



MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF *DICHELYNE* (*NEOCULLANELLUS*) *DAKARENSIS* N. SP. (NEMATODA: CUCULLANIDAE) PARASITIZING AN ECONOMICALLY IMPORTANT MARINE FISH, THE SOMPAT GRUNT, *POMADASYS JUBELINI* (PERCIFORMES: HAEMULIDAE), FROM HANN BAY IN DAKAR, SENEGAL

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KEY WORDS ABSTRACT

Dichelyne dakarensis
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Sompat grunt
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Haemulidae
Dakar
Morphological and molecular
characterization

Nematodes collected from the intestine of sompat grunt *Pomadasys jubelini* Cuvier, 1830 from Hann Bay in Dakar, Senegal represent a new species described herein as *Dichelyne* (*Neocuccanellus*) *dakarensis* n. sp., and investigated with the use of light and scanning electron microscopy. The new species differs from its congeners based on several characteristics, especially because the subgenus *Neocuccanellus* is the only 1 that has 2 ceca. In addition, the new species diagnosis is based on the number and arrangement of the caudal papillae as well as the size of the veil of spicules. Morphological data were supported by molecular analysis. Results obtained using *SSU* rDNA and *COI* distinguished the present specimens from other cucullanids. Molecular data indicated the close relatedness between the new species and *Dichelyne cotylophora* Ward and Magath, 1917.

Nematofauna of marine fish has been the subject of morphological studies in Senegal that resulted in the description of several species (Campana-Rouget, 1957; Vassiliades and Diaw, 1978; Vassiliades and Petter, 1981; Vassiliades, 1982). Research on nematode parasites of marine fish started in the 1950s with the work of Campana-Rouget (1955, 1956, 1957). Later, 2 new species were described by Vassiliades and Diaw (1978) and Vassiliades and Petter (1981). Substantial data on the nematofauna of Senegalese marine fish were obtained with the inventory of Vassiliades (1982). Twenty-two genera belonging to 10 families and 3 orders were identified.

Currently, the family Cucullanidae Cobbold, 1864 comprises 6 genera: *Campanarougetia* Le Van Hoa and Pham-Ngoc-Khue, 1967; *Cucullanus* Muller, 1777; *Dichelyne* Jägerskiöld, 1902; *Neocuccanellus* Travassos, Artigas and Pereira 1928; *Oceanicuccallanus* Schmidt and Kuntz, 1969; and *Truttaedacnitis* Petter, 1974 (Anderson et al., 2009; Gibbons, 2010), parasites of marine, brackish, and freshwater fish and among which the genera *Cucullanus* and *Dichelyne* are the largest groups (Petter, 1974; Li et al., 2014a; Pereira and Luque, 2017). In Senegal, cucullanid parasites of marine fish belong to these groups with, respectively, 9 and 2 species. The

genus *Dichelyne* is represented by *Dichelyne* (*Dichelyne*) *mauritanicus* Gendre, 1928 and *Dichelyne* (*D.*) *pomadasysi* Vassiliades and Petter, 1981, described, respectively, in *Plectorhinchus mediterraneus* Guichenot, 1850 and *Pomadasys jubelini*, both belonging to the family Haemulidae.

In this study, a new species of *Dichelyne* parasite of *P. jubelini* is described using morphological and molecular data.

MATERIALS AND METHODS

Sample collection

Fish were purchased from the fish market in Hann Bay (14°42'58.035"N, 17°25'23.727"W), Dakar, Senegal in April 2018 and identified using morphological keys (Seret and Opic, 1981; Bellemans et al., 1988; Bauchot, 2003). Fish nomenclature and classification follow FishBase (Froese and Pauly, 2022). Nematodes were collected from the intestine of fish, washed in saline, fixed, and stored in 70% ethanol.

Light and scanning electron microscopy

For light microscopy examination, nematodes were cleared in lactophenol. Drawings were made using a Zeiss drawing attachment (Department of Animal Biology, Faculty of Sciences and Technics, Cheikh Anta Diop University).

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Specimens used for scanning electron microscopy were dehydrated through a graded ethanol series (70, 90, and 100%) for 30 min in each concentration and critical-point dried in an Emitech K850 (Quorum Technologies Ltd., Ashford, U.K.); mounted on stubs, coated with gold/palladium in a SC7640 (Quorum Technologies Ltd., Newhaven, U.K.) and examined in a Hitachi S-3400N scanning electron microscope (Hitachi High-Technologies Corporation, Tokyo, Japan) at an accelerating voltage of 10 kV (University of Corsica Pascal Paoli, Corte, France). Measurements are in micrometers unless otherwise stated.

Molecular and phylogenetic study

Specimens were collected and stored in 95% ethanol prior to molecular analysis. Genomic DNA was isolated using EZNA DNA KIT (OMEGA [ref D3396-02], Norcross, Georgia) following the manufacturer's instructions. Target genetic regions included a partial sequence of the mitochondrial cytochrome *c* oxidase subunit I (*COI*) genes, as well as a fragment of the nuclear SSU ribosomal DNA (rDNA). The primers used for *COI* amplification were COI1F (5-TTT TTT GGT CAT CCT GAG GTT TAT-3) and COI1R (5-ACA TAA TGA AAA TGA CTA ACA AC-3) (Xu et al., 2017). The primers used for SSU rDNA were D1-F (5-GCC TAT AAT GGT GAA ACC GCG AAC-3) and D1-R (5-CCG GTT CAA GCC ACT GCG ATT A-3) (Abdel-Ghaffar et al., 2014). Conventional polymerase chain reaction (PCR) was performed in a total volume of 25 ml consisting of 1 µl of gDNA, 1X Colorless GoTaq flexi buffer (Promega, Madison, Wisconsin), 1.5 mM MgCl₂ (Promega), 0.2 mM of each dNTP (Promega [reference U1420]), 0.3 µM forward primer, 0.3 µM reverse primer, and 1U GoTaq G2 hotstart Polymerase (Promega [reference M7405]). DNA amplification consisted of an initial step at 95 C for 3 min, followed by 35 cycles of 95 C for 30 sec, 45 C for 40 sec, and 72 C for 35 sec (*COI*) or 95 C for 40 sec, 56 C for 40 sec, and 72 C for 1 min 40 sec (*SSU* rDNA), and a final extension at 72 C for 2 min. PCR products were visualized by electrophoresis in 5% agarose gel. Positive products were sent to Genoscreen (France) for sequencing in both directions using the same PCR primers. Sequences were assembled and manually edited using Sequencher (GeneCodes Corp) to remove any ambiguities between strands and sequences. *COI* and *SSU* rDNA sequences obtained in the present study were aligned with those of other cucullanids acquired from GenBank, using K2+G (Nei and Kumar, 2000; Tamura et al., 2013). Maximum-likelihood trees were performed using Mega V7.0. Nodal support was assessed by bootstrap resampling with 1,000 replications. Trees were rooted by *Caenorhabditis elegans*.

RESULTS

In total, 79 nematodes, including 60 larvae and 19 adults (8 males and 11 females) were collected. For light and scanning microscopy, 11 (5 males and 6 females) and 4 (1 male and 3 females) nematodes were respectively used. Molecular studies were based on 2 males and 2 females.

DESCRIPTION

Dichelyne (Neocullanellus) dakarensis n. sp.

(Figs. 1–3)

General description: Medium-sized, whitish nematodes. Body elongate, cylindrical, maximum width at midbody. Cuticle with

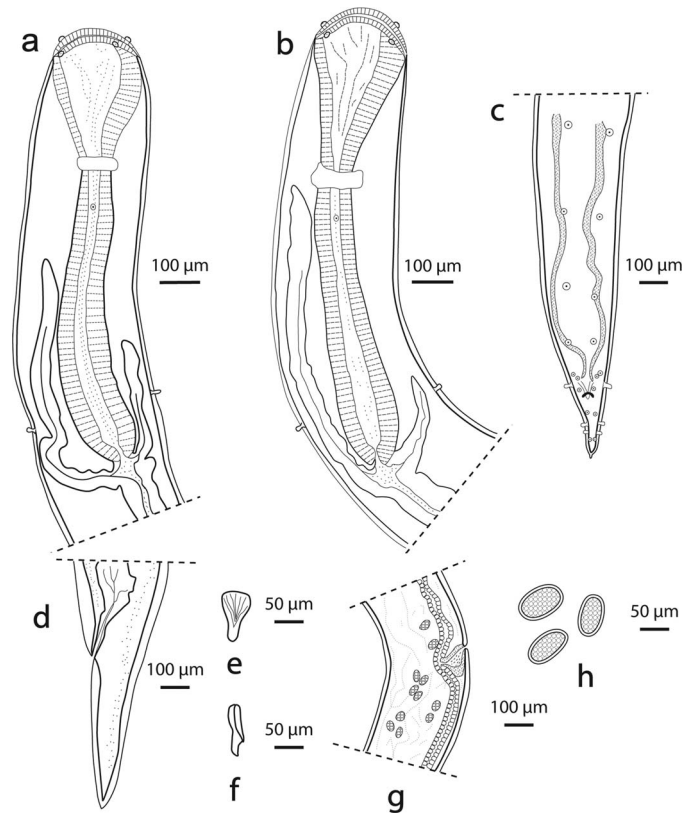


Figure 1. *Dichelyne (Neocullanellus) dakarensis* from *Pomadasyss jubelini* (Perciformes: Haemulidae) in Hann bay in Dakar. (a) Anterior extremity of male (ventral view). (b) Anterior extremity of female (ventral view). (c) Posterior extremity of male (ventral view). (d) Posterior end of female (lateral view). (e) Gubernaculum (lateral view). (f) Gubernaculum (ventral view). (g) Region of vulva (lateral view). (h) Eggs.

fine transverse striations. Lateral alae absent. Oral aperture dorsoventrally elongate, slit-like, surrounded by distinct collarette, bearing row of small denticles on inner surface (Figs. 1a, b, 2a, b, 3a, b); 2 pairs of prominent, sublateral cephalic papillae and pair of median lateral amphids (Figs. 2b, 3c, d). Esophagus muscular, widely expanded anteriorly to form pseudobuccal capsule (esophastome); posterior part of esophagus with a bulge narrower than pseudobuccal capsule (Fig. 1a, b). Nerve ring at about one-third of esophageal length. Two unequal intestinal ceca present, 1 dorsal and 1 ventral, variable in length, reaching nerve-ring level in some specimens (Fig. 1a, b). Small and conical deirids (Fig. 2c) located slightly anterior to esophagus–intestinal junction. Post derides not observed. Excretory pore slightly posterior to the nerve ring (Fig. 1a, b). Tail conical in both sexes, surmounted by a point (Figs. 1c, d, 2d, 3e).

Male (based on 5 specimens; measurements of holotype in parentheses) (Figs. 1, 2): Body length: 2.2–6.8 mm (6.3), maximum width: 110–400 (400) at middle of body, esophagus length: 435–930 (930). Nerve ring, excretory pore, and deirids at 150–310 (310), 160–400 (400), and 410–900 (900) respectively, from anterior extremity. Ventral sucker absent. Spicules subequal, alate, pointed at distal end (Figs. 1c, 2e): left: 610–1,200 (800) long, right: 650–1,210 (875) long, representing 12.70–27.73 (12.7)% and 13.89–29.55 (13.89)% of body length, respectively. Gubernaculum well sclerotized, shovel-like in ventral view (Fig. 1e, f), 65–80 (80) long, and 20–45

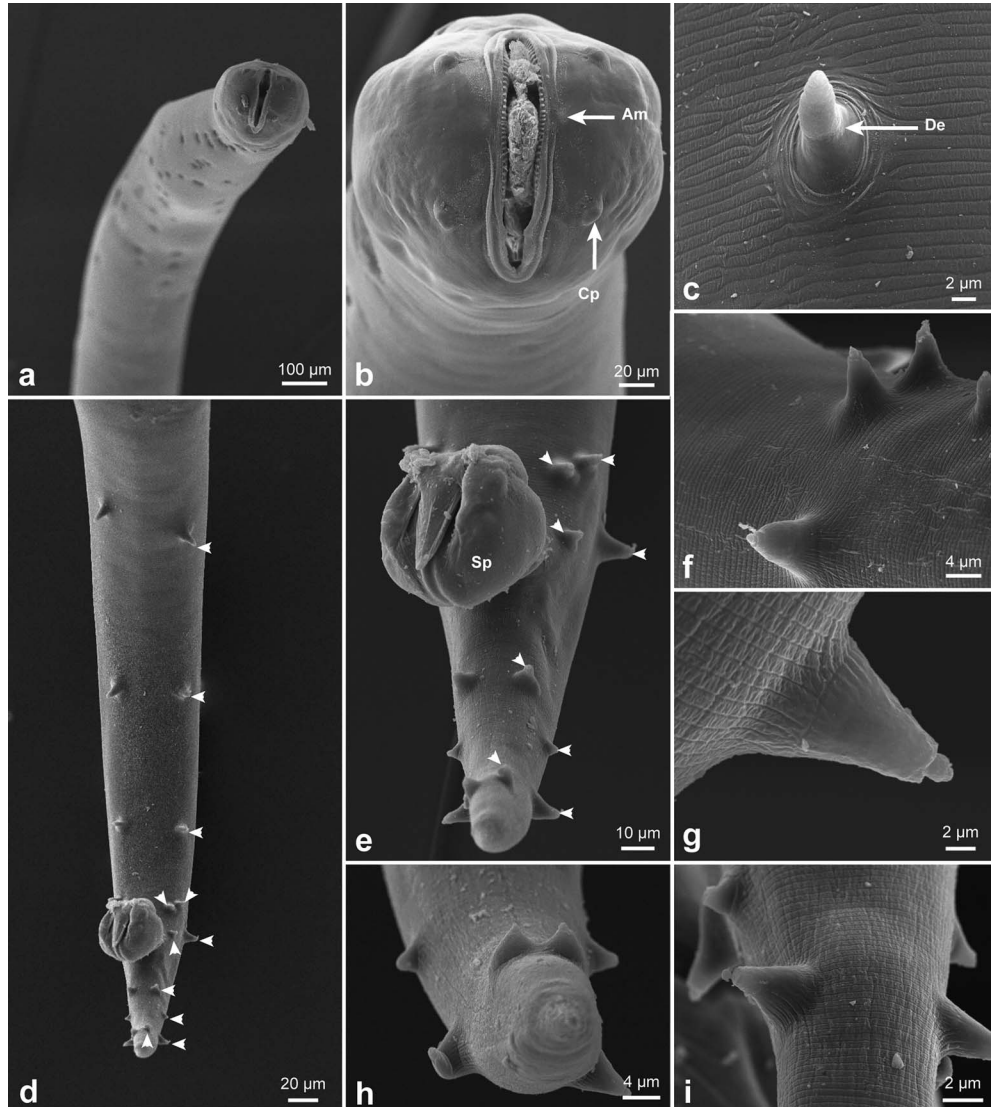


Figure 2. Male specimen of *Dichelyne* (*Neocullanelhus*) *dakarensis*. (a) Anterior extremity. (b) Cephalic end. (c) Deirid. (d) Caudal extremity arrow heads showing caudal papillae. (e) Caudal tail with spicules and ad-post cloacal papillae (arrow heads). (f) Lateral view of preanal papillae. (g) Ventral view of preanal papilla. (h) Ventral view of caudal end. (i) Dorsal view of post cloacal papillae. Abbreviations: Amphide, Am; Cephalic papillae, Cp; Deirid, De; Spicules, Sp.

(45) wide. Caudal papillae, taste buds-like, having an end with a sensory silk that can invaginate (Fig. 2f, g) or stretch (Fig. 2h, i). Twelve pairs of caudal papillae: 4 pairs of subventral preanal papillae (Figs. 1c, 2d, g), 4 pairs of adanal papillae including 3 subventral and 1 lateral (Figs. 1c, 2d, e) and 4 pairs of postanal papillae, of which 2 are subventral and 2 lateral (Figs. 1c, 2d, e, h, i).

Female (based on 6 gravid specimens; measurements of allotype in parentheses) (Figs. 1, 3): Body 3.7–11.6 (9.6) mm long, maximum width of midbody 150–500 (500). Esophagus length: 575–1,300 (1,120) long. Nerve ring, excretory pore, and deirids at 140–425 (370), 250–600 (490), and 600–1,200 (1,050) respectively from anterior extremity. Vulva slit-like, preequatorial, at 1,400–4,500 (1,800) from anterior extremity, 17.24–54.17 (43.90)% of body length. Vulva not protuded. Vagina muscular, anteriorly directed from vulva. Didelphic uterus containing several eggs (Fig. 1g). Eggs oval, not embryonated (Fig. 1h), measuring 70–80 × 50–60. Well-developed anus (Fig. 3f). Tail 100–470 (470) long. Pair of conspicuous papillae-like phasmids at half distance from anus to tail tip (Fig. 3e, g).

Taxonomic summary

Type host: *Pomadasys jubelini*.

Type locality: Hann Bay, Dakar, Senegal (14°42'58.035"N, 17°25'23.727"W).

Site of infection: Intestine.

Prevalence and intensity: 28 out of 40 (70%) *P. jubelini* were infected by *D. (N.) dakarensis* n. sp., with a mean intensity of 2.8 ± 1.25 (1–5).

Type specimens: Holotype: 1 male, allotype: 1 female, paratypes: 5 (3 males, 2 females). Deposited in the National Museum of Natural History, Paris, France: *D. (N.) dakarensis* n. sp. Holotype: HEL1208 and paratype: HEL1209.

ZooBank registration: urn:lsid:zoobank.org:act:29DEB90A-7D2F-4CBE-9C95-4D1FC66D3FA6.

Etymology: The specific name refers to the type locality.

Molecular study

From 4 specimens analyzed, 4 sequences were obtained for the SSU rDNA and 3 for the COI. A unique halotype (MZ297464)

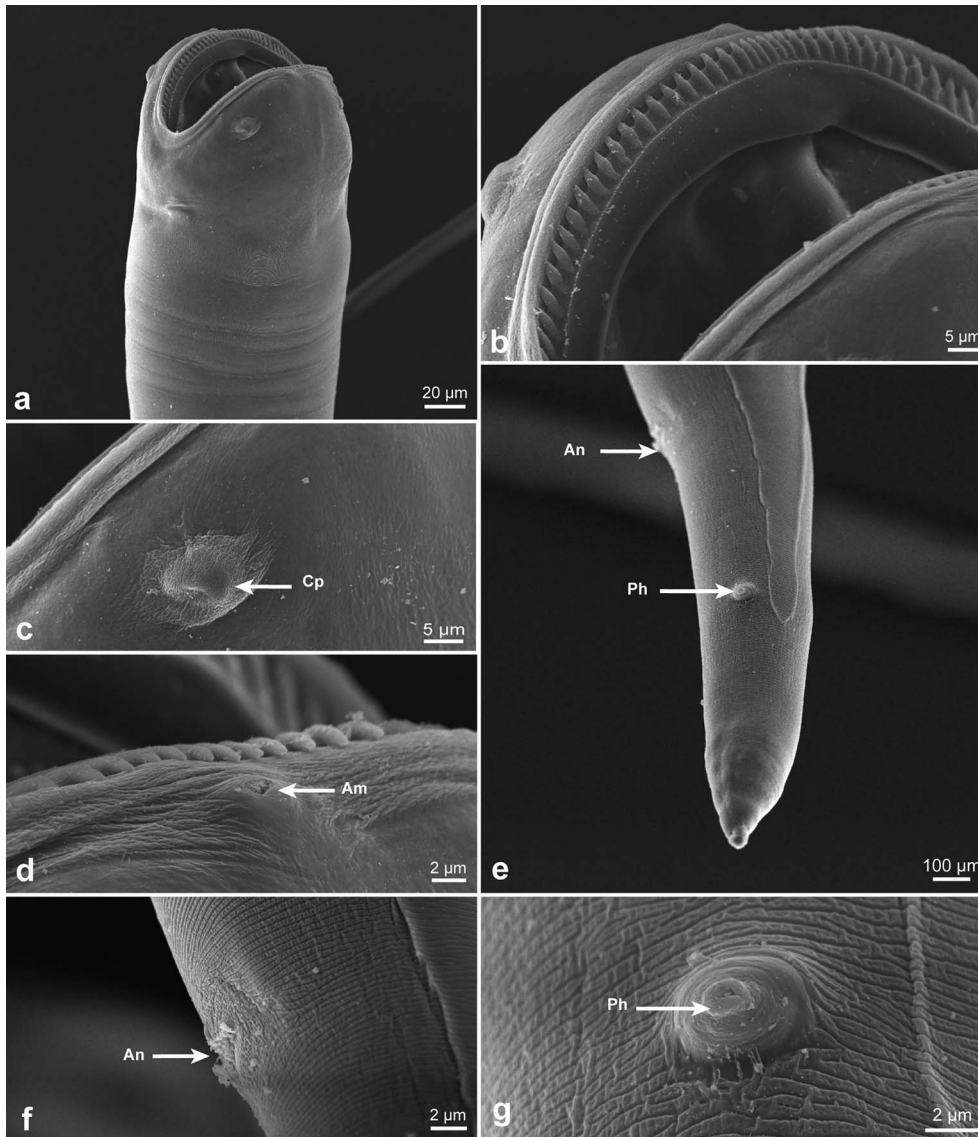


Figure 3. Female specimen of *Dichelyne (Neocucullanellus) dakarensis*. (a) Anterior extremity. (b) Oral aperture with denticles. (c) Cephalic papillae. (d) Amphide. (e) Caudal extremity. (f) Anus. (g) Phasmide. Abbreviations: Amphide, Am; Anus, An; Cephalic papillae, Cp; Denticules, De; Phasmid, Ph.

was obtained for the SSU rDNA and 3 (MZ274397, MZ274398, MZ274399), for the COI.

Tree inferred from analyses of SSU rDNA by using the maximum-likelihood method showed that the new species and *Dichelyne cotylophora* Ward and Magath, 1917 are closely related and constitute a clade supported by significant bootstrap value (Fig. 4). The maximum-likelihood tree obtained with COI gene reveals sister-taxon relationship between *Dichelyne breviculus* Li, Xu and Zang, 2014 and the new species, but with low bootstrap value (Fig. 5).

DISCUSSION

The genus *Dichelyne* is subdivided into 3 subgenera according to the presence or absence of a sucker and the number of pairs of cloacal papillae greater than 11 (Petter, 1974). Species that have a sucker correspond to the subgenus *Cucullanellus* Törnquist 1931, those without sucker form the subgenus *Dichelyne* Jägerskiöld 1902, and those that have more than 11 pairs of cloacal papillae the subgenus *Neocucullanellus*.

According to the number of cloacal papillae (more than 11 pairs) the present specimens were allocated in the subgenus *Neocucullanellus*, with currently 5 species: *Dichelyne (N.) dighaensis* Gupta and Masoodi, 1989 (Gupta and Masoodi, 1989), *Dichelyne (N.) laticeps* Baylis, 1947 (Baylis, 1947; Hasegawa et al., 1991), *Dichelyne (N.) longispiculum* Khan, 1969 (Petter, 1974; Soota, 1983), *Dichelyne (N.) yamagutii* Kalyankar, 1971 (Petter, 1974; Soota, 1983), and *Dichelyne (N.) wallagoni* Chakravarty and Majumdar, 1961 (Chakravarty and Majumdar, 1961; Soota, 1983). Comparisons between species were made based on geographic origin, host family, number and arrangement of caudal papillae, and ratio of spicule length/body length.

No species of the subgenus *Neocucullanellus* from the family of Haemulidae has been described in West Africa. Therefore, *D. (N.) dakarensis* n. sp. is recorded for the first time in Haemulidae in Senegal. However, in addition to features that distinguish the subgenera *Neocucullanellus* from the subgenus *Dichelyne*, it differs from *D. (D.) pomadasysi* and *D. (D.) mauritanicus* by the anterior location of its excretory pore relative to its deirids and the pre-quotational position of its vulva (Table I). It also has 2 intestinal

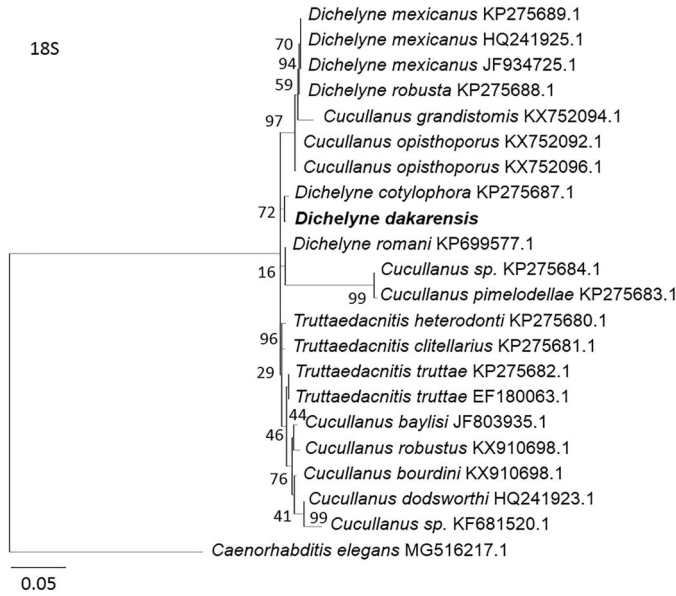


Figure 4. Phylogenetic tree of cucullanids based on SSU rDNA gene. The evolutionary history was inferred by using the maximum-likelihood method based on the Hasegawa–Kishino–Yano model. The clade’s robustness was assessed by bootstrap resampling with 1,000 replications. Trees were rooted by *Caenorhabditis elegans*. All positions containing gaps and missing data were eliminated. There was a total of 704 positions in the final data set. Evolutionary analyses were conducted in MEGA v.7.

ceca, whereas *D. (D.) pomadasysi* and *D. (D.) mauritanicus* have only 1, respectively, present ventrally and dorsally.

The new species can be differentiated from species allocated in the subgenus *Neocucullanellus* based on the presence of 2 intestinal ceca (vs. absent or only 1 present, ventrally or dorsally; Gupta and Masoodi, 1989; Hasegawa et al., 1991; Soota, 1983) and the preequatorial position of its vulva. In addition, it is distinguished by the number and the arrangement of its caudal papillae (Table I). Indeed, *D. (N.) dighaensis* has fewer pairs of papillae than *D. (N.) dakarensis* n. sp.: 11 pairs in *D. (N.) dighaensis* (Gupta and Masoodi, 1989) including 6 preanal, 1 adanal, and 4 postanal pairs. Other species have more pairs of cloacal papillae than the new 1: 13 pairs with an irregular arrangement (4 preanal pairs and 9 postanal) and a single papilla on the upper anal lip in *D. (N.) laticeps* (Baylis, 1947; Hasegawa et al., 1991), 15 pairs including 6 preanal and 9 postanal in *D. (N.) wallagoni* (Chakravarty and Majumdar, 1961, Soota, 1983). *Dichelyne (N.) yamagutii* and *D. (N.) longispiculum* are the only species to have as many pairs of papillae as the new species described, but their arrangement is different: 6 preanal pairs and 6 postanal in *D. (N.) yamagutii* and 7–9 preanal, 1 adanal, and 2–5 postanal in *D. (N.) longispiculum*, unlike our specimens, which have 4 pairs preanal, 4 adanal, and 4 postanal.

The ratio spicule length/body length (Sl/Bl) is considered by many authors to be distinctive (Petter, 1995; Morand and Rigby, 1998; Timi et al., 2009; Moravec and Justine, 2011; Dione et al., 2014; Xu et al., 2014; Li et al., 2014b; Moravec and Scholz, 2017). In this case, our specimens have a Sl/Bl ratio equal to 20.44%. This is much lower than the ratio obtained in *D. (N.) yamagutii* (41.98%), where the spicules occupy almost half of the

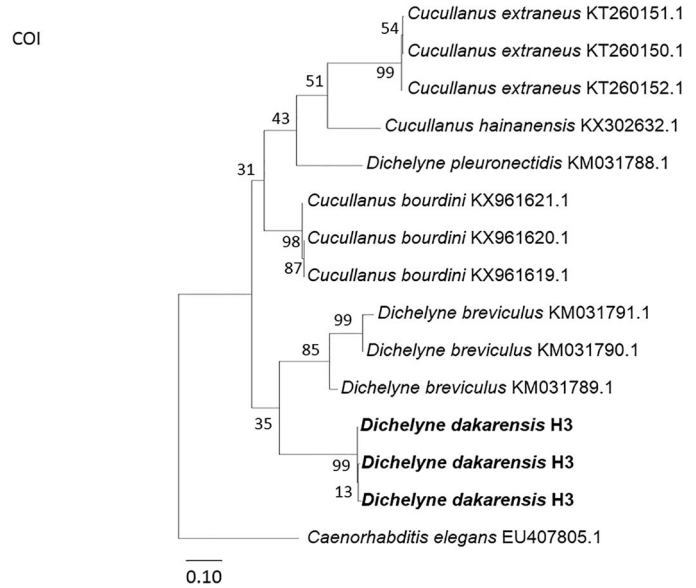


Figure 5. Phylogenetic tree of cucullanids based on COI gene. The evolutionary history was inferred by using the maximum-likelihood method based on the Hasegawa–Kishino–Yano model. The clade’s robustness was assessed by bootstrap resampling with 1,000 replications. Trees were rooted by *Caenorhabditis elegans*. There was a total of 370 positions in the final data set. Evolutionary analyses were conducted in MEGA v.7.

body. On the other hand, this ratio is higher than that observed in *D. (N.) laticeps* (13.3%) and *D. (N.) wallagoni* (14.8%), whereas it is substantially equal to that noted in *D. (N.) dighaensis* (20.41%). However the new species differs from the latter by other criteria such as the number and arrangement of the caudal papillae mentioned in previous paragraph.

Other features help to differentiate our specimens from certain species. These are the absence of gubernaculum and a bifurcated tail in *D. (N.) longispiculum* (Petter, 1974; Soota, 1983) and the presence of sucker in *D. (N.) wallagoni*.

In addition to these comparison criteria, it should be noted that the large size of the veil of spicules also makes it possible to distinguish our specimens from all the species already described in the subgenus *Neocucullanellus*.

The morphological differentiation of nematodes belonging to the family Cucullanidae is based on criteria such as the presence or absence of intestinal cecum, the number and arrangement of caudal papillae, and the presence of a sucker, among others. Genetic characterization has been used as a complement for morphology in taxonomic studies of cucullanid nematodes (Černotíková et al., 2011; Mejía-Madrid and Aguirre-Macedo, 2011; Laetsch et al., 2012; Li et al., 2014a, 2014b, 2016; Xu et al., 2014, 2017; Choudhury and Nadler, 2016; Moravec et al., 2016; Pereira and Luque, 2017). In this sense, the present results represent the first genetic sequences generated for the subgenus *Neocucullanus*, representing an important contribution to the knowledge of these parasites. Thus, the results obtained with rDNA allow us to distinguish our specimens from species belonging to the genera *Cucullanus*, *Truttaedacnitis*, and *Dichelyne*. They corroborate those of Choudhury and Nadler (2016), who argue that no genera within Cucullanidae seems to be monophyletic, based on the current knowledge.

Table 1. Comparison of morphometric characters of the new species with some species of the genus *Dichelyne*. Measurements are given as range (minimum and maximum) or as measurement of the sole type.

<i>Dichelyne</i> species	<i>D. (Neocucullanelus) dakarensis</i> n. sp.	<i>D. (N.) dighaensis</i>	<i>D. (N.) laticeps</i>	<i>D. (N.) longispiculum</i>
Type host	<i>Pomadasys jubelini</i>	<i>Johinus glaucus</i>	<i>Arothron mappa</i>	<i>Protonibea diacanthus</i> <i>Lates calcarifer</i>
Family	Haemulidae	Sciaenidae	Tetraodontidae	Sciaenidae Latidae
Locality	Hann bay, Dakar, Senegal	Digha coast, West Bengal, India	Okinawa, Japan	Karachi, Pakistan
Environment	Marine	Marine	Marine	Marine
Total length males	2.2–6.8	3.09–5.06	11.7	3.103–4.36
Total length females	3.7–11.6	3.10–5.36	9.8–16.8	2.4–6.307
Esophagus length males	0.435–0.93	0.51–0.57	0.88	0.492–0.598
Esophagus length females	0.575–1.3	0.94–1	1.0–1.30	0.46–0.778
Intestinal cecum length males		0.27–0.36	0.63	—
Dorsal lobe	0.325–0.59			
Ventral lobe	0.15–0.26			
Intestinal cecum length females		0.32–0.37	0.66–0.88	—
Dorsal lobe	0.69–1			
Ventral lobe	0.245–0.47			
Nerve ring males	0.15–0.31	0.2–0.22	0.33	0.099–0.291
Nerve ring females	0.14–0.425	0.24–0.26	0.31–0.37	0.35–0.43
Deirids males	0.41–0.9	—	0.86	—
Deirids females	0.6–1.2	—	0.78–0.96	—
Excretory pore males	0.16–0.4	0.36–0.42	0.98	—
Excretory pore females	0.25–0.6	0.52–0.54	0.87–1.03	—
Vulva position	Preequatorial	Postequatorial	Postequatorial	Equatorial or Postequatorial
Spicular length		0.784–0.88		0.864–1.078
Spicular length right	0.65–1.21		1.59	
Spicular length left	0.61–1.2		1.52	
Gubernaculum length	0.065–0.08	—	0.82	—
Papillar arrangement*	12 p: 4 pr/4 ad (3 sv-1 L)/4 po*	11 p: 6 pr/1 ad/4 po	13 p: 9 pr/4 po + 1 ad unpaired	12–13 p: 79 pr/1 ad/2–5 po
Reference	Present work	Gupta and Masoodi (1989)	Hasegawa et al. (1991)	Soota (1983)

<i>Dichelyne</i> species	<i>D. (Dichelyne) pomadasysi</i>	<i>D. (D.) mauritanicus</i>	<i>D. (N.) yamagutii</i>	<i>D. (N.) wallagoni</i>
Type host	<i>Pomadasys jubelini</i>	<i>Plectorhincus mediterraneum</i>	<i>Scoliodon sorrakowah</i>	<i>Wallago attu</i>
Family	Haemulidae	Haemulidae	Carcharhinidae	Siluridae
Locality	Dakar, Senegal	Dakar, Senegal	Bombay, Maharastra	Calcutta
Environment	Marine	Marine	Marine	Marine
Total length males	1.9	—	2.96	15.48
Total length females	5.25	9.6–10	3.12	13.0–15.83
Esophagus length males	0.6	—	0.55	1.37
Esophagus length females	0.8	0.87–1	0.52	1.19–1.24
Intestinal cecum length males	0.4	—	0.18	0.32
Dorsal lobe				
Ventral lobe				
Intestinal cecum length females	0.45	—	0.19	0.26–0.28
Dorsal lobe				
Ventral lobe				
Nerve ring males	0.2	—	0.24	0.57
Nerve ring females	0.3	0.31–0.45	0.22	0.52–0.58
Deirids males	0.33	—	—	—
Deirids females	0.7	0.83–0.95	—	—
Excretory pore males	0.415	—	—	1.0
Excretory pore females	0.8	1.15–1.50	—	0.95–1.0
Vulva position	Postequatorial	Postequatorial	—	Postequatorial
Spicular length	0.55	—	0.88	
Spicular length right				2.17
Spicular length left				2.4
Gubernaculum length	0.06	—	—	0.065
Papillar arrangement*	10 p: 3 pr/4 ad/3 po	—	12 p: 6 pr/6 po	15 p: 6 pr/9 po
Reference	Vassiliades (1982)	Campana-Rouget (1957)	Soota (1983)	Soota (1983)

— = No measurements or information provided.

* Information on papillae: p = pairs of papillae; pr = preanal pair; ad = adanal pair; po = postanal pair; sv = subventral pair, l = lateral pair; ad unpaired = adanal unpaired papilla.

It is based on this morphological as well as molecular information that we consider our specimens as a new species and we name it *D. (N.) dakarensis*.

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