosa were observed under a binocular microscope and the tissue was lacerated with pincers to collect adults and eggs. Slides were immediately prepared from eggs (designated as 'swimbladder fresh eggs' and 'body wall fresh eggs'). A single adult was recovered from the swimbladder of fish JNC2174. Most of the swimbladder and body wall mucosa was then fixed in boiling 70% ethanol (and kept in the same fixative). Three weeks after collection, slides were prepared with this material (designated as 'ethanol-preserved eggs'). In order to observe egg hatching, eggs were removed from the mucosa and placed in a cavity block in saline or seawater at room (23°C) temperature and subdued light; this was done separately for the swimbladder and the mucosa. The cavity blocks were observed daily for larvae. A few eggs were removed every day to observe mobility of larvae. Measurements were performed on eggs removed after 24h in saline (designated as '24h eggs'). The experiment ended after two weeks.

Material from *Bodianus* spp. The infection was detected by observation of dark-brown or black eggs within the gill archbones. The fillets were then removed, and the spinal cord was cut in pieces to detect eggs. The skull was freed of surrounding flesh and was examined for black areas.

Methods. For the preparation of microscope slides, a fragment of tissue was placed on a microscope slide and quickly lacerated with thin pincers; a drop of lactophenol was added and covered with a cover slip; the slide was heated on a flame for a few seconds.

Adult nematodes and tissues with eggs were examined in lactophenol with an Olympus BH2 microscope equipped with Nomarski interference contrast and drawn with a camera lucida. For egg measurements, the extremities of eggs were schematically drawn; for each measurement, two slides from two different egg samples taken from different parts of the infected organ were prepared, and measurements from the two slides were pooled; all egg length measurements include plugs. Measurements were made from the pencil drawings with the help of a custom-made transparent rule, previously calibrated with a scaled microscope slide. All measurements are in micrometers, given as mean \pm standard deviation, with minimum-maximum in parentheses. Pencil drawings were redrawn on a computer using Adobe Illustrator.

X-ray observations were made on *Bodianus perditio* from which all the flesh had been removed, with the spinal cord flat on the observation glass. X-rays photographs were taken on an Eidos 3000 Primax apparatus operated at 45 kV with a focal distance of 1 m. Digital photographs were edited with Adobe Photoshop.

Abbreviations. MNHN, Muséum National d'Histoire Naturelle, Paris, France; HCIP, Helminthological Collection of the Institute of Parasitology, Biology Centre, Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic; BMNH, Natural History Museum, London; USNPC, United States National Parasite Collection, Beltsville, USA; SAMA, South Australian Museum, Adelaide, Australia.

Results

Family Trichosomoididae Hall

Subfamily Huffmanelinae Moravec, 2001

Genus *Huffmanella* Moravec, 1987

Huffmanella balista n. sp.

(Figs 1–3)

Type-host: *Abalistes stellatus* Anonymous (ex Commerson) (Balistidae). Note that *A. stellaris* (Bloch & Schneider) is a junior synonym (Matsuura & Yoshino, 2004).

Host: single infected fish, JNC2163, 02.v.2007, near Récif Toombo, off Nouméa, New Caledonia, 22°32'S, 166°28'E, FL 430, W 1500.

Type-locality: Lagoon off Nouméa, New Caledonia.

Material examined: One complete male (holotype) and several fragments of immature males (paratypes); one incomplete immature female (allotype); laid eggs.

Deposition of specimens: Holotype, allotype and paratypes, MNHN, JNC2163; vouchers of eggs, MNHN, JNC2163; HCIP, N-880; BMNH, 2007.7.12.1; USNPC, 99977; SAMA, AHC 34775.

Site of infection: external mucosa of swimbladder.

Etymology: Latinization of French *baliste*, common name for the host; used as a name in apposition.

Male [mainly from holotype, the single complete specimen]. Body length, holotype 9870, maximum width 30; width of cephalic end 10, of posterior end 17. Two lateral bacillary bands 8–10 wide. Length of entire oesophagus 3890 (39% of body length), length of muscular oesophagus 366, length of posterior part 5980 (in a paratype 4775). Nerve ring not seen. Length of stichosome 3524; stichocytes approximately 25 in number; length of stichocytes 80–130 in middle region of stichosome; terminal stichocyte 160 in length. Single testis reaching anteriorly nearly to oesophago-intestinal junction. Testis hologonic, spermatids and spermatozoa interspersed; spermatids, oval, 23×11 , with oblong nucleus; spermatozoa, 21×6 , more elongate with dense body within cytoplasm, in two parallel lines. Spicule and spicular sheath absent. Caudal end slightly narrowed, rounded; no papillae seen.

Female [from allotype, single female specimen, probably immature, tail lacking]. Body length (incomplete) 4850, maximum width 56, width of cephalic end 16. Length of entire oesophagus 1980, of muscular oesophagus 438, of posterior part (broken) 2870. Muscular oesophagus with anterior cuticularised part, 203 in length with very regular diameter 8–9, and posterior part, not cuticularised, 235 in length. Region of vulva damaged, not illustrated; no vulvar appendage. Immature eggs in uterus, oblong, embryonated with several cells visible, without shell but with regular hyaline peripheral layer, $41\text{--}44 \times 17\text{--}21$.

L1 larvae. Spontaneously hatched larvae, length 260 (245–295, $n = 4$), maximum width 6.5.

Eggs in tissue. Mature eggs yellow-brown to dark-brown. Eggs elongate, with protruding plugs. Size of eggs including polar plug 70.5 ± 2.9 (63–78) \times 34.5 ± 1.5 (32–41) ($n = 132$); egg wall 5–6. Eggs prepared using various methods, see Table 2. Fully developed larvae in most eggs, body width 6–8. Superficial enve-

lopes of eggs often inconspicuous; when visible, envelope thin and unornamented; no filaments. Surface of eggs ornamented with inconspicuous longitudinal ridges.

TABLE 2. Egg measurements of *Huffmanella balista*, JNC2163, from swimbladder.

Methods and characteristics of eggs	Length	Width	n
Dark brown 'fresh eggs'	70.5 ± 2.9 (63–78)	34.5 ± 1.5 (32–41)	132
Dark brown 'ethanol-preserved eggs'	71.3 ± 3.2 (64–78)	34.8 ± 1.6 (31–39)	86
Dark brown 'formalin-preserved eggs'	71.4 ± 2.3 (66–77)	33.7 ± 1.2 (32–37)	76
Yellow 'fresh eggs'	71.2 ± 4.2 (63–81)	35.0 ± 1.7 (30–39)	48
Dark brown 'ethanol-preserved 24h eggs'	74.7 ± 2.2 (71–80)	34.9 ± 1.4 (32–38)	79

Eggs formed thick dark layer within external mucosa of swimbladder (Figure 1). Fibrous mucosa easily removed from rest of swimbladder; no eggs visible in deeper layer of swimbladder wall nor on internal surface of the organ. No other organ infected.

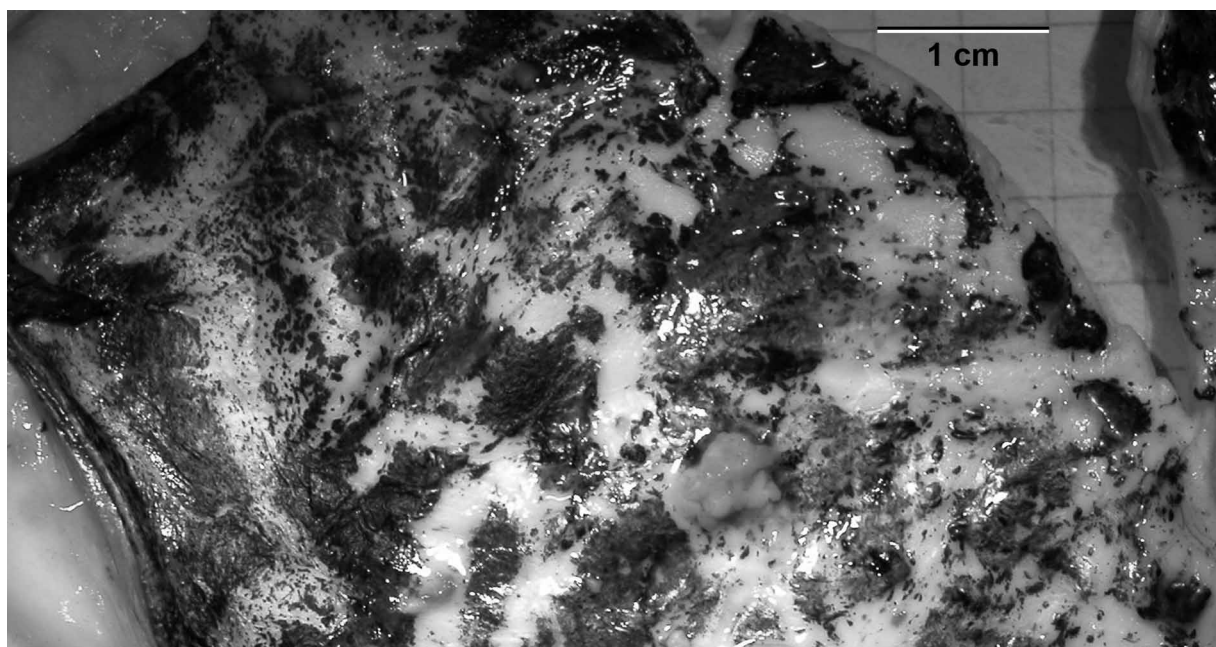
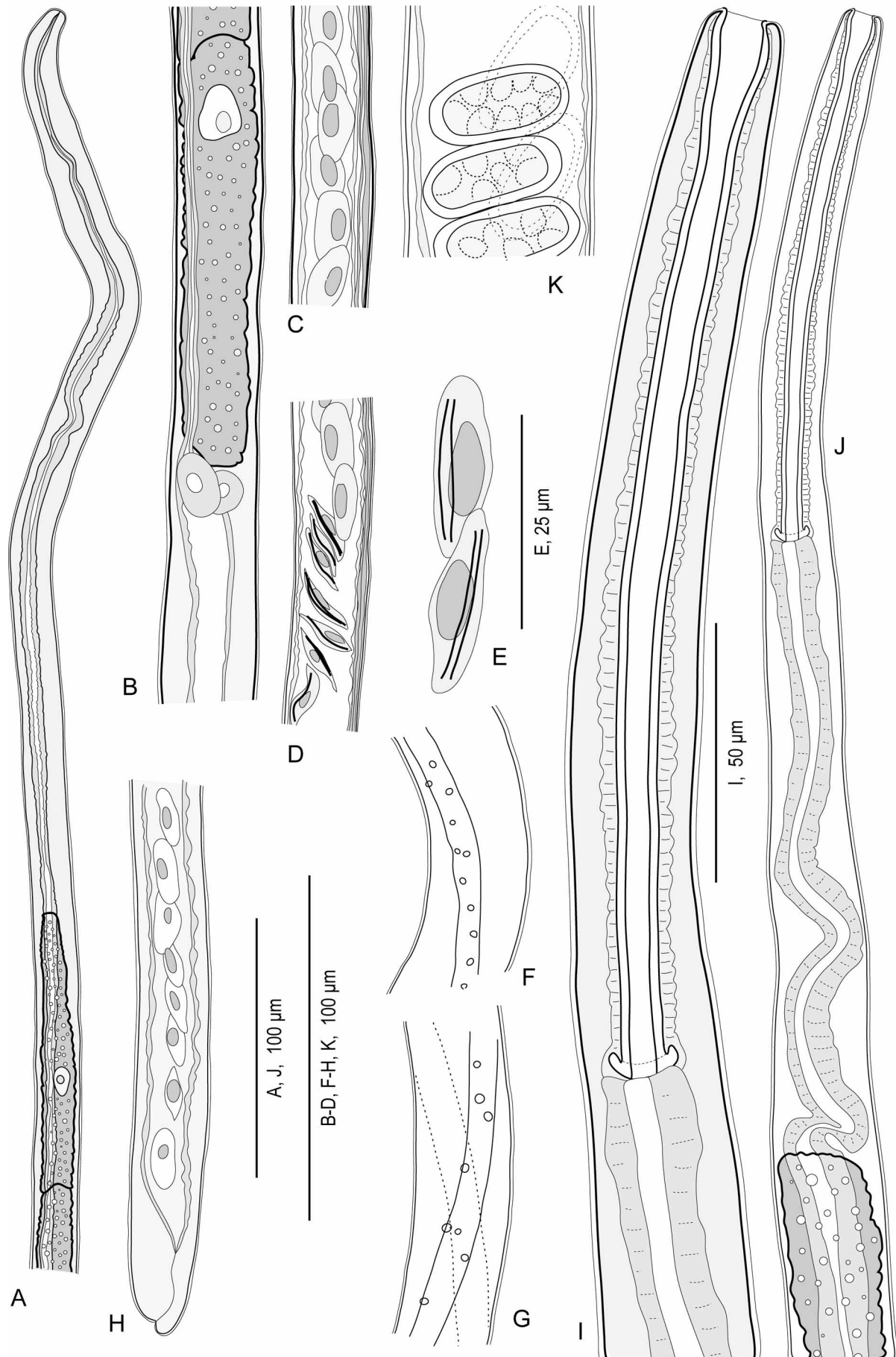


FIGURE 1. Outer surface of the swimbladder of *Abalistes stellatus* (fish JNC2163). Dark zones are accumulations of eggs of *Huffmanella balista* n. sp.

Eggs kept in seawater (ethanol-preserved '24h eggs') showed length measurements (Table 2) different from 'ethanol-preserved eggs' (74.7 vs 71.3, difference 3.6 µm, highly statistically different) width not different (34.8 vs 34.9). Difference due to plugs which protruded at each extremity, in contrast to fresh eggs in which plugs were very flat (all length measurements include the plugs). In many '24h eggs', larvae emerging from eggs were observed. Egg shell does not change when eggs mature, but plugs are modified.

Mobility of larvae and hatching. Larvae feebly motile in laid eggs. Hatching occurred in many eggs after 24h at room and refrigerator temperatures; hatched larvae were mobile.

FIGURE 2. Adults of *Huffmanella balista* n. sp. from *Abalistes stellatus*. **A–H.** Male. **A.** Anterior end. **B.** Oesophago-intestinal junction. **C.** Spermatid in testis, 1800 µm from posterior end. **D.** Spermatids and spermatozoa in testis, 1300 µm from posterior end. **E.** Spermatozoa. **F.** Bacillary band, 200 µm from posterior end. **G.** Bacillary bands, 700 µm from posterior end. **H.** Posterior end. **I–K.** Female. **I.** Cuticularised oesophagus. **J.** Anterior end. **K.** Immature eggs. **A–F,** holotype; **G,** paratype; **I–K,** allotype.



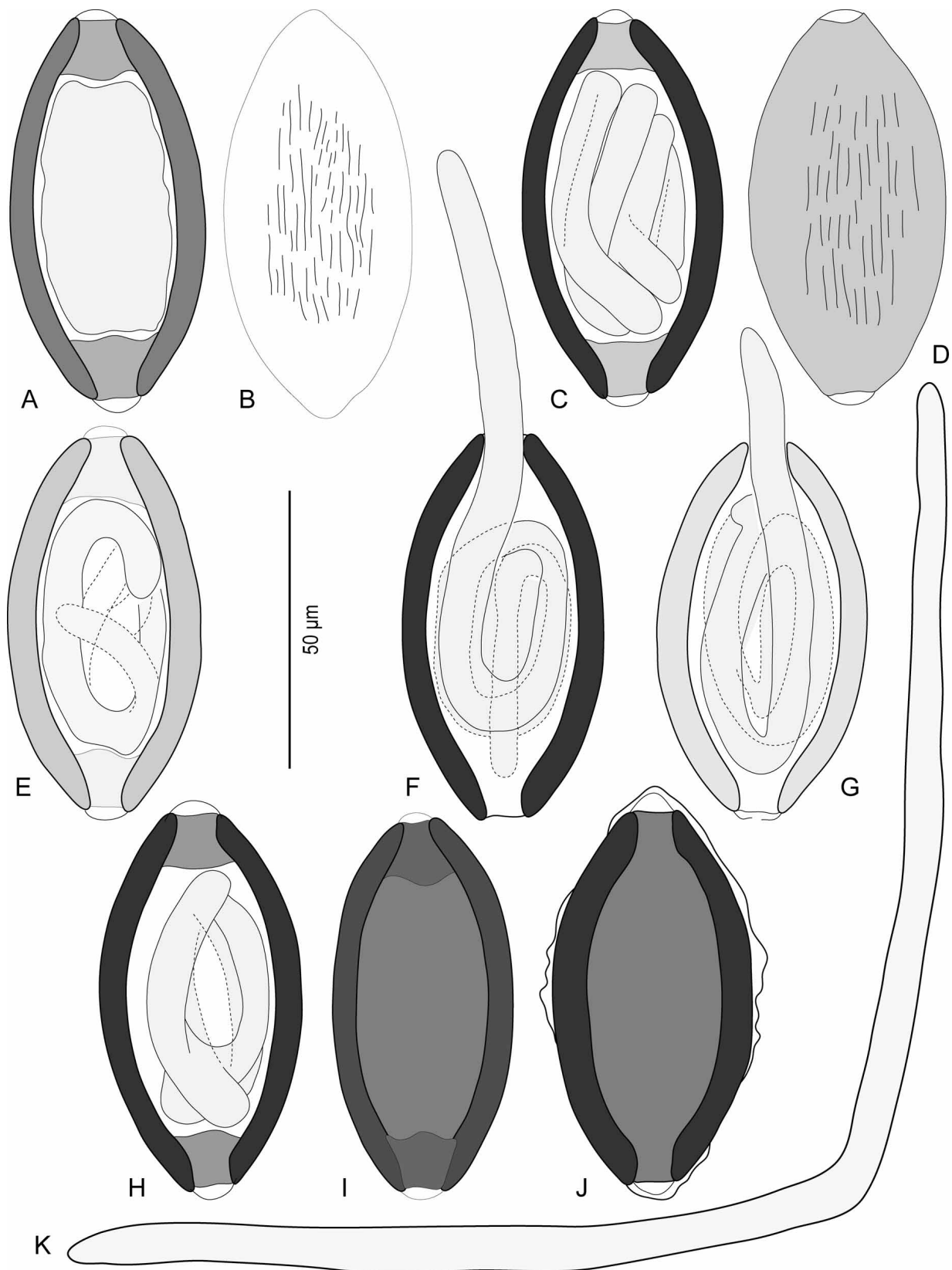


FIGURE 3. Eggs and larvae of *Huffmanella balista* n. sp. from *Abalistes stellatus*. **A–J.** Eggs. **A–B, C–D.** Optical section and surface views of same eggs. **F, G.** Eggs with hatching larvae (note that hatching occurs in both clear and dark eggs). **H.** Dark-brown egg. **I.** Black egg, larva present but not visible. **J.** Black egg, larva present but not visible, envelope visible. **K.** L1 larva, spontaneously hatched.

Differential diagnosis of adults. The male is characterised by its oesophagus length (3.9 mm) and its total length (9.8 mm), both of which longer than all other known species (1.4–1.9 and 4.7–5.1 in *H. huffmanii*, 1.3 and 3.4–3.9 in *H. canadensis* and 1.5–1.6 and 3.8–6.4 in *H. moravecii*). Its maximum width (30 µm) is similar to that of *H. huffmanii* (24–27) but smaller than that of *H. canadensis* (45–63) and *H. moravecii* (60–90).

The female is characterised by its cuticularised oesophagus; no similar structure has been described in other species. The measurement of total oesophagus length is within the range of the three other species, but since the specimen was immature it is possible that mature females are longer than in the three other known species.

Differential diagnosis of eggs. Moravec (2001) proposed a key to the species of *Huffmanella* with two major categories, species with long (>90 µm) eggs and species with shorter eggs. *H. balista* falls into the latter category.

Three species have egg measurements comparable to that of *H. balista* (Table 1): these are *H. mexicana*, *H. schouteni* and *H. longa*.

H. mexicana has eggs 63–69 × 30–33 in the original publication, but measurements taken with the same microscope and methods as for *H. balista* shows that the eggs of *H. balista* are longer (66 × 29 vs 57 × 30). In addition, the eggs of *H. mexicana* have a few superficial filaments (Figure 6), not seen in *H. balista*.

H. schouteni has eggs of similar size (69–75 vs 27–30) but the eggs have superficial protuberances, compared with longitudinal inconspicuous ridges in *H. balista*.

It is noteworthy that both these species have been reported from the same organ, the swimbladder, as *H. balista*. However, in both species (Moravec & Fajer-Avila 2000; Moravec & Garibaldi 2004), as well as in *H. huffmanii* (Moravec 2001) the site was the inner layer of the swimbladder, compared with the outer layer in the case of *H. balista*.

The comparison with *H. longa* is considered under the differential diagnosis of that species.

***Huffmanella longa* n. sp.**

(Figs 4–5)

Type-host: *Gymnocranius grandoculis* (Valenciennes) (Lethrinidae)

Hosts: Three infected hosts were found: JNC1726, 24.i.2006, FL 570, W 3500; JNC2166, 15.v.2007, FL 595, W 3800; JNC2174, 5.vi.2007, FL 365, W 1050. All from near Récif Toombo, off Nouméa, New Caledonia, 22°32'S, 166°28'E.

Type-locality: Lagoon off Nouméa, New Caledonia.

Material examined: a single incomplete female (holotype); laid eggs.

Deposition of material: Holotype, MNHN, JNC2174; vouchers of eggs, MNHN, JNC1726, JNC2166, JNC2174; vouchers of eggs from fish JNC2166, HCIP, N-881; BMNH, 2007.7.12.2; USNPC, 99978; SAMA, AHC 34776.

Site of infection: Eggs laid within mesentery, external mucosa of swimbladder, and mucosa of dorsal wall of abdominal body cavity.

Remarks on infection and prevalence: In fish JNC1726, the eggs formed a dark spot, about 1 cm in diameter, on the mesentery attached to the gonad; no other organ was infected. In fish JNC2166 and JNC2174, the eggs were found on the surface of two organs : the outer mucosa of the swimbladder and the mucosa of the dorsal wall of the abdominal cavity; no other organ was infected. Prevalence was 3/12 (25%).

Etymology: *longa*, long in Latin, for the length of body.

Comparative material examined: syntypes of *Huffmanella filamentosa* Justine, 2004, MNHN JNC892A. A slide with eggs kept in lactophenol for more than 3 years was examined using the same methods and microscope as the new species, and measurements were consistent with those of the original publication (Table 4).

Female [from holotype, immature]. Body length (incomplete) 20730, maximum width 32, width of cephalic end 11. Length of entire oesophagus 6720, length of muscular oesophagus 181, length of posterior part (broken) 14010. Muscular oesophagus without differentiation between cuticularised and non-cuticularised part. Length of stichosome 6510, approximately 35 stichocytes; long stichocytes 217 in length and 22 in width. Lateral bacillary bands 5–6 in width. Vulva situated slightly posterior to oesophago-intestinal junction, 40 from end of oesophagus; vulva not elevated, no vulvar appendage. No immature eggs seen in uterus.

Eggs in tissue. Mature eggs yellow or yellow-brown, with visible larvae; no dark-brown or black eggs found. Eggs elongate, with slightly protruding plugs. Size of eggs including polar plug, from swimbladder, JNC2166, 66.0 ± 2.6 (58–72) \times 26.6 ± 1.7 (23–32) ($n = 121$); egg wall 2–4. For measurements of eggs from other fish and organs, see Table 3. Fully developed larvae in most eggs. Surface of eggs apparently smooth. Superficial envelope of eggs: presence of filaments at both extremities; filaments often tightly packed at the level of plugs; when released, filaments form groups at both extremities and along length of eggs. Few unembryonated eggs, yellow-brown, showed strong longitudinal folds on egg surface; considered as aborted eggs. Aborted eggs, in which extremities do not show plugs, also showed longitudinal folds. Eggs after 24h did not change in length (Table 3).

TABLE 3. Egg measurements of *Huffmanella longa*, JNC2166, from swimbladder and body wall.

Methods and characteristics of eggs	Length	Width	n
Swimbladder ‘fresh eggs’	66.0 ± 2.6 (58–72)	26.6 ± 1.7 (23–32)	121
Body wall ‘fresh eggs’	65.4 ± 2.8 (58–72)	25.5 ± 1.7 (22–29)	58
Swimbladder ‘ethanol-preserved eggs’	64.8 ± 1.8 (62–69)	27.7 ± 1.9 (24–33)	44
Body wall ‘ethanol-preserved eggs’	64.3 ± 1.6 (61–68)	28.5 ± 2.4 (24–32)	36
24h saline body wall ‘fresh eggs’	65.3 ± 2.6 (60–71)	27.3 ± 2.1 (24–34)	97

L1 Larvae. Larvae in eggs, length 151 (140–168, $n = 11$), maximum width 4–5.

Mobility of larvae and hatching. Larvae in fresh laid eggs were immobile in JNC1726 and JNC2166; eggs kept in saline or seawater for four weeks (fish JNC2166) had immobile larvae and no hatching was detected. By contrast, in fish JNC2174, a few mobile larvae were seen within fresh eggs observed in saline; 24h later, the same slides kept in a humid chamber at room temperature showed 10–20% of eggs with motile larvae. Motile larvae were seen in eggs of various colours, i.e. darker eggs did not have more motile larvae than lighter eggs. These experiments show that differences existed between eggs from different individual fish. No hatching was observed.

Differential diagnosis of adults. *H. longa* is characterised by the longest body of all species in which the adult is known (incomplete body of immature specimen 20.7 mm vs adult females 4.9–7.5 in *H. huffmanii*, 7.7–8.2 in *H. canadensis* and 11.1 in *H. moravecii*).

Differential diagnosis of eggs. Eggs of *H. longa* are distinctly lighter (yellow to yellow-brown) than other species, in which darker eggs are dark-brown or black. Three species have egg measurements comparable to that of *H. longa* (Table 1): these are *H. mexicana*, *H. schouteni* and *H. balista*.

H. schouteni has eggs of similar size ($69\text{--}75 \times 27\text{--}30$ vs 66×27) but the eggs have superficial protuberances and no filaments as in *H. longa*.

H. mexicana has eggs with filaments as in *H. longa* but the eggs are shorter (57×30 , this publication).

H. balista has eggs slightly longer and wider (70×34), the eggs have no filaments, the wall is thicker (5–6 vs 2–4).

H. schouteni and *H. mexicana* have been reported from the inner layer of the swimbladder, in contrast to *H. balista* and *H. longa*, both from the outer layer.

H. filamentosa, another species from the same host, is known only from eggs. *H. longa* and *H. filamentosa* share a common morphological characteristic, the presence of thin filaments on the eggs, but have very different measurements (Table 4) and were not found in the same organ. For this reason, the author considered several hypotheses concerning these two infections.

TABLE 4. Measurements of eggs of *Huffmanella filamentosa* and *H. longa*, both from *Gymnocranius grandoculis*. See also histogram in Figure 5.

Fish, organ and methods	Length	Width	n
<i>Huffmanella filamentosa</i>			
JNC892, gills, original publication, 'fresh eggs', syntypes	49.8 ± 1.3 (48–53)	27.3 ± 1.5 (25–30)	54
JNC892, gills, old slide, 'fresh eggs', syntypes	48.9 ± 1.6 (45–52)	25.3 ± 1.3 (23–27)	20
<i>Huffmanella longa</i>			
JNC2166, swimbladder, 'fresh eggs'	66.0 ± 2.6 (58–72)	26.6 ± 1.7 (23–32)	121
JNC2166, body wall, 'fresh eggs'	65.4 ± 2.8 (58–72)	25.5 ± 1.7 (22–29)	58
JNC2174, Swimbladder 'fresh eggs'	64.7 ± 2.2 (61–73)	24.1 ± 1.3 (22–27)	75
JNC2174, Body wall 'fresh eggs'	66.1 ± 2.4 (62–73)	25.4 ± 1.3 (22–28)	52
JNC1726, mesentery, 'fresh eggs'	62.8 ± 2.4 (56–69)	28 ± 2.2 (23–34)	105

—Hypothesis 1. There is a single species of *Huffmanella* in *G. grandoculis*, which inhabits the gills, on the one hand, and the internal abdominal organs, in the other hand. Differences of eggs measurements can be attributed to different trophic sites, with much smaller eggs in the gills.

—Hypothesis 2. Similar to hypothesis 1 with a single species of *Huffmanella* in various organs, but the difference in egg size is related to the parasite life-cycle. Eggs from the gill mucosa are constantly dispersed in the external milieu through natural renewal of the gill mucosa, and thus have a short life-cycle; this is consistent with the presence of small eggs. In contrast, eggs from the internal organs are available for continuation of the life-cycle only at the death of the fish, and thus are longer-lasting eggs; this is consistent with the occurrence of large eggs.

—Hypothesis 3. Two different species are present, one in the gills, *H. filamentosa*, and one in the internal abdominal organs, *H. longa*. The species are differentiated by distinct eggs measurements, with lengths not overlapping (Figure 5).

Hypothesis 1 is contradicted by observations on *H. ossicola*, which show that egg measurements vary only subtly in various parts of the skeletal system (Justine 2004; this paper); however, it might be argued that the different parts of the skeletal system are not as different as between the gills and the internal abdominal organs.

Hypothesis 2 is supported by comparison with other trichinelloid nematodes in which two life cycles are possible, i.e. *Calodium hepaticum* (Bancroft) and *Paracapillaria philippinensis* (Chitwood, Velasquez & Salazar) (see Moravec 2001). However, the absence of observations on the adults of the species studied here precludes the choice of this hypothesis.

Hypothesis 3 would have been chosen without hesitation had the two species been found in two different fish species, because the eggs are extremely different. A possible argument against Hypothesis 3 is the presence in both species of thin filaments on the eggs, apparently a shared character. However, observation of similar thin filaments on eggs of *H. mexicana*, and on *H. ossicola*, probably means that such filaments are widespread in *Huffmanella* eggs and cannot be considered of great differential importance. This hypothesis, the presence of two distinct species, was finally preferred as the most probable, and the one which does not imply speculations on the life cycle which cannot be verified. A consequence is that *G. grandoculis* is the first fish in which two different species of *Huffmanella* have been found. *H. filamentosa* has not been found since its description, meaning that it is possibly rarer than *H. longa*, found in three fish.

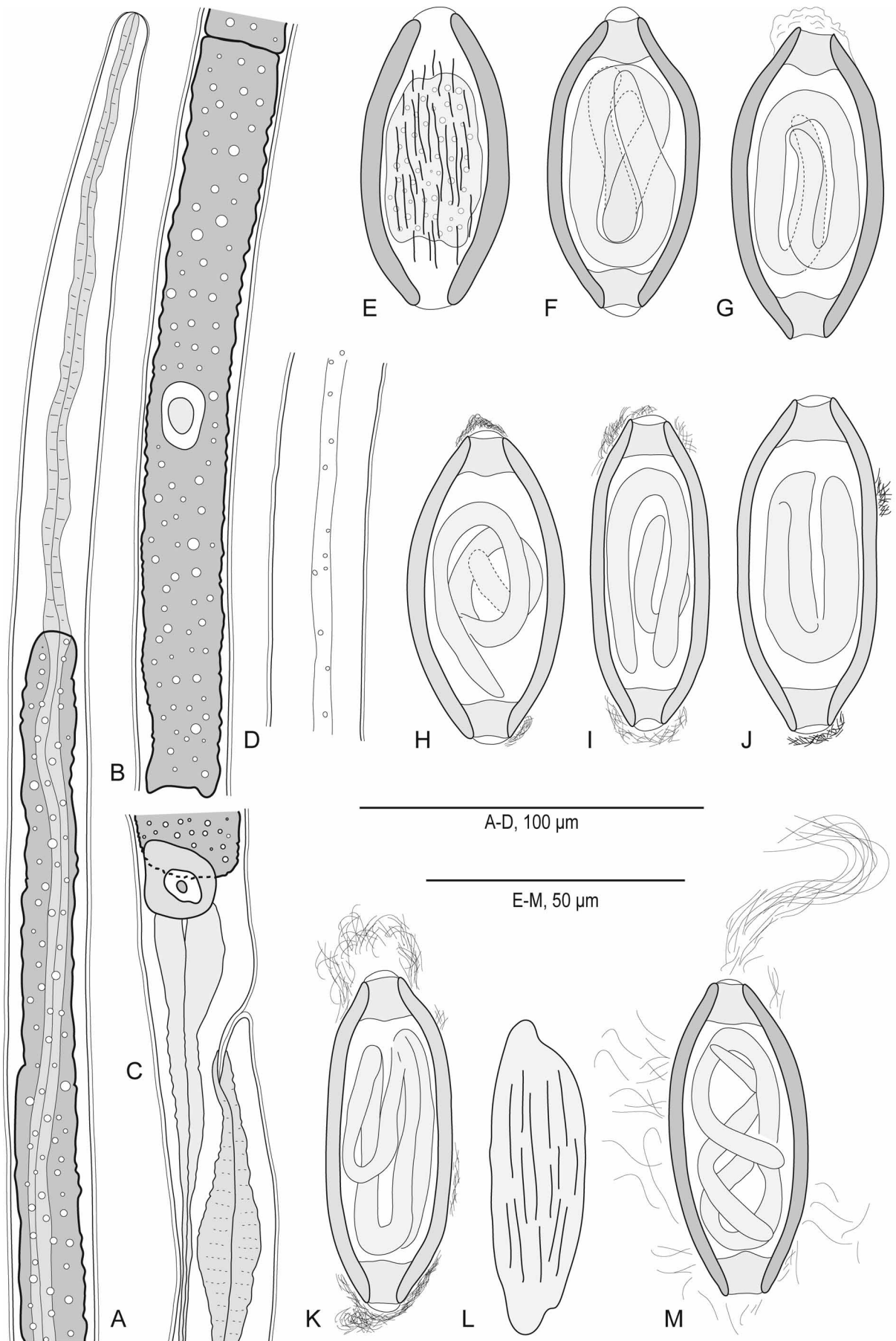


FIGURE 4. Adult female and eggs of *Huffmanella longa* n. sp. from *Gymnocranius grandoculis*. **A–D.** Holotype female. **A.** Anterior end. **B.** Long stichocyte. **C.** Vulva and oesophago-intestinal junction. **D.** Bacillary band. **E–M.** Eggs. **E–G.** Eggs from mesentery, fish JNC1726. **E.** Unembryonated egg with superficial longitudinal folds. **H–I.** Eggs from body wall, fish JNC2166. **J–M.** Eggs from outer layer of swimbladder, fish JNC2166. **L.** Shell of aborted egg with longitudinal folds.

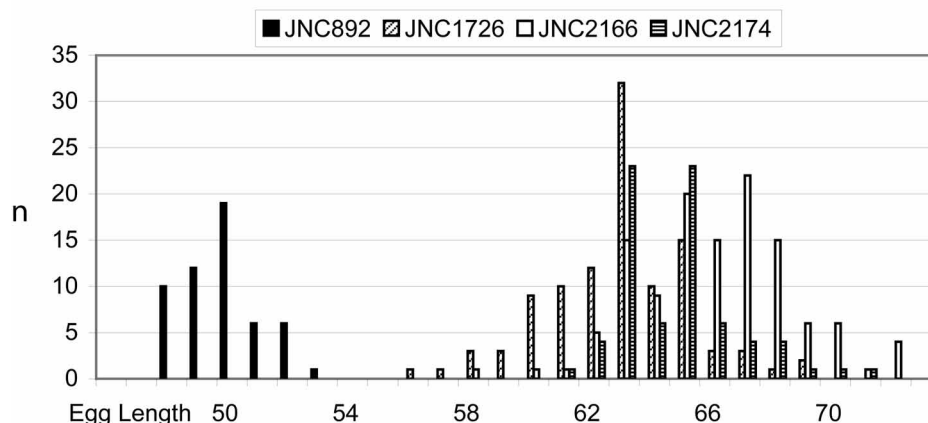


FIGURE 5. Histogram of egg length of the two *Huffmanella* species parasitic in *Gymnocranius grandoculis*. JNC892: eggs from the gills, syntypes of *H. filamentosa* Justine, 2004. JNC1726, JNC 2166, JNC2174, eggs of *H. lata* n. sp. JNC1726: eggs from the mesentery. JNC2166 and JNC2174: eggs from the swimbladder.

More than 15 specimens of the closely related species *Gymnocranius euanus* were examined and none had *Huffmanella* infection. Several species of *Lethrinus* were examined (lists in Justine 2007; Rascalou & Justine 2007) and none had *Huffmanella* infection. This suggests that *H. longa* is specific to *G. grandoculis*.

Huffmanella ossicola Justine, 2004 (Figs 6–7)

New host records and information on prevalence: *Bodianus loxozonus* (type-host): prevalence 4/11 (36 %). Details for additional infected specimens: JNC1087, Récif To, 22°29'30"S, 166°26'E, 28.iv.2004, FL 250, W 319; JNC1468, Récif Le Sournois (RLS), 22°31'S, 166°26'E, 8.iii.2005, FL 605, W 555; JNC1866, Récif Tabou, 15.vi.2006, FL 285, W 524; non infected specimens of similar sizes.

Bodianus busellatus Gomon, 2006: prevalence 1/3 (33%). New host record. Details for infected specimen: JNC1221, near Récif Toombo, 22°32'S, 166°28'E, 10.viii.2004, FL 270, W 371; non infected specimens of similar sizes.

Bodianus perditio (Quoy & Gaimard): prevalence 4/12 (25%). Host record briefly mentioned in Lim & Justine (2007). This contradicts the statement based on examination of two uninfected specimens (Justine, 2004), that *B. perditio* was not a host. Details for infected specimens: JNC1469, RLS, 8.iii.2005, FL435, W 1600; JNC1526, Récif Aboré, 22°21'S, 166°16'E, FL 450, W 1900; JNC1969, RLS, 3.x.2006, FL 375, W 1050; JNC 2171, Récif Toombo, 30.v.2007, FL 253, W 312; non infected specimens of similar sizes.

Only heavy infections with numerous dark eggs were detected by examination of gill arch bones; it is likely that light infections, or recent infections with uncoloured eggs were not detected, and prevalences indicated above are probably underestimates.

Thirteen other species of labrids were examined, including a single specimen of *Bodianus axillaris* (Bennett), and various numbers of *Cheilinus chlorourus* (Bloch), *C. trilobatus* Lacepède, *C. undulatus* Rüppell, *Choerodon fasciatus* (Günther), *C. graphicus* (De Vis), *Coris aygula* Lacepède, *Epibulus insidiator* (Pallas), *Oxycheilinus diagrammus* (Lacepède), *O. unifasciatus* (Streets), *Stethojulis strigiventer* (Bennett), *Thalassoma lutescens* (Lay et Bennett) and *T. nigrofasciatum* Randall, and none was found to harbour *H. ossicola* in the gill archbones. In addition, gills from more than 200 non-labrid fish species were examined and no *H. ossicola* was found.

Gomon (2006) revised *Bodianus*, recognized ten subgenera, and grouped *B. loxozonus*, *B. busellatus* and *B. perditio*, with six other species, within the subgenus *Diastodon* Bowdich; in contrast, *B. axillaris* was classified within the subgenus *Lepidaplois*. Results suggest that *H. ossicola* is specific to members of *Bodianus* (*Diastodon*).

Comparison of eggs between the different hosts. Table 5 shows that measurements were not exactly similar in each individual fish. In the type-host, *B. loxozonus*, length means ranged between 75.6–80.1. Measurements of egg lengths in the two other hosts, *B. busellatus* and *B. perditio*, fall within this range. One of the *B. perditio* specimens had egg widths greater than in other specimens.

TABLE 5. *Huffmanella ossicola*, eggs measurements in three hosts, same organ (the gills).

Species	Fish	Organs	Length	Width	n
<i>B. loxozonus</i>	JNC537*	gills	81.1 ± 3.1 (73–88)	37.4 ± 1.3 (35–40)	29
<i>B. loxozonus</i>	JNC1468	gills	75.6 ± 1.5 (73–79)	38.1 ± 1.1 (36–42)	36
<i>B. loxozonus</i>	JNC1866	gills	80.1 ± 3.9 (73–90)	40.1 ± 2.6 (35–44)	42
<i>B. busellatus</i>	JNC1221	gills	75.5 ± 1.2 (74–78)	36.0 ± 1.0 (34–38)	30
<i>B. perditio</i>	JNC1469	gills	77.0 ± 2.2 (72–83)	38.9 ± 2.1 (34–45)	106
<i>B. perditio</i>	JNC1526	gills	77.5 ± 1.8 (75–82)	42.8 ± 1.4 (39–45)	43
<i>B. perditio</i>	JNC2171	gills	80.1 ± 3.4 (71–87)	37.8 ± 1.5 (35–41)	33

* syntypes.

The morphology of eggs and especially aspects of the egg envelope with numerous thin filaments were similar in all hosts and organs. Length and width variations are interpreted as individual variation and it is concluded that it was the same species in the three hosts.

Comparison of egg measurements according to organ. In the original description (Justine 2004), it was shown that small differences could be detected between eggs from different organs. In *B. busellatus*, measurements of eggs from the gills and the spinal cord were compared to the measurements of the syntypes from *B. loxozonus* (Table 6). In *B. loxozonus*, the eggs were longer in the gills than in the spinal cord (81 vs 77 µm); in *B. busellatus*, in contrast, eggs were longer in the spinal cord (77 vs 75), and eggs were even shorter in the skull. However, all these variations were minor and the species can be recognized whatever the organ of origin, by its characteristic egg envelope and numerous filaments.

Hatching of larvae. Justine (2004) reported that no spontaneously hatched larva was found, even after incubation for four months in seawater. A similar experiment with *B. perditio* (JNC1526) with eggs from arch gill bones and with *B. loxozonus* (JNC1087) with eggs from arch gill bones and skull in saline gave similar negative results, i.e. no hatching was observed. Observation on eggs from several hosts also confirmed the original observation that larvae were immotile within eggs. In contrast, for fish JNC2171 (*Bodianus perditio*), slides prepared with tissue from the gills in saline or seawater showed numerous spontaneously hatched larvae, which showed feeble motility. Free L1 larvae were 228 in length (200–265, n = 6) and 10 in maximum width. Why these specimens behaved differently than the others is unresolved. It is unlikely that these eggs were older and more mature than in other specimens because the fish was one of the smallest examined for this species.

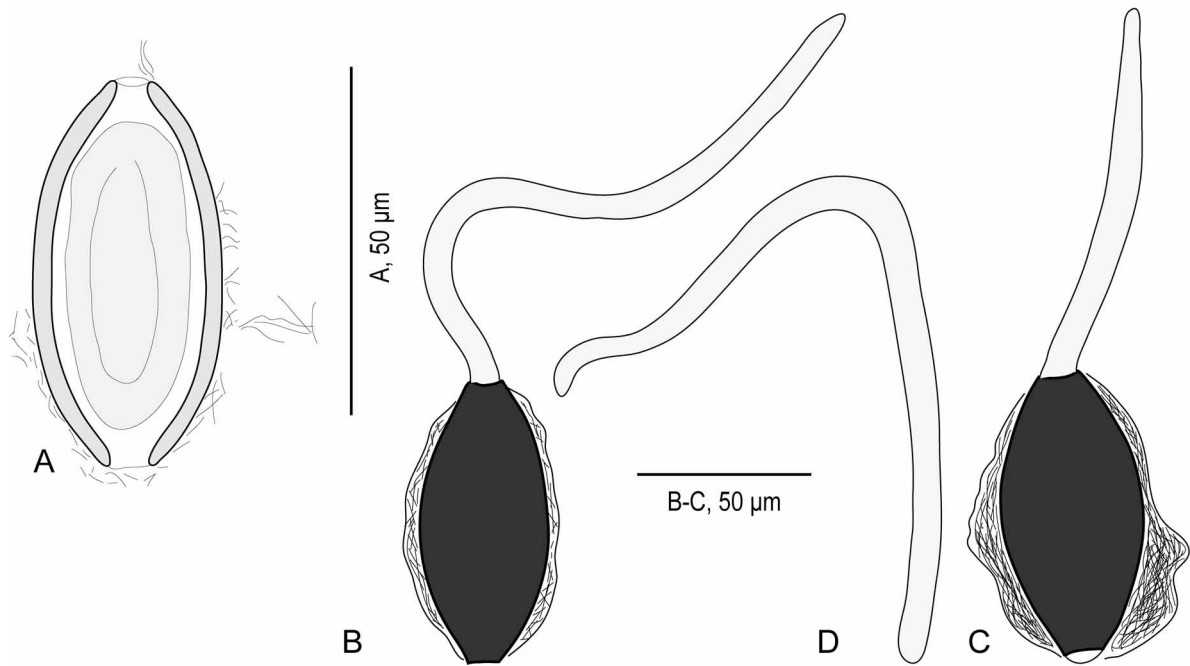


FIGURE 6. A. Egg of *Huffmanella mexicana* Moravec et Fajer-Avila, 2000, for comparison with other species. B–D. Hatching eggs and larvae of *Huffmanella ossicola* Justine, 2004 from the gills of *Bodianus perditio*.

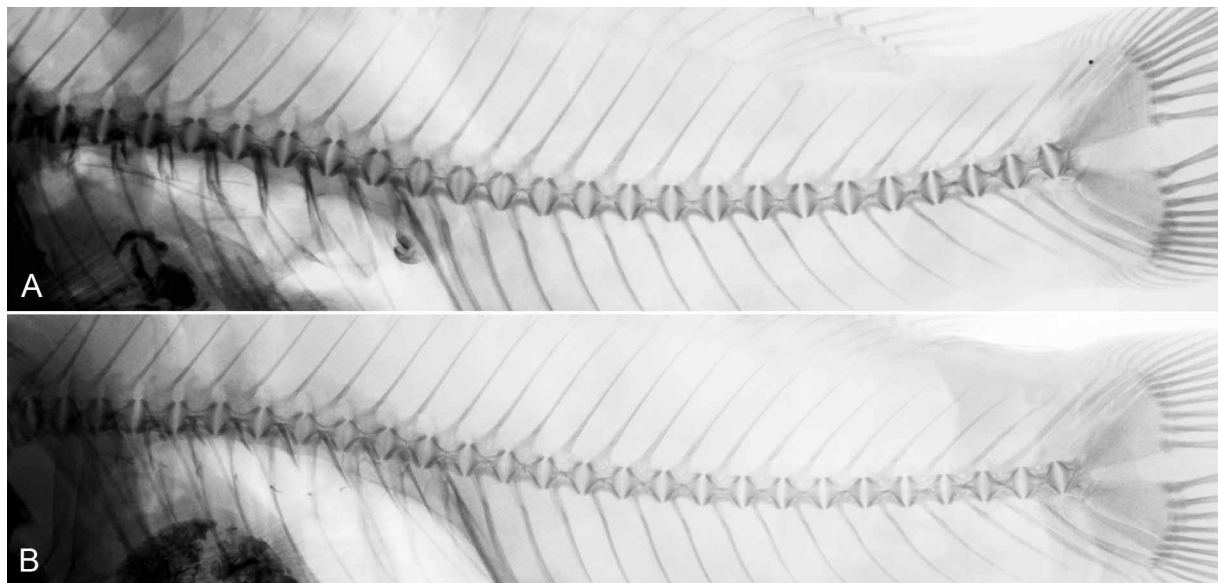


FIGURE 7. X-ray photographs of the spinal cord in two *Bodianus perditio*. A. Specimen not parasitized by *Huffmanella ossicola*. B. Specimen heavily parasitized by *Huffmanella ossicola*. Negative prints.

X-ray examination of the skeleton of infected hosts. Two *B. perditio* of similar size were compared, one heavily infected (JNC1469), and one not infected. X-ray observations were done with the same X-ray apparatus with the same settings. Figure 7 shows the spinal cord in the two fish. No difference could be detected in the aspect or width of the spinal cord. It is concluded that the infection by *H. ossicola* does not alter the structure of the bones of the spinal cord.

It is possible, however, that the bone infection produces pain and thus perturbs the swimming of the infected fish, thus enhancing the probability of the fish being caught by a predator, and thus facilitating completion of the life-cycle.

TABLE 6. *Huffmanella ossicola*, eggs measurements in *B. loxozonus* and *B. busellatus* in different organs.

Species	Fish	Organ	Length	Width	n
<i>B. loxozonus</i>	JNC537*	gills	81.1 ± 3.1 (73–88)	37.4 ± 1.3 (35–40)	29
<i>B. loxozonus</i>	JNC537*	spinal cord	77.3 ± 2.4 (72–82)	34.6 ± 0.8 (32–36)	30
<i>B. loxozonus</i>	JNC537*	all organs	79.1 ± 3.4 (72–88)	35.9 ± 1.7 (32–40)	58
<i>B. busellatus</i>	JNC1221	gills	75.5 ± 1.2 (74–78)	36.0 ± 1.0 (34–38)	30
<i>B. busellatus</i>	JNC1221	spinal cord	77.3 ± 2.0 (70–81)	38.8 ± 1.2 (37–42)	54
<i>B. busellatus</i>	JNC1221	skull	74.4 ± 2.3 (69–80)	37.7 ± 1.2 (35–40)	48
<i>B. busellatus</i>	JNC1221	all organs	75.9 ± 2.3 (69–81)	37.8 ± 1.6 (32–40)	132

* syntypes.

***Huffmanella mexicana* Moravec et Fajer-Avila, 2000**
(Fig. 6)

Material examined. Syntypes, eggs from a fragment of swimbladder of *Sphoeroides annulatus* Jenyns (Tetraodontidae). Original registration number in Helminthological Collection of the Institute of Parasitology, Ceské Budějovice: N-759; material deposited in the collection of MNHN, registration number JNA40.

Because the published measurements of *H. mexicana* eggs (Moravec & Fajer-Avila, 2000) were close to that of *H. balista*, the original material was requested for comparative observation. Eggs, measured with the same microscope and methods as for other species mentioned here, were 57.3 ± 2.7 (48–63) \times 29.7 ± 2.2 (27–37) (n = 83). These measurements were different from that of the original description (63–69 \times 30–33 including plugs). In addition, it was observed that a few thin filaments were present on the surface of most eggs (Figure 6A); this was not reported in the original description but perhaps can be attributed to the use of differential interference microscopy in the present study.

***Huffmanella* sp. of Justine, 2004, from *Pentapodus* sp.**

In a note added in proof, Justine (2004) briefly described *Huffmanella* sp. from *Pentapodus* sp. off New Caledonia.

Additional information: the host is *Pentapodus aureofasciatus* Russel. The parasite was not found in 3 additional fish examined, i.e. prevalence is 1/6 (17%).

Experiments on the validity of egg measurements

Because species differentiation of *Huffmanella* relies largely on eggs measurements, experiments were undertaken to ascertain whether these measurements were affected by various methods or conditions of observations. More than 1600 eggs were measured (length and width) for this study.

Egg colour and measurements. Sometimes eggs of various colours were mixed on a single slide; because eggs mature after deposition, an increase in egg size was possible and could lead to measurement errors.

For *H. balista*, a comparison was made between yellow and dark brown eggs, in ‘fresh’ eggs (Table 2). Only eggs containing larvae were used. Measurements showed that yellow eggs had very similar lengths (71.2 vs 70.5) and widths (35.0 vs 34.5) to dark brown eggs. This means that eggs of various colours can be used for measurements, at least in this species.

Effect of fixation. Slide preparation can be made immediately from fresh eggs, or the tissue with eggs can be preserved in fixative and used later for the preparation of slides. Whether these two different methods could lead to errors in measurement was investigated.

For *H. balista*, a comparison was made between ‘fresh eggs’, ‘ethanol-preserved eggs’ and ‘formalin-preserved eggs’ (Table 2). In each case, only dark brown eggs with a larva inside were used.

For *H. longa*, a comparison was made between ‘fresh eggs’ and ‘ethanol-preserved eggs’ (Table 3). Only eggs with a larva inside were used.

For both species, measurements were very similar, showing that eggs fixed in hot ethanol are unchanged in comparison to fresh material. In certain cases, t-tests showed that differences were statistically significant, but the difference was less than 1 μm , and thus of no consequence for systematics and the identification of species.

In addition, for *H. filamentosa*, examination of an old slide in which eggs had been kept for almost 3 years in lactophenol showed that no significant change was detected when eggs were re-measured (Table 4).

This means that eggs measurements are not affected by various methods of fixation and thus preserved material can be studied and expected to provide reliable egg measurements.

Remarks

Egg counts. Egg counts of *Huffmanella* can be estimated. For *H. balista* in *A. stellatus*, the surface area of an egg, considered similar to that of a rectangle $50 \times 100 \mu\text{m}$, is $5000 \mu\text{m}^2$, i.e. 1 mm^2 of infected tissues corresponds to 200 eggs (single egg layer). The heavily infected surface of the swimbladder was at least 50 cm^2 , thus corresponding to a single layer of 1 million eggs. Eggs are often laid in several layers, so the intensity of egg infection of *H. balista* can be estimated at several million eggs in a fish. For *H. ossicola*, the volume of an egg (including envelope), considered similar to that of a cylinder $45 \mu\text{m}$ in diameter and $80 \mu\text{m}$ in length, is c. $127,000 \mu\text{m}^3$, i.e. 1 mm^3 of infected tissue contains c. 7800 eggs. In a large *B. perditio*, the volume completely filled by eggs in the spinal cord could be a cylinder about 1 mm in width and 40 cm in length, i.e. 300 mm^3 , and infection of the eight gill arch bones and the skull can be of a similar volume, thus a total volume of 600 mm^3 . The intensity of egg infection of *H. ossicola* could thus be estimated at more than 4 million eggs in a fish. A comparable evaluation for *H. huffmanii* in its host was of up to one million eggs per fish (Cox *et al.* 2004).

Characters for the differentiation of *Huffmanella* spp. This study showed that the structure of the oesophagus can be very different in certain species, thus adding a possibly key character for the differentiation of taxa within *Huffmanella*. The measurements of larvae were different in the three species studied here (*H. balista* 260×6.5 , *H. longa* $151 \times 4\text{--}5$, *H. ossicola* 228×10), showing that this could be an additional systematic character for future description of *Huffmanella* species. Free L1 larvae described previously include *H. huffmanii* (225×9), *H. branchialis* (150×9), *H. filamentosa* (150×7) (Moravec 2001, Justine 2004). Spermatids and spermatozoa are described here in *H. balista* and are the first described for a species of *Huffmanella*. Interestingly, their shape is characteristic and different from others known in the Capillariinae (see Justine 1988), and in the few Trichinellida from which they are known. They could represent additional characters for systematics, as has been the case for certain other nematodes (references in Justine & Jamieson 2000; Justine 2002). However, the paucity of characters available from examination of *Huffmanella* adults, which have undifferentiated caudal extremities, enhances the interest of a detailed description of eggs, even when adults are known, because eggs provide a variety of morphological characters, in contrast to the adults.

Biology. Moravec & Garibaldi (2000) remarked that those *Huffmanella* species which inhabit the epidermis or gill epithelium, and thus can be released via the natural turnover of the superficial tissues, were parasites of relatively large fish, and that, in contrast, *Huffmanella* species parasitizing internal organs, for which

the eggs will be available for the continuation of the life cycle only after the death of the host, were parasites of small fish. Although true for several species, this generalisation is partially contradicted by the present study. *G. grandoculis* has both *H. filamentosa* (a gill parasite released by turnover) and *H. longa*, an internal parasite. The hosts of *H. balista* (*Abalistes stellatus*), of *H. longa* (*Gymnocranius grandoculis*) and of *H. ossicola* (*Bodianus perditio*) are relatively large fish (respectively 50, 60 and 80 cm in maximum length; Laboute & Grandperrin 2000, Randall 2005). In the reef environment, the most probable predators of these large fish are sharks. It might be that passage through the shark intestine is an important trigger for the hatching and maturation of *Huffmanella* eggs.

Differences observed for hatching of eggs were often puzzling. Eggs of *H. balista* released larvae within 24h, *H. longa* eggs never produced free larvae and *H. ossicola* exhibited different behaviours in different individual hosts. Intra-specific differences overlapped inter-specific differences.

The present results show that a single species of fish (here *G. grandoculis*) can harbour two different species of *Huffmanella* (*H. filamentosa* and *H. longa*) in different organs. New records of *H. ossicola* show that *Huffmanella* species have a relatively high degree of host specificity (here apparently limited to the subgenus *Diastodon* within *Bodianus*); results from other species suggest a higher degree of specificity. This suggests that the number of species of *Huffmanella* in the marine environment may be considerable. Our knowledge of *Huffmanella* species in marine fish is probably very limited in comparison to the number of species which really exist, thus precluding generalization. However, Table 1 shows that the elasmobranch fish known to be infected with *Huffmanella* all belong to the Carcharhinidae; marine teleosts belong to 14 families (Atherinopsidae, Balistidae, Cynoglossidae, Exocoetidae, Gadidae, Labridae, Lethrinidae, Monacanthidae, Mullidae, Nemipteridae, Ophidiidae, Sebastidae, Tetraodontidae, and Xiphiidae, see Table 1). The teleost families are widely spread in the classification, and, not surprisingly, the Perciformes are well represented. However, it is noteworthy that the Tetraodontiformes (Balistidae, Tetraodontidae and Monacanthidae) are overrepresented in comparison to their importance; fishes of this order are perhaps particularly susceptible to *Huffmanella* infections.

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