A NEW SPECIES OF TRICHOSOMOIDIDAE (NEMATODA) FROM SKIN OF RED SNAPPER, *LUTJANUS CAMPECHANUS* (PERCIFORMES: LUTJANIDAE), ON THE TEXAS–LOUISIANA SHELF, NORTHERN GULF OF MEXICO

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ABSTRACT: Eggs and larvae of *Huffmanela oleumimica* n. sp. infect red snapper, *Lutjanus campechanus* (Poey, 1860), were collected from the Texas–Louisiana Shelf (28°16′36.58″N, 93°03′51.08″W) and are herein described using light and scanning electron microscopy. Eggs in skin comprised fields (1–5 × 1–12 mm; 250 eggs/mm^2) of variously oriented eggs deposited in dense patches or in scribble-like tracks. Eggs had clear (larvae indistinct, principally vitelline material), amber (developing larvae present) or brown (fully developed larvae present; little, or no, vitelline material) shells and measured 46–54 μm ($\bar{x} = 50$; SD ± 1.6; n = 213) long, 23–33 (27 ± 1.4; 213) wide, 2–3 (3 ± 0.5; 213) in eggshell thickness, 18–25 (21 ± 1.1; 213) in vitelline mass width, and 36–42 (39 ± 1.1; 213) in vitelline mass length with protruding polar plugs 5–9 (7 ± 0.6; 213) long and 5–8 (6 ± 0.5; 213) wide. Fully developed larvae were 160–201 (176 ± 7.9) long and 7–8 (7 ± 0.5) wide, had transverse cuticular ridges, and were emerging from some eggs within and beneath epidermis. The new species differs from its congeners by having eggs <65 μm in total length and that have a brown eggshell when fully developed, an envelope throughout development, and irregularly-dispersed eggshell spines plus a larva >110 μm long with transverse cuticular ridges. The eggs lack a spindle-shaped envelope, polar filaments, and eggshell ridges. This is the first report of a species of *Huffmanela* from a snapper (Lutjanidae) or from the Gulf of Mexico. A table of egg and larval characteristics, hosts, and localities for *Huffmanela* spp. is provided.

Species of *Huffmanela* Moravec, 1987 comprise intracellular (Moravec et al., 1998) and intercellular (Moravec, 1987) nematodes that customarily are defined by the morphology of their eggs and larvae (Table I), with only 5 of 17 (29%) accepted species having descriptions that include details of the adult male or female (Huffman and Moravec, 1988; Moravec et al., 2005; Carballo and Navone, 2007; Justine, 2007). Adults and eggs infect extra-intestinal sites such as gill and skin epithelia, skeletal muscle, serosa and mesentery, swim bladder, and bone (Moravec, 1987, 2001; Moravec and Campbell, 1991; Moravec and Garibaldi, 2000; Moravec and Justine, 2010). Adults are seldom collected and difficult to extract from host tissue due to their small size and delicate nature, but also perhaps because the females die and deteriorate soon after depositing eggs (Moravec et al., 1998; Žďárská et al., 2001). It has been stated that few characters exist to discriminate eggs; however, numerous diagnostic features have been developed and employed to differentiate these species based on egg morphology (Table I). Developmental stages of these eggs also contain important taxonomic information and expand our knowledge of their life cycles and general biology (Moravec, 1987; Huffman and Moravec, 1988; Moravec et al., 1998; Moravec and Fajer-Avila, 2000; Moravec and Garibaldi, 2000, 2003; Justine, 2004, 2005, 2007, 2011; Carballo and Navone, 2007; Bullard et al., 2012).

In October 2011, a red snapper, *Lutjanus campechanus* (Poey, 1860), (Perciformes: Lutjanidae), with noticeable skin markings was observed and collected from the Gulf of Mexico off Texas. Herein we describe the markings, identify the etiological agent, describe a new species of *Huffmanela* Moravec, 1987, and thereby also provide the first record of a species of *Huffmanela* from a snapper (Lutjanidae) or from the Gulf of Mexico.

MATERIALS AND METHODS

The infected red snapper (male, 604 mm total length, 3.1 kg) was captured by a bottom long-line set on 15 October 2011 on the Texas–Louisiana Shelf (28°16′36.58″N, 93°03′51.08″W, approximately 200 km off Galveston Bay, Texas), northern Gulf of Mexico, aboard the *F/V Simple Man*. Upon sighting the infected areas of skin, its head, i.e., syncranium without gill arches (Fig. 1), was frozen and later (4 November 2011) delivered to Auburn University where it was subsequently (23 January 2012) thawed in 10% neutral buffered formalin for several days before parasitological examination. The thawed, formalin-fixed head was then carefully bisected along the supraoccipital plane, i.e., cleaved in half using a reciprocating saw, allowing for a clearer view of the luminal surfaces of the buccal cavity. The preopercle, metapterygoid, symplectic, and quadrate then were excised as a single unit with associated soft tissues using a scalpel, allowing for a clearer view of the skin surface covering the urohyal and branchiostegals. These tissue surfaces were examined with a stereomicroscope for the presence of eggs, larval nematodes, and adult nematodes. Foci of infected and non-infected skin were excised using scissors, scalpel, and hemostats and then photographed with the aid of a stereomicroscope or were wet-mounted on coverslipped glass slides (without coverslip pressure) and photographed using a compound light microscope (LM) equipped with differential interference contrast (DIC) optical components (Bullard et al., 2012). All nematode eggs and larvae were measured with an ocular micrometer while viewed using a ×100 oil immersion objective and DIC. Composite illustrations of those eggs and larvae were aided with a drawing tube and supplemented by digital photomicrographs. Skin patches containing eggs and eggs rinsed by gentle pipetting for scanning electron microscopy (SEM) were routinely processed by dehydration through a graded ethanol series, desiccated in hexamethyldisilazane for 1–3 hr followed by evaporation, and mounted on metal stubs using 2-sided sticky tape. Morphometrics are reported in micrometers (μm) as a range followed by the mean ± standard deviation (SD) and sample size (n) in parentheses.

Anatomical terms for the fish skull follow Gregory (1933). Fish nomenclature follows Eschmeyer (2012). The Gulf of Mexico is defined as per Felder et al. (2009): its boundaries span westward from Cabo Catoche, Quintana Roo, Mexico (21°33′00.00″N, 87°00′00.00″W) following a line extending northeast from Cabo Catoche to Cabo de San Antonio, Cuba (21°51′00.00″N, 84°57′00.00″W), further extending along the northern coast of Cuba to Punta Hicacos, Cuba (23°12′00.00″N, 81°08′00.00″W) and west of a line extending from Punta Hicacos to Key Largo, Florida (25°06′00.00″N, 80°26′00.00″W).

DESCRIPTION

Huffmanela oleumimica n. sp. Ruiz and Bullard
(Figs. 2–31)

Diagnosis (based on light microscopy of 213 eggs plus sputter-coated, infected skin, eggs, and larvae): Fields of eggs 1–5 × 1–12 mm, with irregularly-shaped margins, comprising approximately 250 eggs per mm^2, amber or brown in color (eggs with clear shells not visible grossly, see

TABLE I. Eggs, larvae, hosts, sites, and localities for *Huffmanela* spp. (ordered by egg size).

Species	Egg L × W*	Polar plug	Plug W	Shell surface	Shell layers	Shell thick	Envelope type	Larva L × W	Host family	Site	Locality	References†
Huffmanela branchialis	45–52 × 23–30	p	—	smooth	—	2–3	smooth	150 × 7	Nemipteridae	gill	SW Pacific	Justine (2004)
Huffmanela oleumimica	46–54 × 23–33	p	5–8	spinous	ol, id	2–3	smooth	160–201 × 7–8	Lutjanidae	skin	GoM	present study
Huffmanela paronai	48–51 × 21–24	p	6–7	smooth	od, il	3	smooth	—	Xiphiidae	skin	Med	Moravec and Garibaldi (2000)
Huffmanela filamentosa	48–53 × 25–30	p	—	smooth	—	3	absent	150 × 7	Lethrinidae	gill	SW Pacific	Justine (2004)
Huffmanela canadensis	48–63 × 24–27	p	6	ridged	od, il	2–3	absent	—	Scorpaenidae	skin	NE Pacific	Moravec et al. (2005)
Huffmanela moraveci	50–57 × 23–31	p	8–10	spinous	od, il	3–5	smooth	108 × —	Atherinopsidae	skin, gill	SW Atlantic	Carballo and Navone (2007)
Huffmanela japonica	58–69 × 26–30	p	6–9	protuberances	od, il	4–5	smooth	— × 9	Mulidae	me	NW Pacific	Moravec et al. (1998)
Huffmanela longa	58–72 × 23–32	pf	—	smooth	—	2–4	absent	140–168 × 4–5	Lethrinidae	sb, me	SW Pacific	Justine (2007)
Huffmanela huffmani	54–60 × 30–33	p	6–7	smooth	od, il	5–6	spinous	— × 6	Centrarchidae	sb	Texas, USA	Moravec (1987); Huffman and Moravec (1988)
Huffmanela mexicana	63–69 × 30–33	p	6	smooth	od, il	4–5	smooth, exposed plugs	— × 6	Tetraodontidae	sb	NE Pacific	Moravec and Fajer-Avila (2000)
Huffmanela balista	63–78 × 32–41	p	—	ridged	—	5–6	smooth	245–295 × 6–8	Balistidae	sb	SW Pacific	Justine (2007)
Huffmanela plectropomi	64–82 × 29–50	p	6	—	—	3–4	absent	—	Serranidae	m	SW Pacific	Justine (2011)
Huffmanela schouteni	69–75 × 27–33	p	6–9	smooth	od, il	3–5	protuberances	210 × 4–6	Exocoetidae	sb, me	Med, W Atlantic	Moravec and Garibaldi (2003); Moravec and Campbell (1991); Schouten et al. (1968)
Huffmanela ossicola	73–88 × 35–40	p	—	smooth	—	8–10	smooth	250 × —	Labridae	gill, bone	SW Pacific	Justine (2004)
Huffmanela carcharhini	75–110 × 42–63	np	8–13	smooth	ol, id	5–10	absent	188–273 × 7–13	Carcharhinidae	skin	NW Atlantic NC Pacific	MacCallum (1925; 1926); Moravec (1987); Bullard et al. (2012)
Huffmanela lata	77–88 × 52–63	p	—	smooth	—	6–8	absent	220–250 × 8	Carcharhinidae	skin	SE Pacific	Justine (2005)
Huffmanela shikokuensis	78–90 × 36–45	p	15–18	smooth	—	3	smooth, exposed polar plugs	— × 9	Monacanthidae	m	NW Pacific	Moravec et al. (1998)
Huffmanela banningi	99–108 × 42–50	p	9	smooth	—	3	spinous	— × 15	Cynoglossidae	m	NE Atlantic	van Banning (1980); Moravec (1987)

* Abbreviations: length (L), width (W), protruding (p), not specified in the published work (—), outer light eggshell layer (ol), inner dark eggshell layer (id), Gulf of Mexico (GoM), outer dark eggshell layer (od), inner light eggshell layer (il), Mediterranean (Med), mesentery (me), protruding with filament (pf), swim bladder (sb), muscle (m), not protruding (np), North Central (NC).
† Innominate species of *Huffmanela* were reported by Conboy and Speare (2002), Esteves et al. (2009), and Gállego et al. (1993).
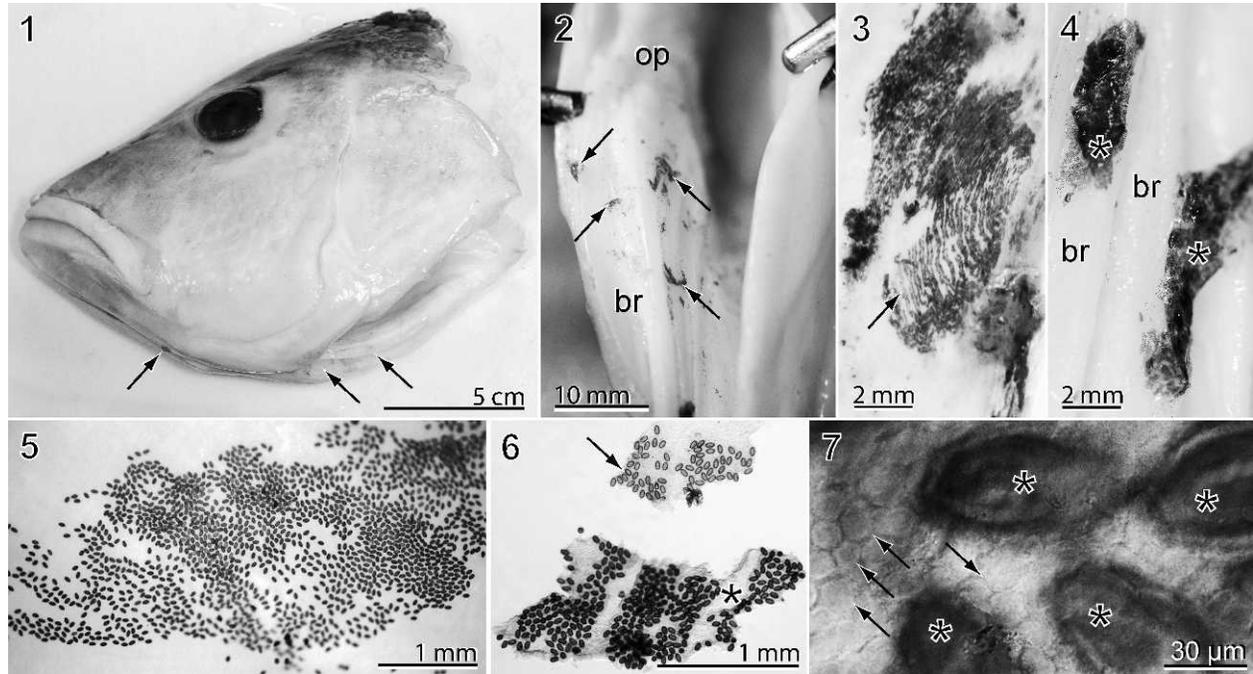
FIGURES 1–7. *Huffmanela oleumimica* n. sp. (Nematoda, Trichosomoididae, Huffmanelinae) from the skin of red snapper, *Lutjanus campechanus* (Poey, 1860), (Perciformes: Lutjanidae). Scale values beside each bar. (**1**) Head of infected red snapper showing locations (arrows) of eggs in skin. (**2**) Ventral view of masses of eggs (arrows) in skin of branchiostegals (br) and operculum (op). (**3**) Eggs in skin of buccal cavity (at level of opercle) showing scribble-like markings (arrow). (**4**) Higher magnification view of eggs from skin of branchiostegal rays (br) showing clumping of eggs (*) within probable sloughed epidermis and mucus. These masses of eggs became detached from the host by gentle irrigation of the skin surface. (**5**) Field of eggs in skin of buccal cavity. Note that the eggs are clearly visible beneath the epidermis covering them and that the eggs are not arranged in any particular orientation with respect to each other. (**6**) Sloughed epidermis enclosing amber eggs (arrow) and brown eggs (*). (**7**) Eggs (*) beneath a contiguous cobblestone-like layer of epidermal cells (arrows).

below), comprising scatters of hundreds of variously oriented eggs (Figs. 2, 4–6), with some fields including regions with eggs distributed in track-like arrays (Fig. 3), principally infecting skin of urohyal, branchiostegals (Fig. 2), and buccal cavity (Figs. 5, 6); covered by epidermis (Figs. 5–7, 23) or not (Figs. 24–26), easily separating–sloughing from fish's body as single sheet within host epidermis (Figs. 6, 23). Eggs 2–3 (3 ± 0.5; 213) in eggshell thickness (Figs. 8–13), elliptical, with envelope surrounding entire egg inclusive of polar plugs (Figs. 8–13, 27–30), lacking thin filaments, spinous (Figs. 14, 15). Markedly protruding polar plugs in more-developed eggs (Figs. 9, 10, 12, 13) with clear, amber, or brown shell depending on stage of development (Figs. 8–13); most possess intact polar plugs, but some lack plug and larva (Fig. 30). Some with probable host cells adhered to surface (Fig. 28), occasionally nearby indented dermal surface; dermal indentations match approximate length and width of eggs (Fig. 25). Probable teratological eggs with amber and brown shells rarely observed, but lack discernible larva. Eggshell obviously bilayered in eggs with amber and brown shell and less so in eggs with clear shell (Figs. 8–13); outer layer approximately 1–2 thick, typically light–translucent; inner layer approximately 1–2 thick, typically dark–optically dense. Eggshell spines best demonstrated in eggs with amber and brown shells (Figs. 14, 15), difficult to distinguish in clear-shelled eggs, irregularly distributed on eggshell surface, minute, each <1 in diameter, numbering approximately 100 per hemisphere of egg, appearing as stub-like projections with LM, beneath envelope and hence not evident in SEM micrographs, but perhaps causing contracted envelope of some specimens to show underlying impression of spines (Fig. 27). Eggs with clear shell (Figs. 8, 11) 47–53 (50 ± 1.5; 71) long, 26–32 (28 ± 1.4, 71) wide, 37–41 (39 ± 0.9; 71) in vitelline mass length, 18–24 (21 ± 1.3; 71) in vitelline mass width, 6–8 (6 ± 0.5; 71) polar plug length, 6–9 (7 ± 0.6; 71) polar plug width, lacking discernible larva. Vitelline mass indented at poles; outer eggshell layer seemingly thinner than inner eggshell layer. Eggs with amber shell (Figs. 9, 12) 46–53 (50 ± 1.6; 71) long, 25–31 (27 ± 1.1; 71) wide, 36–42 (40 ± 1.1; 71) in luminal length, 20–24 (21 ± 0.9; 71) in luminal width, 6–8 (7 ± 0.6; 71) polar plug length, 5–7 (6 ± 0.6; 71) polar plug width, containing

developing larva with cuticle. Less vitelline material (cf. clear eggs), lacking vitelline mass indentations at poles, with outer (translucent) and inner (dark) shell layers approximately equal in thickness. Eggs with brown shell (Figs. 10, 13–15) 46–54 (50 ± 1.6; 71) long, 23–33 (27 ± 1.4; 71) wide, 38–42 (40 ± 1.1; 71) in luminal length, 20–25 (21 ± 1.0; 71) in luminal width, 6–8 (6 ± 0.6; 71) polar plug length, 6–8 (6 ± 0.6; 71) polar plug width. Eggs with fully-developed larvae possess little, or no, vitelline material (cf. clear [Figs. 8, 11] and amber [Figs. 9, 12] eggs), with outer (translucent) and inner (dark) shell layers approximately equal in thickness.

Emerging larva (Figs. 16, 17, 20–22, 31) 160–201 (176 ± 7.9; 30) long, 7–8 (8 ± 0.5; 30) wide, having esophagus approximately 1 wide, with transverse cuticular ridges on body visible by SEM (Fig. 31) but indistinct with LM, emerging from egg anterior-end–first in all cases. Forcibly-hatched larvae subjected to coverslip pressure easily differentiated from emerging larvae by presenting as tightly balled specimens (having been fixed in that position before being forcibly extruded) (Fig. 18), frequently observed to be partly extruding by head and tail from both polar openings simultaneously (Fig. 19; cf. specimens without coverslip pressure, Figs. 16, 17, 20–22).

Taxonomic summary

Type host: *Lutjanus campechanus* (Poey, 1860), (Perciformes: Lutjanidae), red snapper.

Site of infection: Within and beneath epidermis of urohyal, branchiostegals, and buccal cavity.

Type locality: Texas–Louisiana Shelf (28°16′36.58″N, 93°03′51.08″W), approximately 200 km off Galveston Bay, Texas, northern Gulf of Mexico.

Specimens deposited: Syntypes United States National Parasite Collection (USNPC) No. 106453.

Etymology: The Latin specific epithet "*oleumimica*" is from "oleum" (oil) and "mimica" (imitative); intended to indicate that eggs of the new species superficially resemble oil droplets in skin.
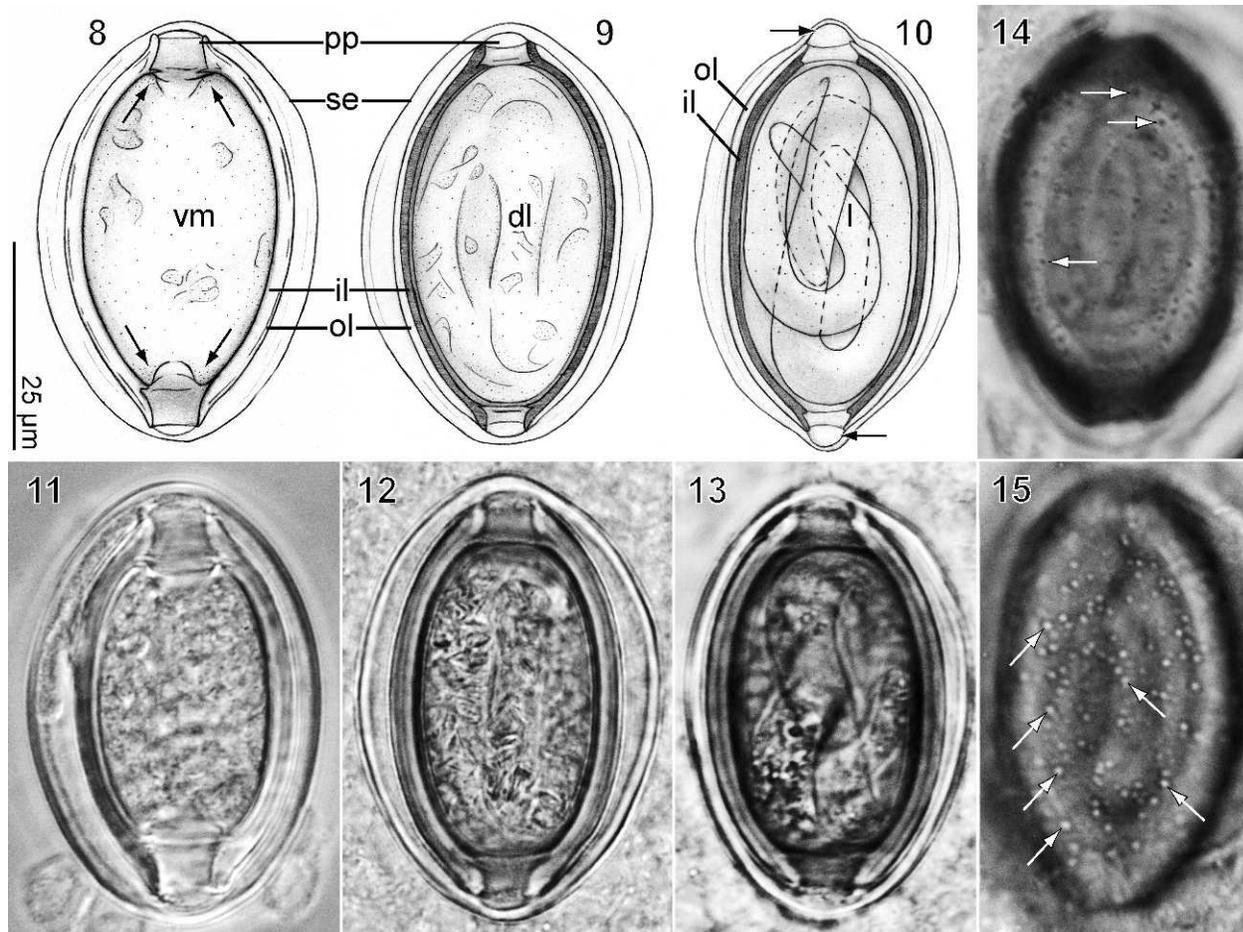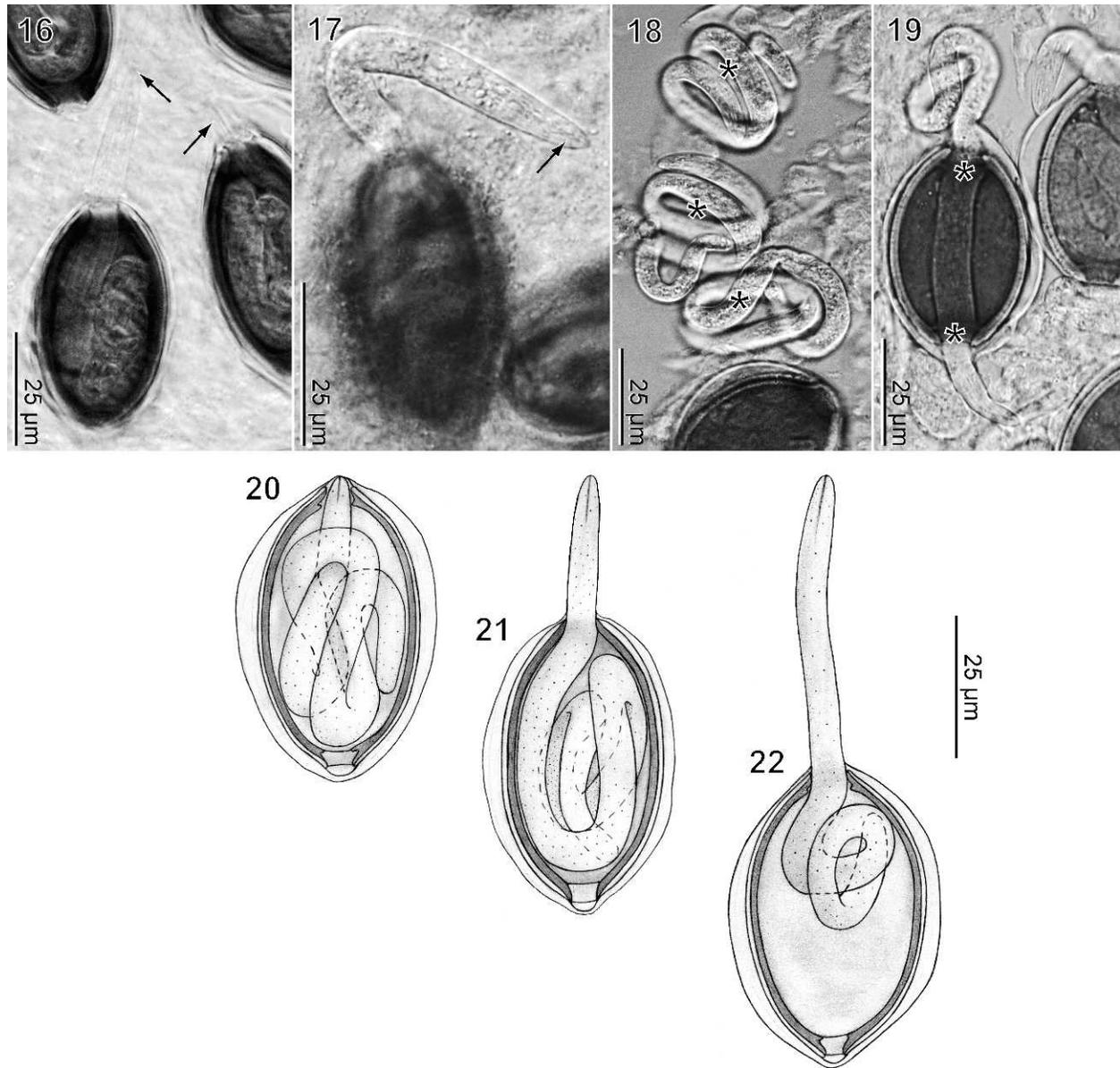
FIGURES 8–15. *Huffmanela oleumimica* n. sp. (Nematoda, Trichosomoididae, Huffmanelinae) from the skin of red snapper, *Lutjanus campechanus* (Poey, 1860), (Perciformes: Lutjanidae). Scale value beside bar represents all Figures. (**8**) Clear egg, showing polar plug (pp), superficial envelope (se), inner eggshell layer (il), outer eggshell layer (ol), vitelline mass (vm), and indentations of vitelline mass (arrows) at polar ends of egg proximal to polar plug; light micrograph of comparable egg below. Eggshell spines not illustrated. (**9**) Amber egg showing developing larva (dl). (**10**) Brown egg showing protruding polar plugs (arrows), translucent outer eggshell layer (ol), dark, optically dense inner eggshell layer (il), and fully-developed larva (l) that is highly folded within egg. Eggshell spines not illustrated. (**11**) Light micrograph of an egg with a clear shell (cf. Fig. 8). (**12**) Light micrograph of an egg with an amber shell (cf. Fig. 9). (**13**) Light micrograph of an egg with a brown shell (cf. Fig. 10). (**14**) Fully-developed egg with brown shell showing apical surface of eggshell in plane of focus and demonstrating unevenly dispersed, minute, stub-like eggshell spines (arrows). (**15**) Comparable view of another brown-shelled egg showing dense field of eggshell spines (arrows).

Remarks

Based on published information, common diagnostic features of the eggs and larvae of *Huffmanela* spp. are compared in Table I. All members of this genus have a larva that is immediately surrounded by an eggshell comprising 2 layers of differing optical densities, light and dark, which are most obvious in fully developed eggs. The egg envelope, which is a pliable, membranous sac-like structure, surrounds the eggshell of all species except *Huffmanela filamentosa* Justine, 2004, *Huffmanela canadensis* Moravec, Conboy, and Speare, 2005, *Huffmanela longa* Justine, 2007, *Huffmanela plectropomi* Justine, 2011, *Huffmanela carcharhini* (MacCallum, 1925) Moravec, 1987, and *Huffmanela lata* Justine, 2005. It covers the eggshell, inclusive of the polar plugs, in all species having an envelope except in *Huffmanela mexicana* Moravec and Fajer-Avila, 2000 and *Huffmanela shikokuensis* Moravec, Koudela, Ogawa, and Nagasawa, 1998, which have polar plugs that are "exposed," i.e., not enveloped. Justine (2004) documented that the envelope is vulnerable to artifactitious expanding and appears spindle-shaped after heating in lactophenol. Envelope ornamentations, i.e., spines and protuberances, have been reported for *Huffmanela huffmani* Moravec, 1987, *Huffmanela schouteni* Moravec and Campbell, 1991, and *Huffmanela banningi* Moravec, 1987, but all other species have a smooth-surfaced envelope, if present. The eggshell of *Huffmanela* spp. can be adorned by spines (*H. oleumimica* n. sp.,

Huffmanela moraveci Carballo and Navone, 2007, and *Huffmanela japonica* Moravec, Koudela, Ogawa, and Nagasawa, 1998) and ridges (transverse and slightly oblique in *H. canadensis*, longitudinal in *Huffmanela balista* Justine, 2007) or co-occur with filamentous structures (*H. filamentosa*, *Huffmanela ossicola* Justine, 2004, *H. longa*, and *H. plectropomi*). Taken together, these features have been variously referred to in the published literature; however, transmission electron microscopy (TEM) details of the eggshell, envelope, and their surfaces exist for only 1 species, *H. huffmani* (Žd'árská et al., 2001). Additional TEM and SEM studies could further enhance our understanding about homology of the various spines, protuberances, filaments, eggshell layers, and envelopes and thereby contribute to a more complete understanding about interrelationships of *Huffmanela* spp.

Moravec and Fajer-Avila's (2000) key for differentiating species of *Huffmanela* included (1) egg total length, width, and color, (2) transparent envelope presence–absence, spinous–aspinous, thickness, and covering–not covering polar plugs, (3) eggshell thickness, (4) polar plug length and width, (5) host species, and (6) tissue site of infection in host. Using that key and subsequent keys, eggs and larvae of *H. oleumimica* could not be identified as a named species. Ten species of *Huffmanela* have been named since 2000 (Table I), but a combination of that key's characters plus additional features reliably distinguishes the new species from all present
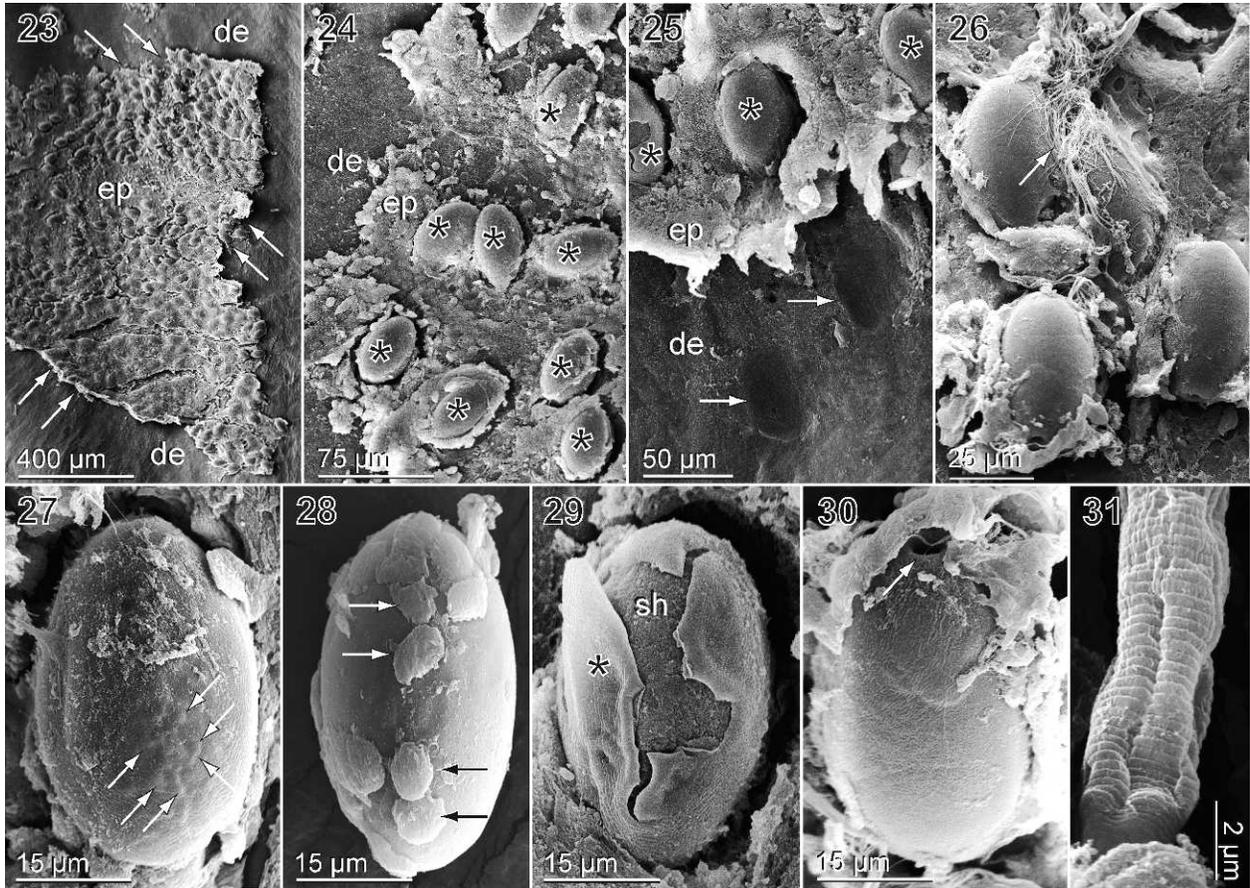
FIGURES 16–19. *Huffmanela oleumimica* n. sp. (Nematoda, Trichosomoididae, Huffmanelinae) from the skin of red snapper, *Lutjanus campechanus* (Poey, 1860), (Perciformes: Lutjanidae). Scale values beside each bar. (**16**) Hatching eggs showing anterior ends (arrows) of emerging nematode larvae projecting into surrounding epidermis. (**17**) Higher magnification view of comparable area of skin where a larva (arrow) is emerging from an egg. Note that nematode larvae emerge from one end of the egg, i.e., anterior-end–first. (**18**) Forcibly-extruded cluster of 3 nematode larvae (*), remaining tightly coiled as they appear within intact eggs (cf. Figure 10). (**19**) Forcibly-extruded larva emerging from both polar openings (*), with anterior end extensively curved in on itself and posterior end extruding in opposite direction.

FIGURES 20–22. All same scale. Rendering of larva emerging from an egg. (**20**) Larva's anterior end punches polar plug and begins to emerge; compare with Figure 10 wherein the anterior end of the larva is not inserted into the polar plug bore. Note also that the opposite end polar plug remains in place throughout hatching. (**21**) Anterior end of larva extends into epidermis. (**22**) Posterior end of body uncoils as larva emerges from egg.

congeners. Egg total length is a typical characteristic used to compare species of *Huffmanela* (see Moravec, 1987) and it is relatively easy to obtain from eggs, does not require electron microscopy, and seems relatively resistant to fixation artifact in specimens preserved and handled variously (Bullard et al., 2012). The new species has eggs that are 46–54 ($\bar{x}$ = 50 µm; SD ± 1.6; n = 213) long, and this feature, among others, differentiates it from *H. japonica* (reported egg length = 58–69), *H. longa* (58–69), *H. mexicana* (63–69), *H. balista* (63–78), *H. plectropomi* (64–82), *H. schouteni* (69–75), *H. ossicola* (73–88), *H.* cf. *carcharhini* (75–95) (see Bullard et al., 2012), *H. lata* (77–88), *H. shikokuensis* (78–90), *H. carcharhini* (90–105), and *H. banningi* (99–108) (Table I).

The remaining nominal species of *Huffmanela*, i.e., *Huffmanela branchialis* Justine, 2004, (45–52), *H. filamentosa* (48–53), *Huffmanela paronai* Moravec and Garibaldi, 2000, (48–51), *H. canadensis* (48–63), *H. moraveci* (50–57), and *H. huffmani* (54–60), each have an egg that, like that of *H. oleumimica*, is reportedly <65 in total length. However, the new species can be most easily differentiated from those 6 species by having the following combination of morphological features: (1) fully-developed eggs with brown shell (shell is black in *H. paronai*) (Figs. 10, 13–15), (2) spindle-shaped envelope absent (present in *H. branchialis*) (Figs. 8–13, 27–30), (3) filaments not associated with eggshell (present in *H. filamentosa*) (Figs. 27–30), (4) larva having transverse cuticular ridges (absent in *H. filamentosa*)

FIGURES 23–31. *Huffmanela oleumimica* n. sp. (Nematoda, Trichosomoididae, Huffmanelinae) from the skin of red snapper, *Lutjanus campechanus* (Poey, 1860), (Perciformes: Lutjanidae). Scanning electron microscopy of infected skin. Scale values beside each bar. (**23**) Region of epidermis (ep) that appears to be sloughing at margins (arrows) and revealing exposed dermis (de) beneath. (**24**) Higher magnification view of Figure 20 showing numerous eggs (*) along margin of sloughing epidermis (ep) and the smooth-surfaced dermis (de) beneath it. (**25**) Dermis (de) beneath sloughing epidermis (ep) showing distinctive indentations (arrows) similar in size and shape to nearby eggs (*), perhaps indicating that eggs have detached. (**26**) Eggs partly embedded in epithelium, including a filamentous matrix associated with skin near eggs (arrow), which are of unknown origin but do not apparently represent filaments associated with eggs. (**27**) Egg showing subtle bumps (arrows) on surface of envelope, potentially associated with eggshell spines beneath envelope. (**28**) Egg with host cells (arrows) adhered to eggshell envelope. (**29**) Egg showing torn eggshell envelope (*) and underlying eggshell (sh) of poorly developed egg. Note that eggshell spines are not clearly developed. (**30**) Egg with absent polar plug (arrow), indicating a larva has emerged. Nearby eggs lacked "sunken" polar plugs and had sealed polar ends. (**31**) Cuticle of forcibly everted larva showing regularly spaced, transverse cuticular ridges.

(Fig. 31), (5) robust envelope present throughout development (absent in *H. filamentosa* and *H. canadensis*) (Figs. 8–13), (6) ridges on eggshell absent (transverse in *H. canadensis*, longitudinal in *H. balista*) (Figs. 8–13), (7) eggshell spines present, minute, irregularly dispersed (cf. "uniformly spaced spines" on envelope, not eggshell, of *H. huffmani* [see Huffman and Moravec, 1988]) (Figs. 14, 15), (8) larva >160 in total length (~108 in *H. moraveci*) (Figs. 20–22), and (9) polar plugs 5–8 wide (8–10 in *H. moraveci*) (Figs. 8–13). The minute spines that we describe from the eggshell of *H. oleumimica* appear similar to those of *H. moraveci* (see fig. 18 of Carballo and Navone, 2007), who reported that these structures were associated with the envelope, not the eggshell, and perhaps *H. schouteni* (see fig. 1 of Moravec and Campbell, 1991), but the density of the spines in the new species appears greater (Figs. 14, 15). Moreover, no previously described species of *Huffmanela* ranges in the Gulf of Mexico nor infects a member of Lutjanidae.

The new species is morphologically most similar to *H. paronai*, which is known from eggs only and infects swordfish, *Xiphias gladius*, in the Mediterranean Sea (Moravec and Garibaldi, 2000) (Table I). Eggs of both species have overlapping measurements and markedly protruding polar plugs (compare Figs. 10 and 13 of present study with fig. 1 of Moravec and Garibaldi [2000]). However, the new species can be most easily differentiated from *H. paronai* by the fully developed egg (Figs. 10, 13)

that, in all specimens observed, has a brown shell and a well-developed, obvious envelope. The mature egg of *H. paronai* has a black shell, which obscures viewing the enclosed fully developed larva, and may lack an envelope (Moravec and Garibaldi, 2000; Justine, 2004). We observed no egg of *H. oleumimica* that was too dark to see its contents, and brown-shelled eggs each had a fully developed larva. Further, the eggshell of *H. oleumimica* has minute, irregularly distributed, nub-like spines (Figs. 14, 15), whereas the eggshell and envelope of *H. paronai* is aspinous and reportedly lacks ornamentation.

With respect to ecological similarities between *H. oleumimica* and *H. paronai*, both infect skin, have shedding eggs that embed in the skin covering the branchiostegals (Fig. 2), infect obligate marine bony fishes of Perciformes, and seemingly elicit a similar host response within fish epidermis. Specifically and uncannily similar to what we observed in eggs of *H. oleumimica* (Figs. 6, 23), Moravec and Garibaldi (2000) showed that the skin of swordfish infected with *H. paronai* presented as an easily sloughed sheet-like layer with multitudes of embedded eggs (see fig. 2.A of Moravec and Garibaldi, 2000). Noteworthy also is that red snapper (type host for *H. oleumimica*) and swordfish (type host for *H. paronai*) both range in the Gulf of Mexico and, whereas red snapper is endemic to the Gulf of Mexico, swordfish is a cosmopolitan species (Chow et al., 1997). Swordfish from the eastern and western North Atlantic Ocean may

represent a single stock (Alvarado Bremer et al., 2005; Kasapidis et al., 2007; Garcia et al., 2011), and its fishery is presently managed as separate North Atlantic and South Atlantic stocks by the International Commission on the Conservation of Atlantic Tunas (ICCAT) (Garcia et al., 2011). Therefore, and although geographic records of these parasites are separated by an ocean basin, the infected individual fish themselves could have co-occurred in the Gulf of Mexico. As such, and underscoring the problem with inferring species identity from host identity and capture locality, sympatry or allopatry of *H. oleumimica* and *H. paronai* is indeterminate, and no record of another species of *Huffmanela* from swordfish has been published (Table I). However, given its population structure (op. cit.), necropsies of swordfish captured from localities outside of the North Atlantic Ocean sensu lato (including the Mediterranean Sea and Gulf of Mexico) could reveal infections by new species of *Huffmanela*.

Noteworthy also is that amber and brown eggs of *H. oleumimica* were consistently observed to have a dark, optically dense, inner eggshell layer and a light, translucent outer eggshell layer (Figs. 9, 10, 12, 13). All other accepted species except *H. carcharhini* (see Bullard et al., 2012) reportedly have a dark outer eggshell layer and a light inner eggshell layer. Like that of *H. paronai*, the outer eggshell layer of *H. oleumimica* is thinnest in the most undeveloped eggs (clear eggs; Figs. 8, 11), becoming proportionally thicker as the egg develops (Figs. 9, 10, 12, 13). Ultrastructural studies are likely needed to address the validity of the light–dark layers of the eggshell as reliably diagnostic for species of *Huffmanela*, and we are unsure about it currently.

DISCUSSION

Although effectively distinguishing it from its congeners, morphometric comparisons of clear, amber, and brown eggs of the new species did not show a statistically significant intraspecific difference (see Description), and the ranges for each measurement overlapped extensively. Eggshell color, however, was concordant with stage of larval development, i.e., eggs with clear shells had no evident larvae and abundant vitelline material, those with amber shells had a developing larva and less vitelline material, and those with brown shells enclosed a fully developed larva and little, or no, vitelline material (Figs. 8–13). A general pattern of increasingly protruding polar plugs was also observed. These results, together, suggest that eggs of the new species, although undergoing obvious morphological changes during development, do not significantly enlarge with age. These results match those of Bullard et al. (2012), who documented egg development of *H. carcharhini* in the skin of a shark. Eggs of other species reportedly do increase in size as the larva develops (e.g., Huffman and Moravec 1988; Moravec et al. 1998, 2005). Determining if the egg enlarges with age is critical regarding the taxonomy of *Huffmanela* because egg measurements themselves are used to describe and differentiate species. Characteristics of developing eggs should complement information from fully developed eggs. For species of *Huffmanela* with eggs that enlarge with age, measurements utilized for taxonomic decisions should be taken principally from fully developed eggs only; but other features of developing eggs, no matter the species, also can be informative. For example, not all species of *Huffmanela* have an envelope surrounding the eggshell throughout development.

Eggs of *H. oleumimica* apparently can hatch before they are sloughed or ejected from the skin of the definitive host (Figs. 16, 17, 20–22). No life cycle of a species of *Huffmanela* has been demonstrated, but several authors have speculated about how eggs or larvae (or both) separate from the definitive host (e.g., Carballo and Navone, 2007; Bullard et al., 2012). In the present study, eggs of *H. oleumimica* were associated with surfaces of the buccal pump, i.e., urohyal, branchiostegals, and buccal cavity,

and typically eggs were associated with sheet-like layers of sloughing epidermis (Figs. 5, 6, 23). Intuitively, water flow generated by the red snapper's respiratory–buccal pump could facilitate the en mass detachment of eggs as constituents of a sloughing sheet of epidermis. Moravec and Garibaldi (2000) discussed this hypothetical relationship between water flow and egg shedding; however, to our knowledge no previous study has documented egg hatching on the definitive host.

In the present study, we observed eggs that had emerging larvae in the epidermis immediately surrounding eggs (Figs. 16, 17, 20–22). Concerned that this may have represented artifact from unintentional, excessive coverslip pressure during wet-mounting, we observed wet-mounted eggs with the compound microscope as we intentionally bled-off water from beneath the coverslip (Figs. 18, 19), causing the coverslip to compress the sample and partially crush it. In some of these eggs, the egg itself was obliterated, resulting in jagged, shattered pieces of eggshell material. Larvae forced from these eggs remained in a tightly coiled ball (Fig. 18). In other eggs, wherein we applied coverslip pressure without crushing the eggs, larvae protruded from both polar openings (Fig. 19). These outcomes were in stark contrast to what we observed in wet-mounted eggs that were not subjected to coverslip pressure (Figs. 16, 17). Based on this primitive operation, we think that non-artifactitious egg hatching within the epidermis is marked by a larva emerging anterior-end–first from 1 polar opening (Figs. 16, 17), then residing outside of the egg as a fully extended, not coiled, individual. This is rather intuitive, as a coiled worm is too broad to emerge through the polar opening. Because formalin-fixed eggs are indeed easily crushed or flattened under coverslip pressure, we think this is a useful observation that can help differentiate "naturally" hatching eggs from forcibly extruded larvae. A seemingly unlikely alternative explanation for our observation of larvae in the epidermis of this red snapper could be that larval emergence was, somehow, stimulated by freezing; thereby representing a kind of artifact, because the fish head we studied was frozen while its nematodes likely were still alive.

Some eggs of *Huffmanela oleumimica* were seemingly randomly distributed in skin (Fig. 5) and others were deposited in scribble-like, linear tracks in the skin (Fig. 3). Assuming that we did not overlook another egg morphotype representing another species of *Huffmanela*, it is interesting that 1 species of *Huffmanela*, or perhaps 1 individual, can exhibit both patterns of egg laying. That of *H*. cf. *carcharhini* seemingly differs, with females of that species depositing eggs in scribble-like skin markings only (Bullard et al., 2012). Sharks have an epidermis that is perforated by placoid scales, and so the female nematode presumably must wind around the bases of the placoid scales as it deposits its eggs. This possibly results in the observed scribble pattern. Yet, even without placoid scales or any scale, i.e., on the surface of the buccal cavity, red snapper infected by *Huffmanela oleumimica* also exhibit this "scribbling," which obviously indicates that the observed pattern of egg laying is not necessarily related to the presence or absence of placoid scales. It also indicates that taxonomic decisions based on the pattern of egg laying in the epidermis may be dubious or at least require further study across more species of *Huffmanela*.

This is the first record of a species of *Huffmanela* from the Gulf of Mexico. In addition, it is the first account of a species of *Huffmanela* infecting a member of Lutjanidae. Species of *Huffmanela* reportedly infect 27 species of marine bony fishes

(Euteleostei) assigned to 17 genera, 14 families (Table I), and 6 orders. One species, *H. huffmani*, infects freshwater fishes (Centrarchidae) in Texas (Huffman and Moravec, 1988) and another 2 species infect sharks (Justine, 2004; Bullard et al., 2012). Considering egg morphology only, we do not see any clear pattern of resemblance among species of *Huffmanela* that infect phylogenetically related fishes, e.g., species of *Huffmanela* infecting fishes of Perciformes, except that the 2 species infecting carcharhinid sharks, *H. carcharhini* and *H. lata*, are relatively large among nominal *Huffmanela* spp. and both infect skin (chondrichthyans lack an air bladder). Nor does there seem to be an obvious pattern of shared morphological features among those species of *Huffmanela* that infect skin versus those infecting swim bladder, muscle, gill, or bone (Table I).

Red snapper is a prized Gulf of Mexico fish (Gillig et al., 2000; Coleman et al., 2004), and it is a politically iconic species for the Gulf's recreational and commercial fishing industries. Periodic media reports have alleged that red snappers captured in the north-central Gulf of Mexico exhibit skin lesions caused by exposure to spilled crude oil from the 2010 *BP Deepwater Horizon* oil spill (DHOS) (pers. obs.; as of 19 June 2012 no peer-reviewed report exists on this topic). The gross appearance of the brown, elliptical, sub-epidermal eggs of *H. oleumimica* in the skin of red snapper could be mistaken by laypersons for "oil droplets" beneath the skin. It should be emphasized that parasites infecting fishes, especially the eggs of species of *Huffmanela* and some adult didymozoids (Digenea: Didymozoidae), superficially can resemble non-biological materials, e.g., oil. Also, these parasites normally infect and are diverse among marine fishes, and their presence does not necessarily indicate a disease condition. The DHOS probably has increased the number of researchers scrutinizing fish diseases in the Gulf of Mexico, and this is good because it will likely broaden our understanding of epidemiology there. However, reports of lesions and abnormalities in and on fish skin could benefit from including parasitological taxonomic expertise.

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