Two new species of nematode parasites, Cucullanus epinepheli sp. n. (Cucullanidae) and Procamallanus (Spirocamallanus) sinespinis sp. n. (Camallanidae), from marine serranid and haemulid fishes off New Caledonia

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Abstract: Based on light and scanning electron microscopical studies, two new species of parasitic nematodes are described from marine perciform fishes off New Caledonia: Cucullanus epinepheli sp. n. (Cucullanidae) from the intestine of the brownspotted groupers Epinephelus chlorostigma (Valenciennes) (Serranidae) and Procamallanus (Spirocamallanus) sinespinis sp. n. from the intestine of the silver grunt Pomadasys argenteus (Forsskål) (Haemulidae). Cucullanus epinepheli sp. n. differs from its congeners mainly in possessing a unique structure of the anterior, elevated cloacal lip with a large posterior outgrowth covering the cloacal aperture and in the presence of cervical alae and two small preanal papillae on the median dome-shaped precloacal elevation. This is the second known nominal species of this genus parasitising fishes of the family Serranidae and the second representative of Cucullanus Müller, 1777 recorded from fishes in New Caledonian waters. Procamallanus (Spirocamallanus) sinespinis sp. n. is mainly characterised by 10–12 spiral ridges in the buccal capsule, the presence of wide caudal alae, three pairs of pedunculate preanal papillae, two unequally long spicules (465–525 µm and 218–231 µm) and by the tail tip with a knob-like structure in the male, and the broad, rounded tail with a terminal digit-like protrusion without cuticular spikes in the female. This is the fifth nominal species of the subgenus Spirocamallanus Olsen, 1952 reported from fishes in New Caledonian waters.

Keywords: Nematoda, helminth parasite, Seuratoidea, Camallanoidea, Epinephelus, Pomadasys, South Pacific
MATERIALS AND METHODS
Fish were caught off Nouméa, New Caledonia, or purchased from the fish market in Nouméa. Fish were identified using standard morphological keys; in addition, specimen JNC3241 [Epinephelus chlorostigma (Valenciennes)] was subjected to a molecular study (Schoolinck et al. 2014) and a tissue sample was deposited in the Muséum National d’Histoire Naturale (MNHN) collection. Parasites were collected by the wash method of Justine et al. (2012). The nematodes were fixed in hot 70% ethanol. For light microscopic examination, they were cleared with glycerine. Drawings were made with the aid of a Zeiss drawing attachment. Specimens used for scanning electron microscopy were transferred to 4% formalin, postfixed in 1% osmium tetroxide (in phosphate buffer), dehydrated through a graded acetone series, critical-point-dried and sputter-coated with gold; they were examined using a JEOL JSM-7401F scanning electron microscope at an accelerating voltage of 4 kV (GB low mode). All measurements are in micrometres unless otherwise indicated. The fish nomenclature and classification adopted follows FishBase (Froese and Pauly 2016).

RESULTS
Family Cucullanidae Cobbold, 1864

Cucullanus epinepheli sp. n. Figs. 1–3

ZooBank number for species: urn:lsid:zoobank.org:act:C16237C7-781C-4BA1-BA36-D3D5F09A6216

Description of male (three specimens; measurements of holotype in parentheses). Medium-sized nematodes. Body whitish, elongate, 7.9–9.0 mm (7.9 mm) long, maximum width 326–449 (326) at middle of body; width at level of oesophastome 218–258 (218), at middle of oesophagus 204–258 (204). Narrow lateral alae present, extending from approximately base of level of pseudobuccal capsule to level of posterior oesophagus (Figs. 1B, 2E,F). Length of alae in one paratype 898, width 24. Cephalic end somewhat asymmetrical in lateral view (Figs. 1A, 2A). Oral aperture dorsoventrally elongate, surrounded by raised narrow membranous ala (collarette) supported by row of ca 100 minute basal teeth. Four submedian cephalic papillae and pair of lateral amphids present (Figs. 1C, 2A–C). Oesophagus muscular, expanded at anterior end to form bulbous pseudobuccal capsule (oesophostome); posterior part of oesophagus also expanded, broader than oesophastome (Fig. 1A,B). Length of entire oesophagus 1.1–1.2 mm (1.1 mm), representing 13–14% (14%) of whole body length; length of oesophastome 313–354 (313), its width 190–218 (190); minimum width of oesophagus 95–109 (95); maximum width of posterior part of oesophagus 218–258 (218). Oesophagus opens into intestine through large valve (Fig. 1A,B).

Distance of nerve ring from anterior extremity 408–422 (408), representing 35–37% (37%) of oesophageal length. Deirids small, slightly asymmetrical, situated short distance anterior to end of oesophagus (Figs. 1A,B,D, 2D–F). Postdeirids not found. Excretory pore just posterior to oesophago-intestinal junction (Fig. 1A). Deirids and excretory pore 891–979 (891) and 1,197–1,360 (1,197), respectively, from anterior end of body. Posterior end of body curves ventrally. Ventral precloacal sucker and subventral oblique muscle bands in region between sucker and cloacal aperture well developed (Figs. 1F, 2G, 3A); distance of sucker from cloaca 870–884 (870). Anterior lip of cloaca elevated, forming large posterior transverse-oval outgrowth covering cloacal aperture; large median dome-shaped formation containing 2 papillae present just anterior to anterior cloacal lip (Figs. 1E–G, 2G, 3A–D); elevated anterior cloacal lip and median dome-shaped formation 15–18 (18) high and both together 63–72 (63) long in lateral view.

Spicules equal, alate, 748–789 (748) long, with pointed distal ends (Fig. 1F,H), representing 9–10% (10%) of body length; width of distal end of spicule in holotype (20) in lateral view. Gubernaculum small, rod-like in lateral view, well sclerotised, 150–163 (163) long (Fig. 1E,F). Genital papillae 10 pairs: 4 pairs of subventral preanal papillae, 1 pair of subventral anal papillae and 5 pairs of postanal papillae, of which 3 pairs subventral and 2 pairs dorsolateral. First pair of subventral postanal papillae just posterior to cloacal aperture, second and third pairs of subventral postanals in posterior half of tail; dorsolateral pairs of postanals at level of first and second pairs of subventrals (Figs. 1E,F,G, 2G, 3A–C). Phasmids small, lateral, slightly anterior to level of second pair of subventral postanal papillae (Figs. 1E–G, 3B). Tail conical, 204–286 (245) long, with rather obtuse tip (Figs. 1E–G, 3A–C).

Female: Not known.

Type host: Brownspotted grouper Epinephelus chlorostigma (Valenciennes) (Perciformes: Serranidae). The three fish specimens examined ranged between 380–570 mm in fork length and 719–2,700 g in weight. Tissue sample of fish JNC3141 deposited in the collections of the MNHN as MNHN-IC-IC-TI-2145.

Site of infection: Intestine.

Type locality: Along slope off the barrier reef, off Nouméa, New Caledonia, coordinates 22°34’S; 166°26’E (collected 1 December 2009).

Prevalence and intensity: 1 fish infected/3 fish examined; 3 nematodes.


Etymology: The specific name of this nematode relates to the generic form of the generic name of the host.

Remarks. Existing taxonomic problems of the numerous species of Cucullanus due to a rather uniform morphology and often inadequate descriptions of these nematodes make a detailed comparison among all of these species impossible (Moravec et al. 2005). Therefore, these parasites are mostly dealt with according to their host groups or their zoogeographical region, as has been described by Petter (1974) and Moravec et al. (1997), respectively.

Cucullanus epinepheli sp. n. can be easily distinguished from all its congeners by the unique structure of the an-
Fig. 1. *Cucullanus epinepheli* sp. n., male from *Epinephelus chlorostigma* (Valenciennes). A, B – anterior end, lateral and dorsoventral views; C – cephalic end, apical view; D – deirid and part of cervical ala, dorsoventral view; E – tail, lateral view; F – posterior end of body, lateral view; G – tail, ventral view; H – distal end of spicule.

terior cloacal lip. Moreover, of the many species of *Cucullanus*, the presence of cervical lateral alae, as found in *C. epinepheli*, was described only in *Cucullanus* (*Truttaedacnitis*) *truttae* Fabricius, 1794, a parasite mainly of freshwater salmonids (Salmonidae) in the Holarctic (see Moravec and Malqvist 1977, Moravec 2013). The experimental study of the larval morphogenesis of *C. truttae* showed that the third- and fourth-stage larvae of this species possessed rather wide cervical alae, whereas only narrow alae remained in conspecific adults after the last larval moult (Moravec 1979). Nevertheless, *C. truttae* differs considerably from *C. epinepheli* in other morphological features, such as the structure of the cephalic end, a more anterior location of deirids and the excretory pore relative to the oesophago-intestinal junction, the posterior portion of the oesophagus distinctly narrower than the pseudobuc-
cal capsule, and the absence of the median precloacal papilla and the markedly elevated anterior cloacal lip with a posterior outgrowth (Berland 1970, Moravec 2013).

Another feature of *C. epinepheli* is the presence of two small papillae on the median precloacal elevation. The presence of a single median precloacal papilla was found in several *Cucullanus* spp., e.g. *C. australiensis* Baylis, 1927, *C. baylisi* Campana-Rouget, 1961, *C. bonaerensis* Lanfranchi, Timi et Sardella, 2004, *C. bourdini*, *C. costaricensis* López-Caballero, Osorio-Sarabia et Garcia-Pri-

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**Fig. 2.** *Cucullanus epinepheli* sp. n., scanning electron micrographs of male from *Epinephelus chlorostigma* (Valenciennes). A–C – cephalic end, lateral, apical and subdorsal views, respectively; D – deirid; E, F – anterior end of body with distinct cervical alae, lateral and dorsal views, respectively (arrows indicate deirids); G – posterior end of body, ventral view (arrows indicate papillae of first three preanal pairs. Abbreviations: a – amphid; b – cephalic papilla; c – ventral sucker.
Fig. 3. Cucullanus epinepheli sp. n., scanning electron micrographs of male from Epinephelus chlorostigma (Valenciennes). A – posterior end of body, lateral view (arrows indicate papillae of first three preanal pairs); B – tail, lateral view (arrow indicates phasmid); C – same, ventral view; D – region of cloaca, ventral view. Abbreviations: c – ventral sucker; d – cloaca; e – precloacal median dome-shaped elevation containing two small papillae; f – outgrowth of anterior cloacal lip covering cloacal aperture.


To date, C. mycteropercae, a parasite of Mycteroperca bonaci Poey (Serranidae) from off the coast of Yucatán, Mexico, has been the only nominal species of Cucullanus described from a serranid fish host (Mejía-Madrid and Guilién-Herrández 2011). Accordingly, C. epinepheli is the second species known to parasitise hosts of this fish family. Both of these species are characterised by the presence of the elevated anterior cloacal lip with a distinct bearing an outgrowth has also been described for C. mycteropercae, but, in this case, the outgrowth is small, oriented ventrally and not covering the cloacal aperture (see Mejía-Madrid and Guilién-Herrández 2011). However, as mentioned above, the presence of the elevated anterior cloacal lip provided with a conspicuously large posterior outgrowth covering the cloacal aperture of males of C. epinepheli is unique among species of Cucullanus described to date. The elevated anterior cloacal lip
outgrowth (see above), in which they differ from all other congeneres. In addition to these nominal species, Justine et al. (2010) mentioned the presence of an unidentified Cucullanus sp. in Variola louti (Forskal) and Variola albimarginata Baissac off New Caledonia.

Cucullanus stossichi Barreto, 1922 was reported from a North American serranid fish Mycteropeca venenosa (Linnaeus), but this inadequately described species was based on data of Linton (1905, 1907) (see Barreto 1922), who had evidently misidentified his specimens, collected from hosts belonging to several families, as Heterakis foveolata (= Cucullanus foveolatus Rudolph, 1809, a junior synonym of C. cirratus Müller, 1777, a parasite of gadiform fishes). However, in view of the improbability of a single cucullanid species occurring in such a wide range of hosts, it is probable that C. stossichi represents several species and should be considered a species inquirenda. Bharathalakshmi and Sudha (1999) reported Cucullanus fastigatus (= Dichelyne fastigatus Chandler, 1935) from Epinephelus undulosus (Quoy et Gaimard) (Serrinidae) and two other host species belonging to the Haemulidae and Scaenidae off India, but it was a probable misidentification of these nematodes (Moravec et al. 2011).

Cucullanus epinepheli is the second known nominal species of this genus reported from marine fishes in New Caledonian waters.

Although only three specimens of E. chlorostigma were collected off New Caledonia for parasitology, identifications and descriptions of new species were obtained for copepods (Justine et al. 2010), digeneans (Bray and Justine 2011, 2012, 2013), cestodes (Beveridge et al. 2014), monogeneans (Justine and Henry 2010, Schoelinc et al. 2012) and nematodes (the present paper).

Camallanidae Railliet et Henry, 1915

Procamallanus (Spirocamallanus) sinespinis sp. n.

Figs. 4, 5

ZooBank number for species: urn:lsid:zoobank.org:act:583E0B28-A95D-4339-8A29-08D794FD9E89

Description: Medium-sized nematode with finely transversely striated cuticle. Mouth aperture oval, surrounded by 12 submedian cephalic papillae arranged in three circles, each formed by four papillae; papillae of outer circle distinctly larger; each of four small inner papillae located near margin of oral aperture accompanied by distinct proximal pore; pair of small lateral amphiids present (Figs. 4D, 5A,B,D). Buccal capsule orange-brown, thick-walled, slightly longer than wide, with simple, well-developed basal ring. Maximum width/length ratio of buccal capsule 1 : 1.16–1.33. Inner surface of capsule provided with 10–12 spiral ridges in lateral view, 2–3 of them being incomplete (Fig. 4A–C). Muscular oesophagus somewhat shorter than glandular oesophagus; both parts of oesophagus slightly expanded near their posterior ends (Fig. 1A). Intestine brown, narrow. Deirids small, simple, rounded, situated at short distance posterior to base of buccal capsule (Figs. 4B–C,H, 5A). Excretory pore approximately at level of junction of both parts of oesophagus (Fig. 4A).

Male (three specimens; measurements of holotype in parentheses). Length of body 19.7–23.2 mm (23.2 mm), maximum width 313–340 (313). Buccal capsule including basilar ring 90–96 (96) long, its width 78–81 (81); basilar ring 9–15 (12) long and 57–60 (60) wide. Maximum width/length ratio of buccal capsule 1 : 1.16–1.19 (1 : 1.19). Spiral ridges 10–12 (12), 2–3 (2) of which incomplete. Length of muscular oesophagus 530–585 (585), maximum width 90–105 (90); length of glandular oesophagus 734–843 (734), maximum width 102–163; length ratio of muscular and glandular oesophagus 1 : 1.25–1.59 (1 : 1.25). Length of entire oesophagus and buccal capsule representing 6–7% (6%) of body length. Deirids, nerve ring and excretory pore 159–177 (159), 326–340 (340) and 571–653 (653) from anterior extremity, respectively.

Posterior end of body ventrally bent, provided with wide, vesiculated caudal alae supported by pedunculate papillae; anteriorly alae interconnected, forming a kind of pseudosucker, and posteriorly reaching caudal terminal knob (Figs. 4F,G, 5C,E,F). Preanal papillae: 3 pairs of subventral pedunculate papillae, of which second and third pairs closer to each other than first and second pairs; postanal papillae: 6 pairs of pedunculate papillae, 4 subventral and 2 lateral (last pair representing phasmids); additional 2 pairs of small, transversely-elongate sessile ventral papillae surrounding cloacal opening (Figs. 4F,G, 5C,E,F).

Spicules similar in shape, unequal, with sharply pointed distal ends; large (right) spicule 465–525 (465) long; small (left) spicule less well sclerotised, 218–231 (218) long. Length ratio of spicules 1 : 2.13–2.27 (1 : 2.13). Gubernaculum absent. Tail conical, 150–177 (163) long, with small terminal cuticular knob (Figs. 4F,G, 5C,E,F).

Female (one ovigerous specimen, allotype). Length of body 35.7 mm, maximum width 612. Buccal capsule including basilar ring 120 long and 90 wide; basilar ring 12 long and 96 wide. Maximum width/length ratio of buccal capsule 1 : 1.33. Number of spiral ridges 12, of which two incomplete. Length of muscular oesophagus 571, maximum length 95; length of glandular oesophagus 1,006, maximum width 150; length ratio of muscular and glandular oesophagus 1 : 1.76. Length of entire oesophagus and buccal capsule representing 5% of body length. Deirids, nerve ring and excretory pore 225, 408 and 789, respectively, from anterior extremity. Vulva equatorial, 17.7 mm from anterior extremity, at 50% of body length. Vulval lips not elevated. Uterus filled with numerous eggs. Tail broad, rounded, its posterior end abruptly narrowed to form terminal digit-like protrusion with smooth, rounded tip; length of entire tail 270; digit-like protrusion 60 long, 21 wide (Fig. 4E).

Type host: Silver grunt Pomadasys argenteus (Forskål) (Perco- formaes: Haemulidae). The two fish examined ranged between 230–250 mm in fork length and 227–262 g in weight.

Site of infection: Digestive tract.

Type locality: Off New Caledonia (collected 29 April 2010).

Prevalence and intensity: 1 fish infected/2 fish examined; 4 nematodes.
Fig. 4. Procamallanus (Spirocamallanus) sinespinis sp. n. from Pomadasys argenteus (Forskål). A – anterior, oesophageal part of male body, lateral view; B – anterior end of subgravid female, lateral view; C, D – cephalic end of male, lateral and apical views, respectively; E – tail of subgravid female, lateral view; F – posterior end of male, lateral view; G – tail of male, ventral view; H – shape of deirid.


Etymology: The specific name of this nematode sinespinis consists of the Latin preposition sine (= without) and the ablative plural of the substantive spina (= thorn, spike), i.e. sine spinis (= without spikes); this relates to the fact that there are no cuticular spikes on the female tail tip.

Remarks. The general morphology of the new species shows that it belongs to the subgenus Spirocamallanus Olsen, 1952 of the genus Procamallanus in the conception of Moravec and Thatcher (1997). By its morphology, it should be assigned to the group of Spirocamallanus species characterised by the presence of wide caudal alae, three pairs of pedunculate preanal papillae and two unequal spicules, that are mostly parasites of marine fishes (Petter 1979). A great majority of species of this group is
characterised by the presence of two caudal spikes, one dorsal and one ventral, on a digitiform projection of the female tail (González-Solís et al. 2002, Yooyen et al. 2011), whereas these are lacking in only a few species. As pointed out by Petter et al. (1977), Rigby and Adamson (1997) and Moravec et al. (2006), the shape and structure of the female tail appears to be constant within species of Procamallanus (Spirocamallanus).

Based on the shape of the female tail and the absence of any terminal spikes, P. (S.) sinespinis sp. n. resembles only P. (S.) macaensis (Vicente et Santos, 1972) from several species of marine fishes off Brazil (Vicente and San-
tos 1972, Luque et al. 2011), *P. (S.) mexicanus* Moravec, Salgado-Maldonado et Caspeta-Mandujano, 2000 from the freshwater cichlid *Cichlasoma geidlisi* (Regan) (Cichlidae) in Mexico (Moravec et al. 2000) and *P. (S.) murrayensis* Johnston et Mawson, 1940 from freshwater perciform fishes *Pseudaphritus urvilli* (Valenciennes) (Pseudaphritidae), *Macquaria colonorum* (Günther) and *M. ambigua* (Richardson) (both Percichthyidae) in Australia (Johnston and Mawson 1940).

However, in contrast to the new species, *P. (S.) macaen-sis* has the right spicule markedly longer (610–620 µm vs 465–525 µm), whereas the same spicule is distinctly shorter (290 µm) in *P. (S.) murrayensis*. The right spicule of *P. (S.) mexicanus* is rather similar (456–480 µm vs 465–525 µm) to that of *P. (S.) sinespinis* and both of these species have the same number (10–12) of spiral ridges in the buccal capsule, but the arrangement of their postanal papillae is different. The male tail tip of *P. (S.) mexicanus* bears a conical cuticular spike (vs a knob-like structure in *P. (S.) sinespinis*) and the female tail of the former species is shorter (147 µm vs 270 µm) and has a different shape (its anterior portion is narrow and conical vs broad and posteriorly rounded). Moreover, *P. (S.) mexicanus* and *P. (S.) murrayensis* are parasites of freshwater fishes, whereas the host of *P. (S.) sinespinis* is the marine fish.

Moravec et al. (2006) reported a subgravid female nemato-de, designated as *Procamallanus (Spirocamallanus) sp. 3*, from the marine fish *Scolopsis bilineata* (Blok) (Nemipteridae) from off New Caledonia. The shape of its tail is similar to that of *P. (S.) sinespinis*, but since the number of spiral ridges in the buccal capsule is different (13 vs 10–12), as well as the location of deirids (at the level of the nerve ring vs at a short distance posterior to the buccal capsule), this specimen clearly represents another species.

*Procamallanus (S.) sinespinis* is the fifth nominal species of the subgenus *Spirocamallanus* reported from marine fishes in New Caledonian waters.

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