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VERSITA

A new species of *Ascarophis* (Nematoda, Cystidicolidae) from the stomach of the marine scorpaeniform fish *Hoplichthys citrinus* from a seamount off the Chesterfield Islands, New Caledonia

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Abstract

A new species of parasitic nematode, *Ascarophis* (*Similascarophis*) *richeri* sp. nov. (Cystidicolidae), is described from the stomach of the marine scorpaeniform fish *Hoplichthys citrinus* Gilbert (Hoplichthyidae) (prevalence 19%, intensity 1–8 nematodes per fish) collected in the region of the Chesterfield Islands (Coral Sea, west of New Caledonia) in October 2005. The new species, studied using both light and scanning electron microscopy, is characterized mainly by the structure of the mouth (highly reduced pseudolabia, high pseudolabial terminal projections, no submedian labia, well-developed, bilobed sublabia), bifurcate deirids, the length of the spicules (663–729 µm and 105–108 µm) and the presence of filaments on both egg poles (2–5 on each). *Similascarophis* Muñoz, González et George-Nascimento, 2004 is considered a subgenus of *Ascarophis* van Beneden, 1871 to accommodate the species characterized by highly reduced pseudolabia. Presence of the new species in fish from Seamount Nova (Lord Howe Rise) but not from the Chesterfield plateau suggests that it is endemic to this seamount, a case already encountered for many benthic invertebrates.

Résumé

Une nouvelle espèce de Nématode parasite, *Ascarophis* (*Similascarophis*) *richeri* sp. nov. (Cystidicolidae), est décrite de l'estomac du poisson marin *Hoplichthys citrinus* Gilbert (Scorpaeniformes, Hoplichthyidae) (prévalence 19%, intensité 1–8 nématodes par poisson) collecté en octobre 2005 dans la région des Îles Chesterfield (Mer du Corail, Ouest de la Nouvelle-Calédonie). La nouvelle espèce, étudiée au microscope photonique et au microscope électronique à balayage, est caractérisée principalement par la structure de la bouche (pseudolabia très réduites, terminaison des pseudolabia haute, pas de labia submédiane, sublabia bien développées et bilobées), des deirides bifurquées, la longueur des spicules (663–729 µm et 105–108 µm) et la présence de 2–5 filaments sur chaque pôle des œufs. *Similascarophis* Muñoz, González et George-Nascimento, 2004 est considéré comme un sous-genre de *Ascarophis* van Beneden, 1871, et inclut les espèces caractérisées par des pseudolabia très réduites. La présence de la nouvelle espèce dans des poissons provenant du mont sous-marin Nova (Ride de Lord Howe) mais pas du plateau des Chesterfield suggère qu'elle est endémique à ce mont, un cas déjà rencontré chez de nombreux invertébrés benthiques.

Key words

Parasitic nematode, *Ascarophis*, *Similascarophis*, marine fish, *Hoplichthys*, Chesterfield Islands, New Caledonia

Introduction

During recent helminthological investigations of marine fishes of New Caledonia, South Pacific, conspecific cystidicolid nematodes referable to *Ascarophis* van Beneden, 1871 were recovered from the stomach of *Hoplichthys citrinus* Gilbert (Hoplichthyidae, Scorpaeniformes) collected in the region of the Chesterfield Islands, situated about 1000 km west of New Caledonia (about half-way between New Caledonia and Australia), in October 2005. Their detailed study using the light

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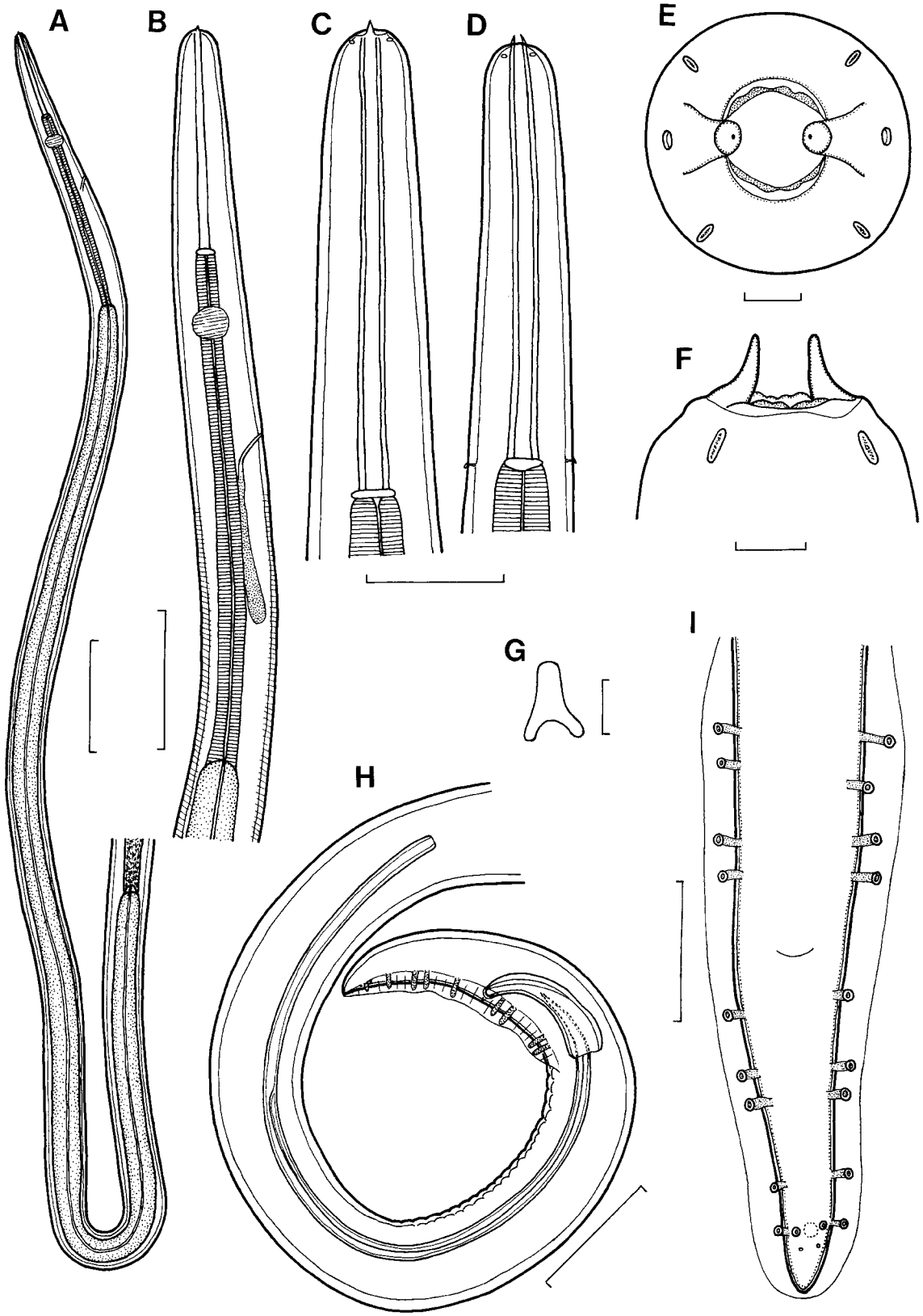

Fig. 1. *Ascarophis* (*Similascarophis*) *richeri* sp. nov.: **A** – oesophageal part of male body, lateral view; **B** – anterior end of male, lateral view; **C** and **D** – anterior end of gravid female, lateral and dorsoventral views; **E** and **F** – cephalic end, apical and dorsoventral views (reconstructed from SEM micrographs); **G** – deirid; **H** and **I** – posterior end of male, lateral and ventral views. Scale bars = 200 µm (A); 100 µm (B, H); 50 µm (C, D, I); 3 µm (E, F); 2 µm (G)

and scanning electron microscopy have shown that they represent a previously undescribed species, which is described below.

Hoplichthys citrinus is a small-sized marine fish (maximum body length 24.5 cm) distributed in the Western Central Pacific (Australia) and the Southeast Pacific (Nazca and Salay-Gomez) (Froese and Pauly 2007).

Materials and methods

The fish were collected on board R/V 'Coris' during the EBISCO Campaign off the Chesterfield Islands, New Caledonia, and immediately deep frozen. In October 2006, the fish were examined for the presence of helminth parasites in the laboratory of the Institut pour le Développement (IRD) in Nouméa, New Caledonia. The nematodes for morphological studies were fixed in 4% formaldehyde solution. For light microscopical examination, they were cleared with glycerine. Drawings were made with the aid of a Zeiss microscope drawing attachment. Specimens used for scanning electron microscopy (SEM) were postfixed in 1% osmium tetroxide, dehydrated through a graded ethanol series, critical point dried and sputter-coated with gold; they were examined using a JEOL JSM-6300 scanning electron microscope at an accelerating voltage of 15 kV. All measurements are in micrometres unless otherwise stated. Fish names follow FishBase (Froese and Pauly 2007).

Results

Family Cystidicolidae Skryabin, 1946

***Ascarophis* (*Similascarophis*) *richeri* sp. nov.** (Figs 1–4)

General: Small, whitish nematodes. Maximum width of body near posterior end. Cuticle thick, with transverse striations distinctly visible particularly in middle part of body, some anastomosing (Fig. 3E). Cephalic end rounded, with two conspicuous conical pseudolabial terminal protrusions with pointed tips. Oral aperture almost circular (Figs 1E and 3A). Subme-

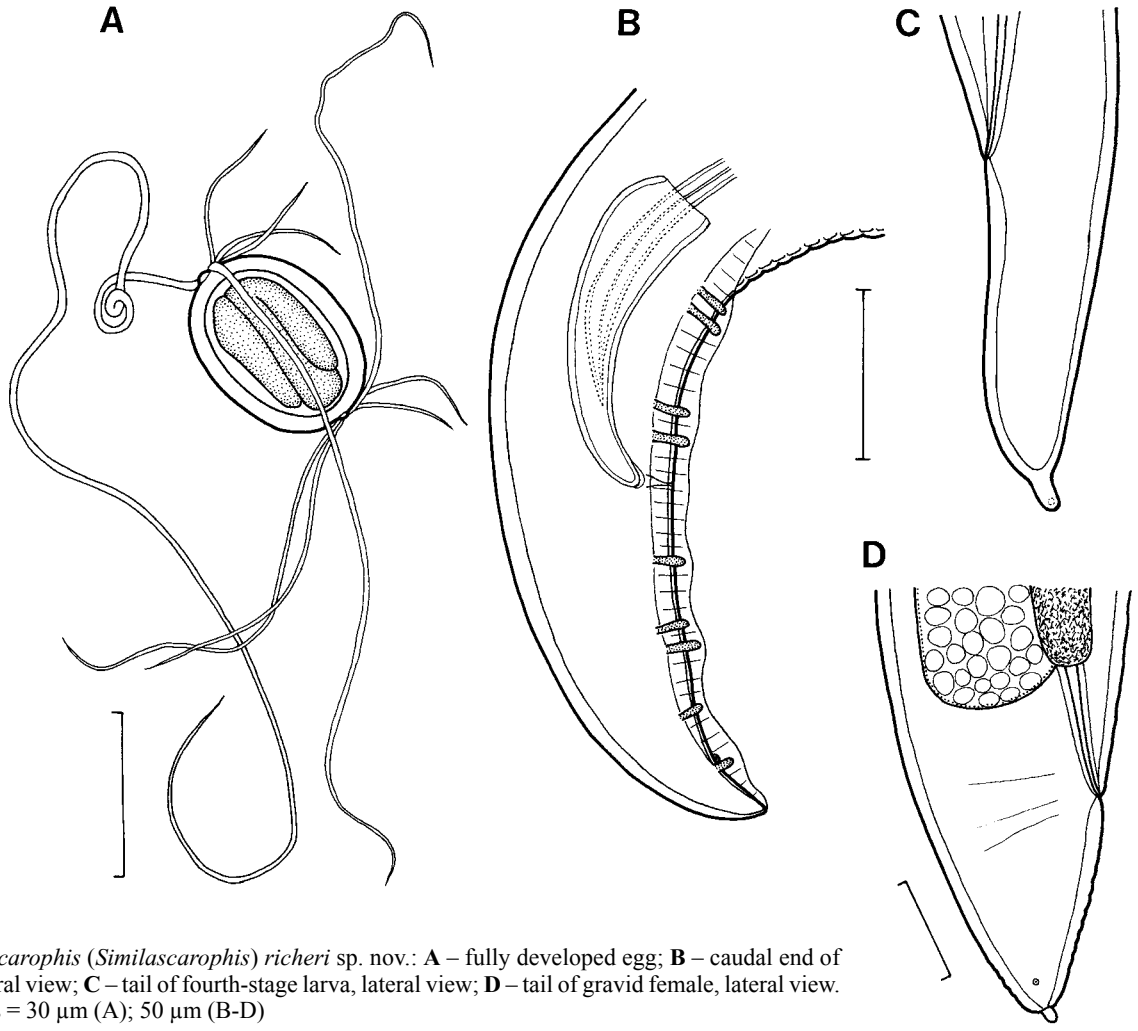

Fig. 2. *Ascarophis* (*Similascarophis*) *richeri* sp. nov.: **A** – fully developed egg; **B** – caudal end of male, lateral view; **C** – tail of fourth-stage larva, lateral view; **D** – tail of gravid female, lateral view. Scale bars = 30 μm (A); 50 μm (B-D)

dian labia absent. Lateral pseudolabia small, without inner extensions, each bearing one conspicuous conical terminal protrusion (Figs 1E, F and 3B, D). Submedian sublabia well developed, bilobed (Fig. 3A-D). Four elongate submedian cephalic papillae and pair of lateral amphids present (Figs 1E and 3A, B). Vestibule (stoma) long, cylindrical, without usual

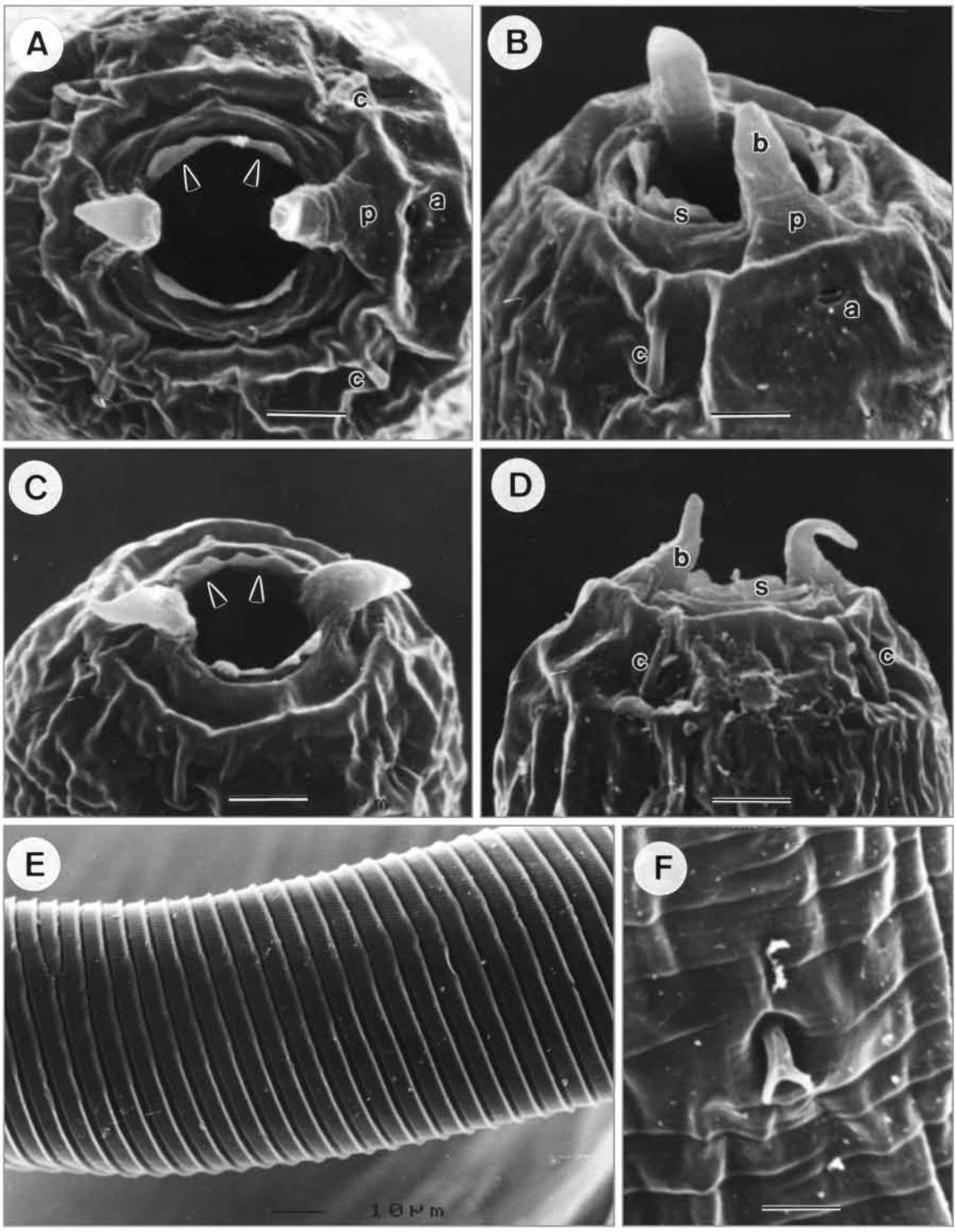

Fig. 3. *Ascarophis* (*Similascarophis*) *richeri* sp. nov., SEM micrographs: **A** – cephalic end, apical view (arrowheads indicate sublabia); **B** – cephalic end, sublateral view; **C** – cephalic end of another specimen with distinct bilobed sublabia (see arrowheads), subapical view; **D** – cephalic end, lateral view; **E** – striations of cuticle at middle part of body; **F** – bifurcate deirid, lateral view. Scale bars = 2 µm (A-D, F); 10 µm (E). **Abbreviations:** a – amphid, b – pseudolabial protrusion, c – cephalic papilla, p – pseudolabium, s – sublabium

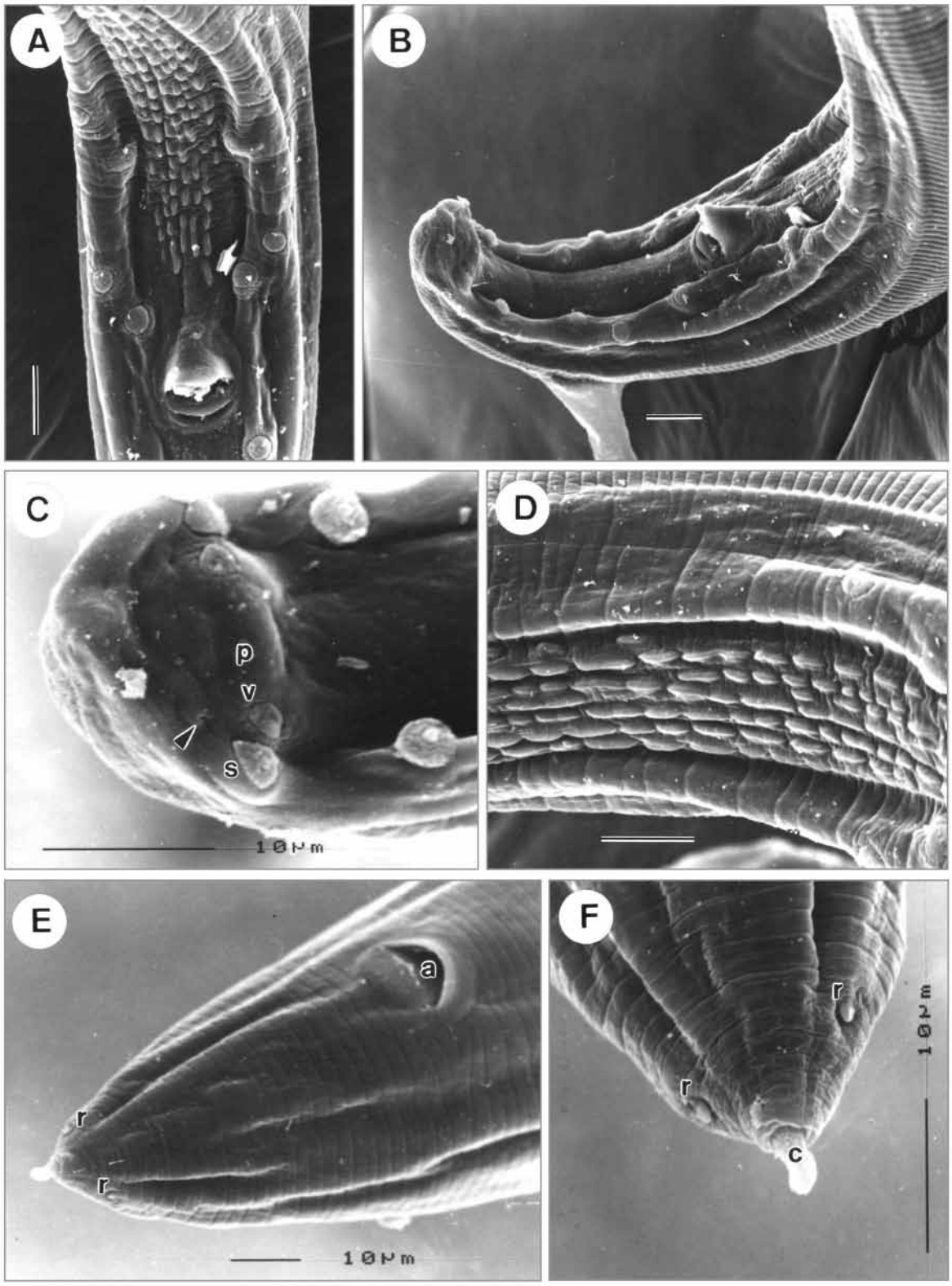

Fig. 4. *Ascarophis* (*Similascarophis*) *richeri* sp. nov., SEM micrographs: **A** – precloacal region of male, ventral view; **B** – caudal end of male, sublateral view; **C** – posterior end of male tail, ventral view (arrowhead indicates phasmid); **D** – ventral precloacal cuticular tessellated ridges (area rugosa) of male, ventral view; **E** – tail of gravid female, sublateral view; **F** – tail tip of gravid female, ventral view. Scale bars = 10 µm (A, B, D). **Abbreviations:** a – anus, c – caudal appendage, p – ventral caudal protuberance, r – phasmid, s – postanal papilla of last subventral pair, v – ventral postanal papilla

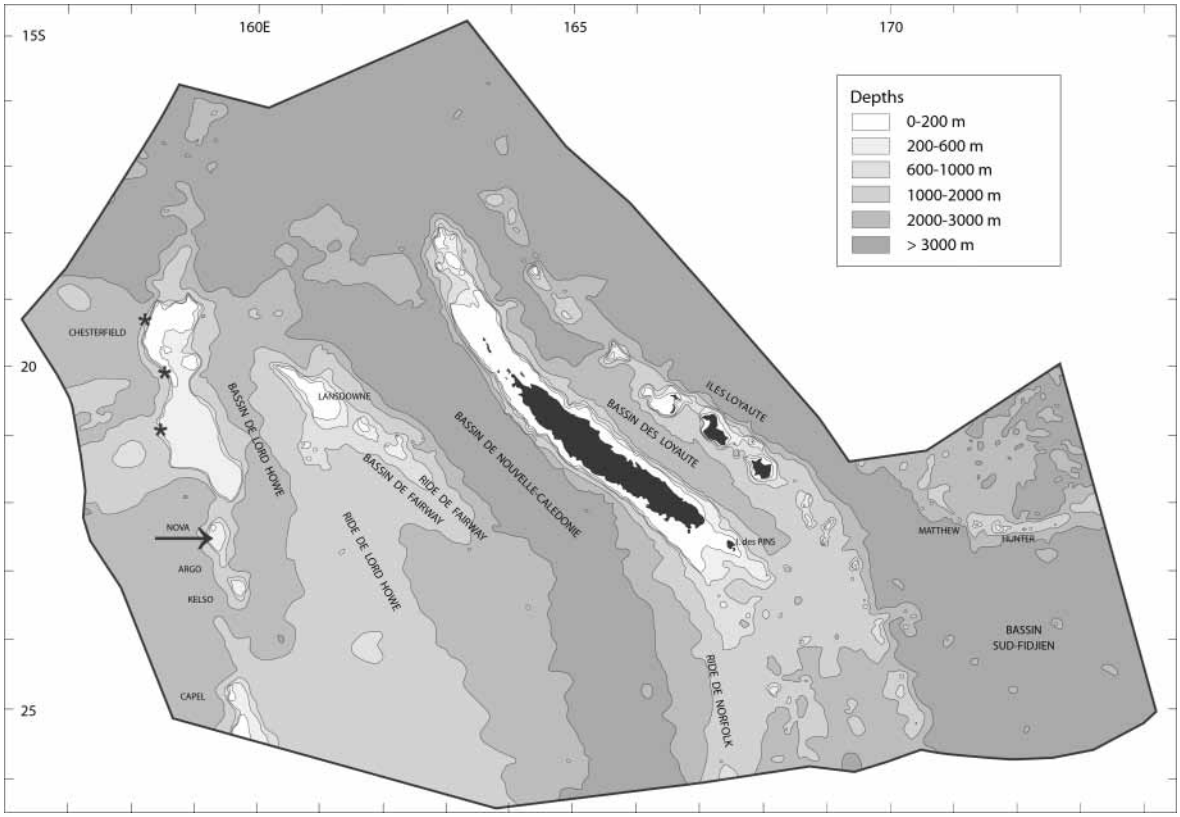

Fig. 5. Map of the Exclusive Economic Zone of New Caledonia indicating the localities. Asterisks: negative records from off the Chesterfield plateau; arrow: Seamount Nova, where the fish harboured the new species

funnel-shaped prostom in lateral view (Fig. 1C, D). Glandular oesophagus 5–7 times longer than muscular; both parts of oesophagus distinctly separated from each other (Fig. 1A, B). Nerve ring encircles muscular oesophagus approximately at border of its first and second sixths; excretory pore located somewhat posterior to level of nerve ring; deirids very small, situated at about level of posterior end of vestibule (Figs 1D and 3F).

Male (4 specimens; measurements of holotype in parentheses): Length of body 7.52–9.72 (9.72), maximum width 75–87 (87). Height of cephalic protrusions 3 (3). Vestibule 144–165 (165) long. Length of muscular oesophagus 330–399 (399), maximum width 15 (15); length of glandular oesophagus 1.74–2.39 (2.39) mm, maximum width 33–51 (51); length ratio of muscular and glandular parts of oesophagus 1:5.3–6.0 (1:6.0). Length of entire oesophagus and vestibule represents 29–32 (30)% of total body length. Nerve ring, excretory pore and deirids 183–222 (222), 258–321 (321) and 129–162 (162), respectively, from anterior extremity. Posterior end of body spirally coiled, provided with narrow caudal alae. Preanal papillae: 4 pairs of subventral pedunculate papillae present, of which first and second and third and fourth are shifted in relation to each other, alternating along margins of tail (Fig. 4A). Postanal papillae: 6 pairs present, including 5 pairs of pedunculate subventral papillae and 1 pair of minute ventral sessile papillae located approximately at level of last pair of subventrals (Figs 1I and 4C). In addition, a pair of very small ventral phasmids present just posterior to ventral papillae (Fig. 4C). Small median ventral caudal protuberance present between both ventral papillae (Fig. 4C). Ventral cuticular ridges (area rugosa) anterior to cloaca well developed, consisting of about seven longitudinal tessellated ornamentations (Fig. 4A, D). Large (left) spicule 663–729 (726) long, with pointed distal tip; its shaft 240–279 (279) long, forming 34–38 (38)% of overall length of spicule (Fig. 1H). Small (right) spicule broad, 105–108 (105) long, with rounded distal end provided with small cuticular membrane. Length ratio of spicules 1:6.1–6.9 (1:6.9). Tail conical, 111–135 (135) long, with rounded tip.

Female (5 gravid specimens with mature eggs; measurements of allotype in parentheses): Length of body 15.22–21.12 (21.12) mm, maximum width 95–163 (163). Height of cephalic protrusions 3 (3). Vestibule 150–174 (174) long. Length of muscular oesophagus 351–471 (429), maximum width 21 (21); length of glandular oesophagus 2.30–3.26 (3.06) mm, maximum width 51–66 (66); length ratio of muscular and glandular parts of oesophagus 1:6.4–7.1 (1:7.1). Length of entire oesophagus and vestibule represents 17–20 (17)% of total body length. Nerve ring, excretory pore and deirids 195–225 (225), 270–300 (300) and 153–168 (153), respectively, from anterior extremity. Tail conical, short, 90–111

(111) long, with two lateral papilla-like phasmids near its tip, and a distinct terminal appendage 3–9 (6) long and 3–9 (6) wide (Figs 2D and 4E, F). Vulva somewhat postequatorial, situated 8.75–13.19 (13.19) mm from anterior end of body, at 56–62 (62)% of body length; vulval lips not elevated. Vagina directed posteriorly from vulva. Amphidelphic. Anterior ovary and uterus not extending anteriorly to oesophageal part of body. Uterus filled with numerous eggs, occupies major part of body, reaching posteriorly level of posterior end of intestine. Mature eggs (containing larvae) oval, thick-walled, size 42–45 × 24–27 (45 × 27); thickness of egg wall 3–4 (4). Each pole of egg bears small knob provided with 2–5 thread-like filaments up to about 300 long; knob on one pole somewhat larger than that on opposite pole, bearing usually two markedly longer and thicker filaments and 2–3 short, fine filaments; filaments growing up from smaller knob relatively short and fine (Fig. 2A).

Fourth-stage larva (1 specimen): Body length 5.01 mm, maximum width 57. Height of cephalic protrusions 1. Vestibule measuring 162. Muscular oesophagus 285 long, maximum width 15; glandular oesophagus 1.01 long, maximum width 39; length ratio of both parts of oesophagus 1:3.5. Nerve ring and excretory pore 204 and 270, respectively, from anterior extremity. Tail conical, 114 long, with large terminal appendage 9 long and 6 wide (Fig. 2C).

Type host: *Hoplichthys citrinus* (Hoplichthyidae, Scorpaeniformes) (body length 20.5–24.5 cm).

Site of infection: Stomach and rarely intestine (1 case).

Type locality: Seamount Nova (22°48´S, 159°22´E), depth 330 m, south of the Chesterfield Islands, New Caledonia, 9 October 2005 (Fig. 5).

Prevalence and intensity: 19% (7 fish infected/37 fish examined); intensity 1–8 (mean 3) nematodes per fish. See also Table I.

Deposition of type specimens: Holotype and allotype (JNC2015; jar N 759), 5 paratypes (JNC2004, JNC2005, JNC2007, JNC2015; jar N 759) and 1 voucher specimen (JNC2103; jar N 562) in the Muséum National d'Histoire Naturelle, Paris; 2 paratypes in the Institute of Parasitology, Biology Centre of the ASCR, České Budějovice (N-872).

Etymology: This species is named in honour of Dr. Bertrand Richer de Forges, who contributed greatly to the knowledge of the diversity of benthic fauna in the southwest Pacific.

Discussion

Ascarophis includes many species parasitic as adults in the gastrointestinal tract of marine and estuarine fishes. Taking into account Ko's (1986) re-diagnosis of the genus, Ferrer *et al*. (2005) considered 33 *Ascarophis* spp. (of 53 nominal species) to be valid. They also included the species with reduced pseudolabia, considering this character a variation in the fine morphology of the cephalic structures within *Ascarophis*. In contrast, Muñoz *et al*. (2004) erected *Similascarophis* Muñoz, González *et* George-Nascimento, 2004 to accommodate the *Ascarophis*-like species with reduced pseudolabia, to which they assigned *S. maulensis* Muñoz, González *et* George-Nascimento, 2004 (type species), *S. chilensis* Muñoz, González *et* George-Nascimento, 2004, *S. marina* (Szidat, 1961) and *S. nasonis* (Machida, 1981). However, Moravec and González-Solís (2007) followed the conception of Ferrer *et al*. (2005) and they synonymized *Similascarophis* with *Ascarophis*, reporting that the relationships among the species assigned to *Ascarophis* can only be resolved when substantial SEM data on the cephalic structures and molecular data accumulate (see also Moravec and Justine 2007, Moravec and Klimpel 2007, Moravec *et al*. 2007).

It is apparent from SEM studies that the shape and size of pseudolabia may somewhat differ in different *Ascarophis* spp. (e.g., Appy 1981, Ko 1986, Fagerholm and Berland 1988); also the shape of mouth may be from elongate oval to almost hexagonal or circular and the submedian lips, well developed in some species, may be indistinct or absent in others (e.g., in *A. extalicola* Appy, 1981 or *A. minuta* Ko, 1985). Even in the species with reduced pseudolabia, listed in *Similascarophis* by Muñoz *et al*. (2004) (see above), the shape and structure of pseudolabia may differ considerably: whereas the pseudolabia of *S. maulensis* are without inner extensions, each bearing a marked conical terminal projection (Muñoz *et al*. 2004), those of *S. marina* have distinct inner extensions slightly dorsoventrally expanded in an apical view, each with a rather small terminal projection (Ivanov *et al*. 1997). Therefore, in view of considerable variations in the shape and size of pseudolabia in these nematodes, we follow Moravec and González-Solís (2007) in considering the genus *Similascarophis* a junior synonym of *Ascarophis*. However, we suggest to retain *Similascarophis* Muñoz, González et George-Nascimento, 2004 as a subgenus of the genus *Ascarophis* van Beneden, 1871 for the morphological group of species with highly reduced pseudolabia. In addition to the four species listed in *Similascarophis* by Muñoz *et al*. (2004) (see above), *Ascarophis ayalai* Caballero, 1975, *A. mexicana* Moravec, Salgado-Maldonado et Vivas-Rodríguez, 1995 and *A. valentina* Ferrer, Aznar, Balbuena, Kostadinova, Raga *et* Moravec, 2005 should also be assigned to this subgenus based on data by González-Solís *et al*. (2002), Ferrer *et al*. (2005) and Moravec and González-Solís (2007).

In having highly reduced pseudolabia and no submedian labia, the new species belongs to the group of *Ascarophis* spp. now assigned to the subgenus *Similascarophis*. However, the cephalic structures of the majority of *Ascarophis* spp. have not yet been studied by SEM, so that their belonging to subgenera remains unknown. Of the *Ascarophis* spp. considered by Ferrer *et al*. (2005) to be valid, 22 have not been examined by SEM. Of them, only *A. litoralica* Zhukov, 1960, a parasite of some perciform fishes in the Far East (South-Kurile shallow waters), resembles *A. richeri* sp. nov. in the length of the left spicule and in that both egg poles are filamented (Zhukov 1960); all other species have the left spicule less than 500 µm or more than 800 µm long, or filaments are lacking on their eggs. However, in contrast to the new species, the females of

Table I. Localities where the fish were collected and prevalence of *Ascarophis richeri*

Locality	Station	Date	Latitude (S)	Longitude (E)	Depth (m)	Prevalence
Seamount Nova	DW2526	9/10/05	22°47.492	159°22.890	330–340	0/1
Seamount Nova	CP2529	9/10/05	22°48.63	159°22.98	330–340	3/8
Seamount Nova	CP2531	9/10/05	22°48.083	159°22.867	330–340	3/8
Seamount Nova*	CP2542	10/10/05	22°16.081	159°25.89	335–338	1/20
West Bellona, external slope	CP2545	11/10/05	21°10.040	158°37.400	765–778	0/2
North Bellona, external slope	DW2578	14/10/05	20°19.785	158°39.714	440–505	0/10
East Chesterfield, external slope	CP2596	17/10/05	19°43.784	158°36.221	382–386	0/5

*3 fish from this station also had anisakid larvae.

A. litoralica are much smaller (length 6.6–12.0 mm vs. 15.2–21.1 mm), their glandular oesophagus and tail are distinctly shorter (1.15–1.88 vs. 2.30–3.26 mm and 29–33 µm vs. 90–111 µm, respectively), and each egg pole bears only two filaments; the male has allegedly only five pairs of postanal papillae, the left spicule is usually somewhat shorter (590–670 µm vs. 663–729 µm) and both species also differ in the host orders (Perciformes vs. Scorpaeniformes) and the geographical distribution (Far East vs. New Caledonia).

Of the *Ascarophis* species already studied by SEM, *A. arctica* Polyansky, 1952, *A. extalicola* Appy, 1981, *A. filiformis* Polyansky, 1952, *A. minuta* Ko, 1985, *A. morrhuae* van Beneden, 1871, *A. nototheniae* Johnston *et* Mawson, 1945 and *A. sebastodis* Olsen, 1952 are characterized by unreduced pseudolabia (Appy 1981, Ko 1986, Fagerholm and Berland 1988) by which they distinctly differ from *A. richeri*; all of them are representatives of the nominotypical subgenus *Ascarophis*. The pseudolabia of the six remaining species are highly reduced and the species are assigned to the subgenus *Similascarophis* (see above).

The only species of *Ascarophis* (*Similascarophis*) resembling *A. richeri* by the length of the left spicule is *A. maulensis* (Muñoz, González *et* George-Nascimento, 2004), a parasite of the marine perciform fish *Bovichtus chilensis* Regan off the coast of Chile; in all other species this spicule is distinctly shorter (*A. ayalai*, *A. chilensis*, *A. marina*, *A. valentina*) or much longer (*A. mexicana*). *Ascarophis maulensis* seems to be similar to the new species also by some other features, such as, for example, the numbers and arrangement of egg filaments or the situation of deirids. However, both species differ substantially in the structure of the mouth: whereas the oral aperture is dorsoventrally elongate, the sublabia are reduced to simple narrow folds and the terminal pseudolabial protrusions are small in *A. maulensis*, in *A. richeri* the oral aperture is circular, the sublabia are well developed, bilobed and the pseudolabial protrusions are conspicuously high. Moreover, the eggs of *A. maulensis* are somewhat smaller (36–41 × 20–24 µm vs. 42–45 × 24–27 µm) and of a somewhat different shape (more elongate), there are allegedly only five pairs of postanal papillae in this species, and the caudal appendage is present only in some females. The shape of deirids and the presence of the median caudal protuberance in the male were not reported for *A. maulensis*. Taking into account different host orders (Perciformes vs. Scorpaeniformes) and different geographical regions (Chile vs. New Caledonia), we consider nematodes of the present material to represent a new species. After *Metabronema magnum* (Taylor, 1925) (see Moravec and Justine 2007), *Ascarophis richeri* is the second cystidicolid nematode recorded from New Caledonian waters. It is the second fish parasite recorded from the region of the Chesterfield Islands, after an isopod (Trilles and Justine 2006).

Possible endemism of Ascarophis richeri

Table I shows that contrasting results were obtained for prevalence of this nematode in its host. In three stations along the Chesterfield plateau, no nematode was found in a total of 17 fish observed (prevalence 0%). In contrast, in fish collected from Seamount Nova, prevalence was 19% (7/37). Previous analyses of the benthic fauna of sea mounts in the South Pacific, including Seamount Nova (Richer de Forges *et al*. 2000), have demonstrated a high level of endemicity of benthic invertebrates in seamounts; very low species overlap was observed between samples from seamounts separated by only a few kilometres (but see also Samadi *et al*. 2006). Our results, although based on a limited (n = 54) number of fish specimens (but collection of deep-sea fish from a remote seamount is extremely expensive), suggest that the new *Ascarophis* species is located only in Seamount Nova and not in various places along the Chesterfield plateaus. Since depth is higher in the stations investigated in along the Chesterfield plateaus, it might be that depth is responsible for this difference, but an alternative explanation is that the parasite is endemic to Seamount Nova. The fish is evenly distributed in the Western Central and Southeast Pacific but it might be that a benthic crustacean involved in the life-cycle of the *A. richeri* is present only in Seamount Nova and absent on the external slope of the Chesterfield plateau. The present findings could be the first example of a fish parasite species endemic of a seamount; additional collection is needed to confirm this hypothesis.

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