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Dichelyne (Cucullanellus) romani n. sp. (Nematoda: Cucullanidae) in notacanthid fishes from the Northeast Atlantic and Western Mediterranean

Wolf Isbert · Francisco Esteban Montero ·
Maite Carrassón · David González-Solís

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Abstract A new cucullanid, *Dichelyne (Cucullanellus) romani* n. sp. (Nematoda: Cucullanidae), is described from the digestive tract of two notacanthid fishes, *Notacanthus chemnitzii* Bloch and *N. bonaparte* Risso (Notacanthiformes: Notacanthidae), from the Northeast Atlantic and Western Mediterranean. The presence of a preloacal sucker and ten pairs of caudal papillae in males allocates it to the subgenus *Cucullanellus* Törnquist, 1931. The new species differs from other members of this subgenus in its larger body size, smaller spicule/body length ratio, the distribution of caudal papillae, and the position of

deirids and excretory pore. Both notacanthid fishes act as definitive hosts with slightly larger nematode specimens detected in *N. chemnitzii*. A wide intraspecific variability was found in the distribution of caudal papillae, and in some specimens the position of deirids, excretory pore and length of intestinal caecum also varied. A complete list of all assigned species of the subgenus *Cucullanellus* is presented and discussed as there are several uncertainties regarding the validity and synonymy of some species. This is the first species of *Dichelyne* Jägerskiöld, 1902 in a notacanthid fish and one of the only two records in deep-sea fish species.

W. Isbert · F. E. Montero
Unidad de Zoología Marina, Institut Cavanilles de
Biodiversitat i Biologia Evolutiva, Parc Científic,
Universitat de València, PO Box 22085, Valencia 46071,
Spain

M. Carrassón
Departament de Biologia Animal, Biologia Vegetal i
Ecologia, Universitat Autònoma de Barcelona, Barcelona,
Spain

D. González-Solís (✉)
Institute of Parasitology, Biology Centre of the Czech
Academy of Sciences, Branišovská 31,
370 05 České Budějovice, Czech Republic
e-mail: dgonzale@ecosur.mx

D. González-Solís
Laboratorio de Necton, El Colegio de la Frontera Sur
(ECOSUR), Unidad Chetumal, Chetumal, Quintana Roo,
Mexico

Introduction

The fish family Notacanthidae (Elopomorpha: Notacanthiformes) has a global distribution and includes species inhabiting deep waters between 200–3,500 m (Froese & Pauly, 2014). These benthopelagic fishes, commonly known as deep-sea spiny eels, feed on different small benthic invertebrates (crustaceans, echinoderms, polychaetes, bryozoans, hydrozoans) and small nektonic crustaceans (Macpherson, 1981; Coggan et al., 1998; Carrassón & Matallanas, 2002). Two of the nominal species belonging to this family are the snub-nosed spiny eel *Notacanthus chemnitzii* Bloch with a worldwide distribution, except in tropical waters and the Mediterranean, and the shortfin spiny

eel *N. bonaparte* Risso which is restricted to the Eastern Atlantic and Western Mediterranean (Froese & Pauly, 2014).

The knowledge about the parasite fauna of these fish species is scarce. Currently, the known helminth fauna of *N. chemnitzii* comprises seven metazoan parasites: two monogeneans (*Atlanticotyle notacanthi* Mamaev & Zubchenko, 1978 and *Syncoelicutyle polyorchis* Mamaev & Zubchenko, 1978), four trematodes (*Antorchis spinosus* Zubchenko, 1978; *Lecithophyllum euzeti* Gibson & Bray, 2003; *Koiea notacanthi* Bray & Campbell, 1995 and *Panopula spinosa* Zubchenko, 1979) and one nematode (*Cucullanus* sp.) (Gibson et al., 2005; Soares, 2007). One cestode (*Steringovermes notacanthi* Bray, 2004) and one trematode species (*Bathycetus brayi* Kuchta & Scholz, 2004) have been recorded in *N. bonaparte* in the Atlantic (Bray, 2004; Kuchta & Scholz, 2004) and there exist no records of parasites of this fish from the Mediterranean.

During recent studies on the parasitic helminths of marine fishes from the Northeast (NE) Atlantic and Western Mediterranean several nematodes were recovered from the digestive tract of *N. chemnitzii* and *N. bonaparte*. On the basis of a detailed morphological study of these specimens by using light and scanning electron microscopy, a new species of *Dichelyne* (*Cucullanellus*) is proposed and described herein. Moreover, the number of species in the subgenus *Cucullanellus* Törnquist, 1931 was revised and a comprehensive list comprising valid, invalid and questionable species assignments is provided.

Materials and methods

Ten individuals of *N. chemnitzii* [total body length (TL) 40.0–100.0 cm] and 21 *N. bonaparte* (TL 25.0–50.0 cm) from the NE Atlantic were collected during a survey off Outer Hebrides in August and September 2004 at depths between 400 and 1,800 m. A total of 156 specimens of *N. bonaparte* (TL 13.5–29.0 cm) was sampled during two projects (BIOMARE, ANTROMARE) carried out in the Western Mediterranean.

All fish specimens were measured and weighed; fishes from the NE Atlantic collected by R. Kuchta (Institute of Parasitology, Biology Centre of the Czech Academy of Sciences) were immediately examined for parasites; nematodes collected were washed in

physiological saline and preserved in buffered 4% formalin. Fish samples from the Western Mediterranean were frozen at -25°C ; examination for the presence of parasites was later conducted in the laboratory. Nematodes isolated from these fishes were washed in physiological saline and preserved in 70% ethanol for morphological examination and in 100% ethanol for molecular analysis. For light microscopy, nematodes were cleared in a mixture of glycerine and water in different concentrations. For scanning electron microscopy (SEM), specimens were post-fixed in 1% osmium tetroxide (in phosphate buffer), dehydrated through a graded acetone series, critical-point-dried and sputter-coated with gold and examined using a JEOL JSM-7401F scanning electron microscope (JEOL, Tokyo, Japan) at an accelerating voltage of 4 kV (GB low mode). Drawings were made with the aid of a drawing tube. All measurements are given in micrometres, unless otherwise indicated. Nomenclature of the caudal papillae follows Petter (1974).

After morphological identification, the mid-body parts of three adult individuals were dissected out and used for molecular analysis. DNA from all samples was extracted with QiagenTM (Valencia, California) DNeasy[®] Blood & Tissue Kit. The partial 18S rRNA gene fragment was amplified by polymerase chain reaction (PCR) using the primers ERIB1 (forward: 5'-ACC TGG TTG ATC CTG CCA G-3') and ERIB10 (reverse: 5'-CTT CCG CAG GTT CAC CTA CGG-3') (Barta et al., 1997). PCR was performed in an Applied Biosystems Veriti 96 Well thermal cycler under the following conditions: initial denaturation at 95°C for 3 min, followed by 35 amplification cycles of 50 s at 94°C (denaturation), 50 s at 56°C (annealing), 80 s at 72°C (extension) and a final extension step for 4 min at 72°C . The rDNA internal transcribed spacer cluster ITS1-5.8S-ITS2 was amplified by PCR using the primers A (forward: 5'-GTC GAA TTC GTA GGT GAA CCT GCG GAA GGA TCA-3') and B (reverse: 5'-GCC GGA TCC GAA TCC TGG TTA GTT TCT TTT CCT-3') (D'Amelio et al., 2000). PCR was performed under the following conditions: initial denaturation at 94°C for 5 min, followed by 30 amplification cycles of 30 s at 94°C , 30 s at 55°C , 70 s at 72°C and a final incubation for 7 min at 72°C . PCR products were purified using a QiagenTM (Valencia, California) MinElute[®] PCR Purification Kit. PCR fragments were sequenced by Macrogen (Amsterdam,

Netherlands) for both strands using the PCR primers. Sequences were aligned using BioEdit 7.0.1 (Hall, 1999) and variable sites were checked visually for accuracy.

Family Cucullanidae Cobbold, 1864
Genus *Dichelyne* Jägerskiöld, 1902

***Dichelyne (Cucullanellus) romani* n. sp.**

Type-host: Snub-nose spiny eel *Notacanthus chemnitzii* Bloch (Notacanthiformes: Notacanthidae).

Other hosts: Shortfin spiny eel *Notacanthus bonaparte* Risso (Notacanthiformes: Notacanthidae).

Type-locality: Northeast Atlantic, off Outer Hebrides (56°10'N, 09°34'E–58°44'N, 08°10'E).

Other localities: Western Mediterranean, off Spain (40°10'N, 01°30'E–41°12'N, 02°26'E).

Site in host: Digestive tract (pyloric caeca, stomach, intestine).

Prevalence and mean intensity: Ex *N. chemnitzii* from the NE Atlantic: 40% (4 infected out of 10 examined); 4 nematodes per infected fish. Ex *N. bonaparte* from the NE Atlantic: 29% (6 infected out of 21); 4 nematodes per infected fish. Ex *N. bonaparte* from the Western Mediterranean: 46% (71 infected out of 156); 2 nematodes per infected fish.

Specimens deposited: Holotype and allotype ex *N. chemnitzii* and two paratypes ex *N. bonaparte* (collected in August 2004, depth range: 1,400–1,500 m) are deposited in the Helminthological Collection of the Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice (Cat. No. IPCAS N-1066); two paratypes ex *N. bonaparte* are deposited in the National Helminthological Collection, UNAM, Mexico (CNHE No. 9369).

Representative sequences: 18S rDNA (KP699577); ITS1-5.8S-ITS2 (KP699576).

Etymology: The new species is named in honour of Dr Roman Kuchta from the Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice.

Description (Figs. 1–3)

General. Large-sized nematodes. Cuticle finely transversely striated. Lateral alae absent. Anterior end rounded, dorsoventrally expanded (Figs. 1A–C, 2D).

Cephalic extremity with 2 pairs of prominent submedian cephalic papillae and a pair of lateral amphids surrounding mouth. Mouth slit-like, dorsoventrally elongated, with an inner collarette armed with numerous triangular denticles (89–108 in number) (Fig. 2A–C). Oesophagus expanded in its anterior part, forming a distinct buccal capsule (oesophastome), with internal cuticular lining and dorsal denticulate surface (Fig. 1A–C). Nerve-ring surrounding oesophagus at its first third. Posterior end of oesophagus broad, opens into intestine through small valve. Ventral intestinal caecum anteriorly directed, extends below level of excretory pore (Fig. 1A, B). Caecum absent or poorly-developed in some specimens. Deirids relatively large, posterior to nerve-ring (Figs. 1A, B, 2D, E), at level of nerve-ring in one specimen. Excretory pore posterior to deirids, clearly anterior to posterior end of oesophagus (Fig. 1A, B). Postdeirids asymmetrical, right postdeirid almost equatorial, left postdeirid close to tail. Tail conical, with sharp tip (Figs. 1G–K, 2G, 3B, E).

Male [Based on 4 specimens ex *N. chemnitzii*; measurements of holotype in parentheses. Measurements for 17 specimens ex *N. bonaparte* are provided in Table 1.] Body 18.46–21.98 (20.53) mm long, with maximum width 313–395 (360). Oesophagus 1.78–2.00 (1.98) mm long, 8–10% (10%) of body length, 177–219 (200) wide at base; buccal capsule 196–221 (215) wide. Intestinal caecum 520–636 (586) long, intestinal caecum to oesophagus length ratio 29–32% (30%). Nerve-ring, deirids and excretory pore 570–649 (649), 840–971 (889) and 1,109–1,176 (1,176), respectively, from anterior extremity. Right and left postdeirid 8.6–10.79 (8.63) mm and 3.33–7.28 (3.33) mm, respectively, from posterior extremity. Ventral precloacal sucker present (Fig. 1G, H), difficult to observe in smaller specimens, variable in size according to the development of males. Distance from ventral precloacal sucker to posterior end 1.56–1.94 (1.78) mm. Ventral muscular bands well developed, starting posterior to ventral precloacal sucker, almost reaching cloacal opening (Fig. 1G, H, J, K). Caudal alae absent. Cloaca with slightly elevated lips (Fig. 3B, C). Ten pairs of caudal papillae (Fig. 1H, J, K): 3 subventral precloacal pairs (pair 1 slightly anterior to ventral precloacal sucker, pair 2 posterior to it, pair 3 between precloacal sucker and cloaca), 5 adcloacal pairs (pair 4 lateral and slightly posterior to cloaca, located between pairs 7 and 8; pairs 5–6

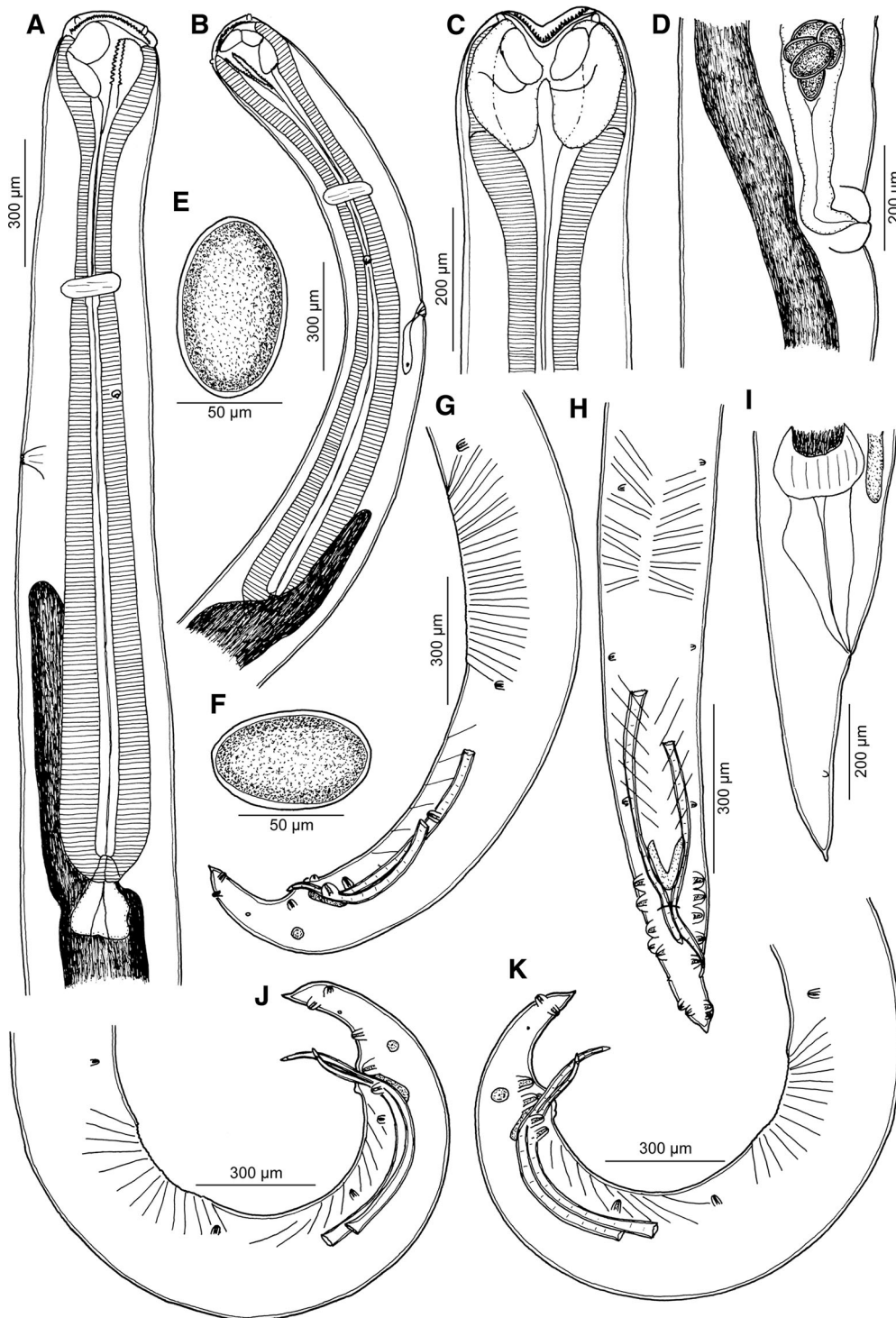


Fig. 1 *Dichelyne* (*Cucullanellus*) *romani* n. sp. ex *Notacanthus chemnitzii*. A, B, Anterior end of body, lateral views, different specimens; C, Cephalic end, dorsoventral view; D, Region of vulva, lateral view; E, F, Egg; G, H, Posterior end of male, lateral and ventral views, respectively; I, Posterior end of female, lateral view; J, K, Posterior end of male, lateral views, both sides of the same specimen to illustrate the variability of papillae

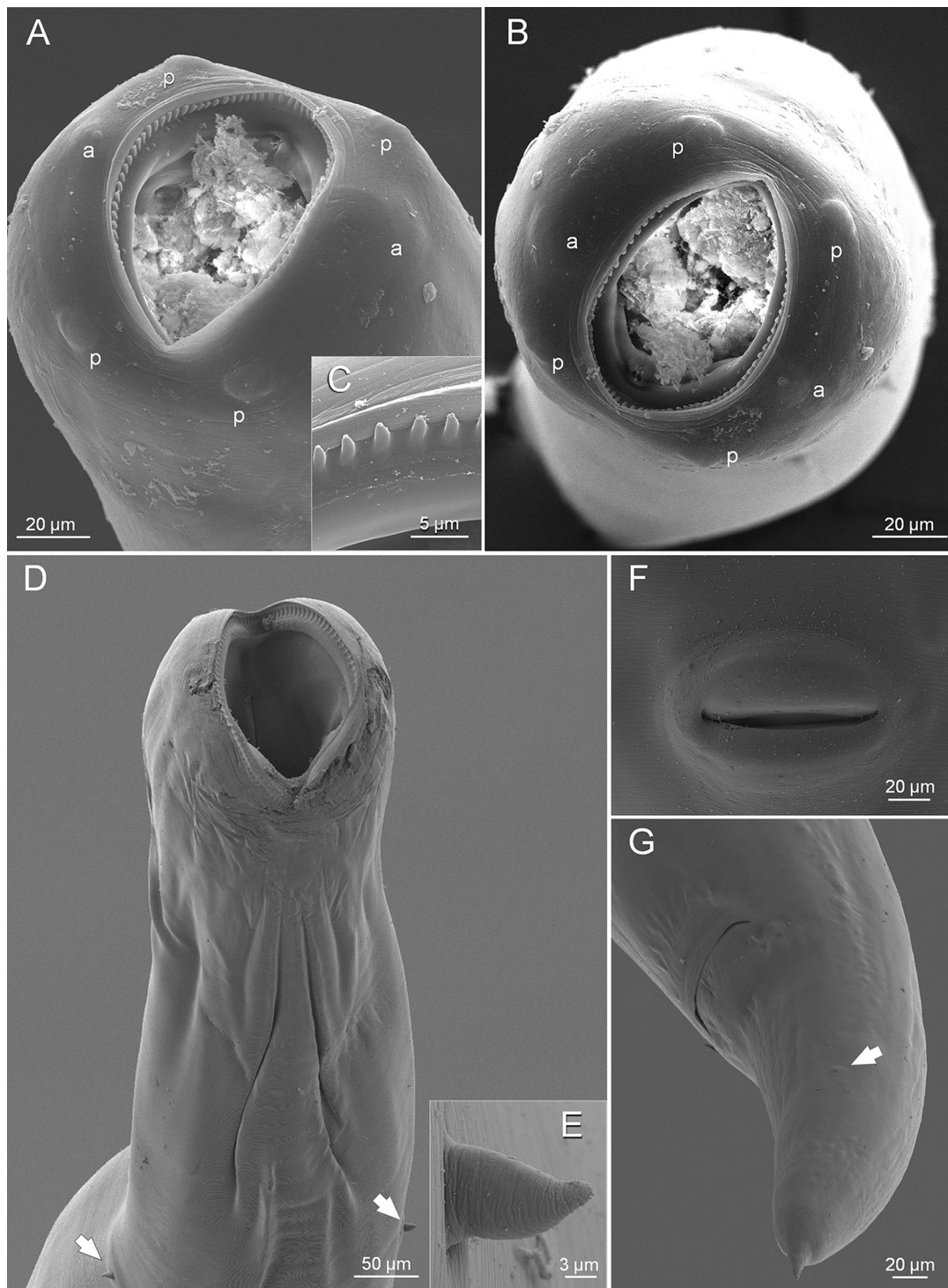


Fig. 2 *Dichelyne* (*Cucullanellus*) *romani* n. sp., scanning electron micrographs (A–C ex *Notacanthus bonaparte*; D–G ex *Notacanthus chemnitzii*). A, Cephalic end, sub-apical view; B, Cephalic end, apical view; C, Detail of teeth; D, Anterior end of body, dorsoventral view (arrows indicate deirids); E, Deirid; F, Vulva, ventral view; G, Tail of female, sublateral view (arrow indicates phasmid). Abbreviations: a, lateral amphid; p, cephalic papillae

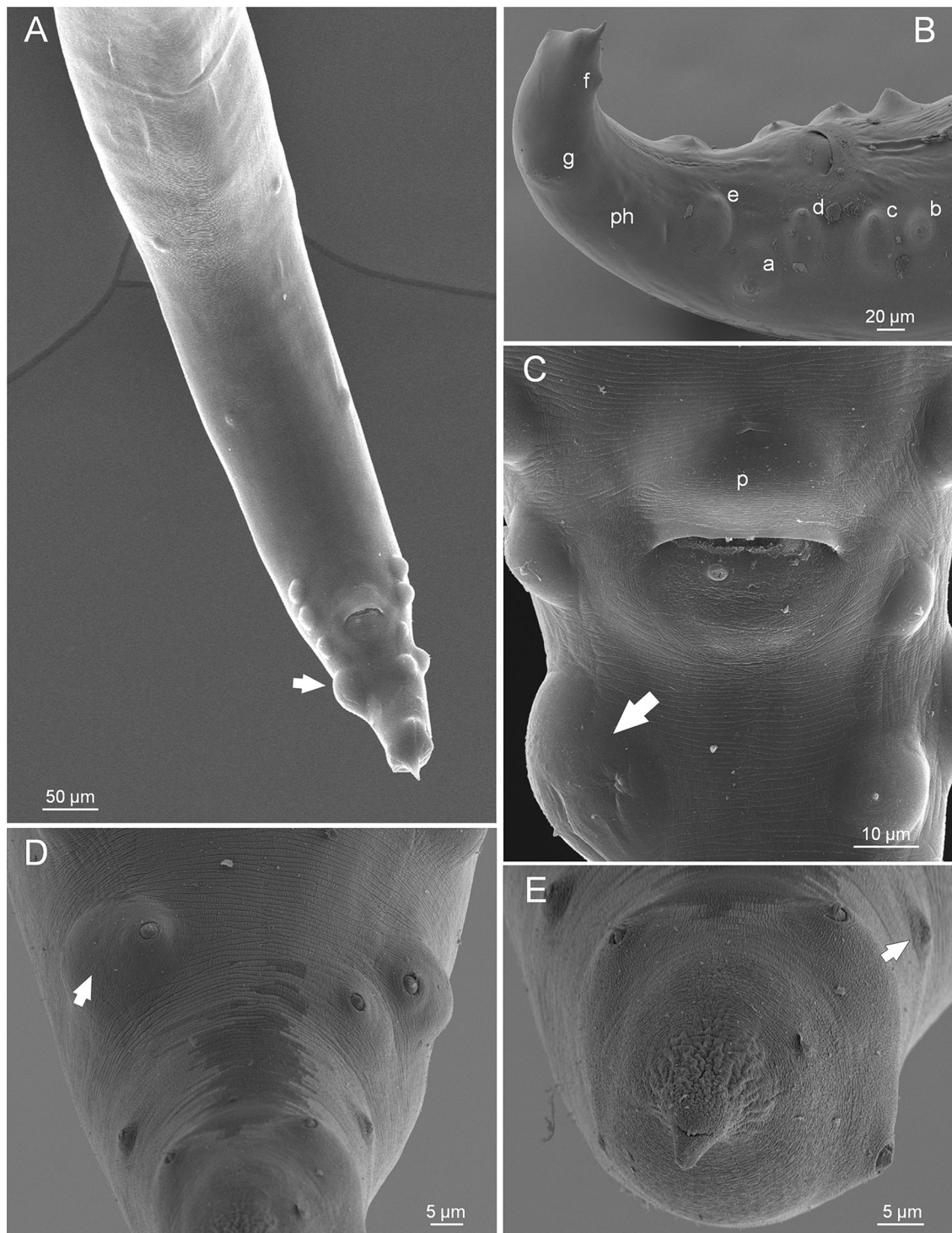


Fig. 3 *Dichelyne* (*Cucullanellus*) *romani* n. sp., scanning electron micrographs of male (A, C–E ex *Notacanthus bonaparte*; B ex *Notacanthus chemnitzii*). A, Posterior end, ventral view (arrow indicates fused papillae); B, Tail, subventral view (a–g, papillae 4–10; ph, phasmid); C, Region of cloaca, ventral view (arrow indicates fused papillae; p, unpaired medioventral papilla); D, Caudal region, ventral view (arrow indicates fused papillae); E, Tail tip, apical view (arrow indicates phasmid)

Table 1 Morphometric data for *Dichelyne* (*Cucullanellus*) *romani* n. sp.

Host	<i>N. chemnitzii</i>		<i>N. bonaparte</i>		<i>N. bonaparte</i>	
Locality	NE Atlantic		NE Atlantic		Western Mediterranean	
	Males (n = 4)	Females (n = 5)	Males (n = 2)	Females (n = 6)	Males (n = 15)	Females (n = 14)
Total length (mm)	18.46–21.98	16.94–25.21	14.83–15.71	17.55–24.95	3.53–12.01	3.96–14.16
Maximum width	313–395	306–462	263–290	89–433	127–268	103–323
Oesophagus length (mm)	1.78–2.00	1.75–2.04	1.80–1.93	1.84–2.29	0.56–1.28	0.48–1.30
Oesophagus length/total length (%)	8.45–9.65	7.38–10.34	11.47–12.98	9.04–10.46	9.56–17.73	8.77–14.79
Oesophagus width at base	177–219	153–238	161–170	131–230	56–174	52–167
Buccal capsule width	196–221	187–253	182–191	180–243	66–177	60–180
Buccal capsule length	255–302	225–331	317–319	283–376	121–215	126–241
Intestinal caecum length	520–636	532–821	410–675	533–830	136–414	153–524
Intestinal caecum width	89–105	73–113	43–89	69–94	30–64	23–69
Intestinal caecum length/oesophagus length (%)	29.21–31.93	29.18–42.74	22.75–35.06	29.03–36.32	13.76–38.98	16.13–46.25
Nerve-ring to anterior end	570–649	590–618	541–565	574–691	225–446	209–451
Excretory pore to anterior end	1,109–1,176	1,092–1,189	908–941	1,005–1,369	404–890	416–992
Right deirid to anterior end	866–971	880–952	754–755	901–1,021	335–636	306–669
Left deirid to anterior end	840–940	855–1,093	753–781	674–961	339–684	348–663
Right postdeirid to posterior end (mm)	8.63–10.79	9.07–15.13	6.54	9.58–12.20	2.07–5.80	2.27–7.53
Left postdeirid to posterior end (mm)	3.33–7.28	3.81–6.94	3.21	4.56–6.33	0.9–3.62	1.32–6.81
Ventral precloacal sucker to cloaca (mm)	1.08–1.33	–	0.77–0.90	–	0.33–0.80	–
Ventral precloacal sucker to posterior end (mm)	1.56–1.94	–	1.11–1.32	–	0.47–1.11	–
Right spicule length	608–1,187	–	728–744	–	253–597	–
Left spicule length	527–1,063	–	675–727	–	255–546	–
Spicule length/total body length (%)	2.63–6.09	–	4.46–4.96	–	2.70–7.79	–
Gubernaculum length	171–254	–	163–178	–	52–118	–
Vulva to anterior end (mm)	–	10.99–16.03	–	11.06–15.93	–	2.76–9.60
Vulva length/total body length (%)	–	57.88–67.62	–	63.00–65.63	–	56–73
Ovary to anterior end (mm)	–	2.48–3.35	–	2.65–8.36	–	1.21–4.98
Ovary to posterior end (mm)	–	0.46–1.09	–	0.51–3.75	–	0.32–1.58
Egg length	–	62–94	–	62–74	–	65–66
Egg width	–	43–55	–	48–54	–	45–50
Tail length	446–594	357–630	315–433	332–481	139–311	180–375
Phasmid to posterior end	217–295	170–385	160–163	141–211	63–145	81–188

subventral and anterior to cloaca, pairs 7–8 subventral and posterior to cloaca), and 2 postcloacal pairs (pair 9 subdorsal, pair 10 subventral, both near tail tip). In some specimens, pair 1 far anterior to ventral precloacal sucker, almost on anterior border or above it (Fig. 1G, H, J); pair 2 near or above posterior border

of precloacal sucker (Fig. 1H, J, K). Adcloacal papillae in some specimens close to each other on one side and spread out on opposite side (Fig. 1J, K). Pairs 4 and 8 fused together in some specimens (Fig. 3A, C, D); pair 7 lacking on left side of one specimen (Fig. 1G). Pair 9 anterior or posterior to pair

Table 2 Nominal species of *Dichelyne* (*Cucullaneilus*) along with their type-hosts, host families and original geographic distribution

Species	Type-host	Host family	Locality	Reference
<i>D. (C.) abbreviatus</i> (Rudolphi, 1819)	<i>Umbrina cirrosa</i> (Linnaeus)	Sciaenidae	Mediterranean, off Italy	Petter (1974)
<i>D. (C.) adriaticus</i> (Törnquist, 1931)	<i>Sparus aurata</i> (Linnaeus)	Sparidae	Mediterranean, off Italy	Petter (1974)
<i>D. (C.) amaruincai</i> (Freitas, Ibañez & Vicente, 1969)	<i>Paralichthys peruanus</i> (Steindachner)	Sciaenidae	Southeast Pacific, off Peru	Petter (1974)
<i>D. (C.) branchiostegi</i> (Yamaguti, 1941)	<i>Branchiostegus japonicus</i> (Houttuyn)	Malacanthidae	West Pacific, off Japan	Paschoal et al. (2014)
<i>D. (C.) bullocki</i> Stromberg & Crites, 1972	<i>Fundulus heteroclitus heteroclitus</i> (Linnaeus)	Fundulidae	Northwest Atlantic, off USA	Baker (1984)
<i>D. (C.) cnidoglanis</i> (Johnston & Mawson, 1945)	<i>Cnidoglanis macrocephalus</i> (Valenciennes)	Plotosidae	South Pacific, off South Australia	Paschoal et al. (2014)
<i>D. (C.) cotylophora</i> (Ward & Magath, 1917)	<i>Perca flavescens</i> Mitchell	Percidae	Northwest Atlantic, off USA	Moravec et al. (2011)
<i>D. (C.) dichelyneformis</i> (Szidat, 1950)	<i>Eleginops maclovinus</i> Cuvier	Eligonosidae	South Atlantic, off Argentina	Zdzitowiecki & Cielecka (1996)
<i>D. (C.) diplocaecum</i> Chandler, 1935	<i>Ictalurus furcatus</i> (Valenciennes)	Ictaluridae	Gulf of Mexico, off Texas, USA	Moravec & Justine (2011)
<i>D. (C.) elongatus</i> (Törnquist, 1931)	Unknown sciaenid fish	Sciaenidae	South Pacific, off Chile	Petter (1974)
<i>D. (C.) fastigatus</i> Chandler, 1935	<i>Sciaenops ocellatus</i> (Linnaeus)	Sciaenidae	Gulf of Mexico, off Texas, USA	Moravec et al. (2011)
<i>D. (C.) fraseri</i> (Baylis, 1929)	<i>Chaenocephalus aceratus</i> (Lönnberg)	Channichthyidae	West Antarctic, off South Georgia	Petter (1974)
<i>D. (C.) hardellus</i> Khera, 1954	<i>Hardellus thurjii</i> (Gray)	Geomyridae	Uttar Pradesh, off North India	Petter (1974)
<i>D. (C.) kanabus</i> Walder & Arai, 1974	<i>Cymatogaster aggregata</i> Gibbons	Embiotocidae	Northeast Pacific, off Canada	Walder & Arai (1974)
<i>D. (C.) jialaris</i> Luo, Guo, Fang & Hang, 2004	<i>Pagrus major</i> (Temminck & Schlegel)	Sparidae	Taiwan Strait, off China	Luo et al. (2004)
<i>D. (C.) mariajuliae</i> Alarcos, Timi, Etchegoin & Sardella, 2006	<i>Pogonias cromis</i> (Linnaeus)	Sciaenidae	Mar Chiquita, off Argentina	Alarcos et al. (2006)
<i>D. (C.) minutus</i> (Rudolphi, 1819)	<i>Platichthys flesus</i> (Linnaeus)	Pleuronectidae	Baltic Sea	Moravec (1994)
<i>D. (C.) pleuronectidis</i> (Yamaguti, 1935)	<i>Pleuronichthys cornutus</i> (Temminck & Schlegel)	Pleuronectidae	West Pacific, off Japan	Li et al. (2014)
<i>D. (C.) rodriguezi</i> (Pinto, Fábio & Noronha, 1970)	<i>Micropogonias</i> sp. (probably <i>M. furnieri</i> ; Desmarest)	Sciaenidae	Southwest Atlantic, off Brazil	Petter (1974)
<i>D. (C.) romani</i> n. sp.	<i>Notacanthus chemnitzii</i> Bloch	Notacanthidae	Northeast Atlantic, off Scotland	Present study
<i>D. (C.) sciaenidicola</i> Timi, Lanfranchi, Tavares & Luque, 2009	<i>Umbrina canosai</i> Berg	Sciaenidae	Southwest Atlantic, off Argentina and Brazil	Timi et al. (2009)
<i>D. (C.) sheardi</i> (Johnston & Mawson, 1944)	<i>Chironemus maculosus</i> (Richard)	Chironemidae	South Pacific, off Australia	Paschoal et al. (2014)
<i>D. (C.) szidati</i> Timi & Sardella, 2002	<i>Acanthisthius brasiliensis</i> (Cuvier)	Serranidae	Southwest Atlantic, off Argentina	Timi & Sardella (2002)
<i>D. (C.) travassosi</i> (Guimarães & Cristofaro, 1974)	<i>Balistes vetula</i> (Linnaeus)	Balistidae	Southwest Atlantic, off Brazil	Vicente et al. (1989)

Table 2 continued

Species	Type-host	Host family	Locality	Reference
<i>D. (C.) tritonyxi</i> Chakravarty & Majumdar, 1961	<i>Nilssonina gangetica</i> (Cuvier)	Trionychidae	Indian Ocean, off Eastern India	Chakravarty & Majumdar (1961)
<i>D. (C.) tripapillatus</i> (Gendre, 1927)	<i>Diplodus cervinus</i> (Lowe)	Sparidae	East Atlantic, off Mauritania	Petter (1974)
<i>D. (C.) tornquisti</i> Paschoal, Vieira, Cezar & Luque, 2014	<i>Orthopristis ruber</i> (Cuvier)	Haemulidae	Southwest Atlantic, off Brazil	Paschoal et al. (2014)
<i>D. (C.) wallagoni</i> Chakravarty & Majumdar, 1961	<i>Wallago attu</i> (Bloch & Schneider)	Siluridae	Indian Ocean, off Eastern India	Chakravarty & Majumdar (1961)
<i>D. (C.) yvonnecampanae</i> Timi, Lanfranchi, Tavares & Luque, 2009	<i>Umbrina canariensis</i> Valenciennes	Sciaenidae	Eastern Atlantic, off West Africa	Timi et al. (2009)

10, the latter near phasmids in one specimen. Additional medioventral unpaired papilla present anterior to cloacal opening (Figs. 1G, J, 3C). Spicules similar, equal, with proximal end expanded and distal end pointed (Fig. 1G, H, J, K); right spicule 608–1,187 (1,187) long, left spicule 527–1,063 (985) long, spicule length 3–8% (5%) of body length. Gubernaculum Y-shaped (Fig. 1H), well sclerotised, 171–254 (224) long. Tail 446–594 (540) long. Small, lateral papilla-like outlets (probably representing phasmids) posterior to last pair of adcloacal papillae, situated at 217–295 (295) from posterior extremity (Figs. 1G, H, J, K, 3D, E).

Female [Based on 5 specimens ex *N. chemnitzii*; measurements of allotype in parentheses. Measurements for 20 specimens ex *N. bonaparte* are provided in Table 1.] Body 16.94–25.21 (23.13) mm long, with maximum width 306–462 (421). Oesophagus 1.75–2.04 (2.04) mm long, 7–10% (9%) of body length, 153–238 (217) wide at base; buccal capsule 187–253 (246) wide. Intestinal caecum 532–821 (693) long, intestinal caecum to oesophagus length ratio 29–43% (34%). Nerve-ring, deirids and excretory pore 590–618 (614), 855–1,093 (935) and 1,092–1,189 (1,164), respectively, from anterior extremity. Right postdeirid anterior to vulva, left postdeirid posterior to it, at 9.07–15.13 (13.68) mm and 3.81–6.94 (5.56) mm, respectively, from posterior extremity. Vulva with elevated lips (Figs. 1D, 2F), slightly postequatatorial, 10.99–16.03 (14.91) mm from anterior extremity, representing 58–68% (64%) of body length. Vagina anteriorly directed from vulva (Fig. 1D). Uteri amphidelphic. Ovaries extending from just posterior to oesophago-intestinal junction to near anus (Fig. 1I). Eggs in utero oval, partly embryonated, 62–94 × 43–55 (94 × 43) (Fig. 1E, F). Tail 357–630 (384) long, with pair of caudal papillae (phasmids) at its mid-length, situated at 170–385 (192) from posterior extremity (Figs. 1I, 2G).

Discussion

The nematode family Cucullanidae Cobbold, 1864 is mainly characterised by the presence of a buccal capsule (Moravec, 1994) and includes parasites of marine, brackish- and freshwater fishes throughout the globe, occasionally occurring in turtles (Petter, 1974).

To date, six genera are considered in this family, namely: *Cucullanus* Müller, 1777, *Dichelyne* Jäger-skiöld, 1902, *Neocucullanus* Travassos, Artigas & Pereira, 1928, *Campanarougetia* Le Van Hoa & Pham-Ngoc-Khue, 1967, *Oceanicucullanus* Schmidt & Kunz, 1969 and *Truttaedacnitis* Petter, 1974 (Petter, 1974; Anderson et al., 2009). However, some authors consider the latter genus a subgenus of *Cucullanus* (e.g. Moravec, 1979). The genus *Dichelyne* includes three subgenera: *Dichelyne*, *Cucullanellus*, and *Neocucullanellus* Yamaguti, 1941 (Petter, 1974; Anderson et al., 2009). The present nematodes possess a precloacal sucker, ten pairs of caudal papillae, and an intestinal caecum, typical features for species belonging to *Dichelyne* (*Cucullanellus*).

To date, the number of recognised species within the genus *Dichelyne* is not completely clarified, but there is an estimation of over 40 nominal species (Li et al., 2014) of which 28 have been assigned to the subgenus *Cucullanellus* (Table 2). Of these, *D. (C.) abbreviatus* (Rudolphi, 1819) should be considered a *species inquirenda* due to its poor description (see Moravec & Justine, 2011), *D. (C.) diplocaecum* Chandler, 1935 cannot be assigned to the subgenus because males are unknown (Petter, 1974; Crites & Overstreet, 1997; Moravec & Justine, 2011) and *D. (C.) amaruincai* (Freitas, Ibañez & Vicente, 1969) was synonymised with *D. (C.) elongatus* (Törnquist, 1931) by Vicente et al. (1989), but recent studies considered it as a valid species (Alarcos et al., 2006; Timi et al., 2009). Timi et al. (2009) indicated that this applies only to the nematodes detected in *Paralonchurus peruanus* (Steindachner) from Brazil (Pinto et al., 1992).

Even though the status of *D. (C.) rodriguesi* (Pinto, Fábio & Noronha, 1970) was confirmed by Vicente et al. (1989), re-examination of the type-material by Timi et al. (2009) revealed that an intestinal caecum is lacking, thus indicating that the specimens may belong to the genus *Cucullanus*, although the bad condition of the material did not allow to clarify its identity (see Timi et al., 2009 for details).

Zdzitowiecki & Cielecka (1996) mentioned that *D. (C.) dichelyneformis* (Szidat, 1950) shows apparently high similarity and might be conspecific with *D. (C.) fraseri* (Baylis, 1929), but the type-specimens were not examined; therefore we consider these species distinct. The nomination of *D. (C.) yvon-necampanae* Timi, Lanfranchi, Tavares & Luque, 2009 is rather questionable since it was not based on

the examination of the type-specimens, but just on the brief original description provided by Campana-Rouget (1957). Timi et al. (2009) differentiated this species from its congeners by the closeness of pairs 2 and 3 of the caudal papillae. However, as we have shown, the variability of the caudal papillae might be very high. Therefore, the number of recognised species within the subgenus *Cucullanellus* should be 26, although a careful revision of the type-specimens of several species should be carried out in order to elucidate their status.

Out of the recognised species, none has so far been described for notacanthid fishes (Table 2). Apparently, there is only one record of a cucullanid nematode in *N. chemnitzii* from the NE Atlantic (Soares, 2007). In general, records of species of *Dichelyne* are scarce in the North Atlantic and especially in the Mediterranean, since most studies have been carried out on shallow-water perciform fishes of different families and geographical regions, such as the Southwestern and Western Atlantic (e.g. Szidat, 1950; Zdzitowiecki & Cielecka, 1996; González-Solís et al., 2002; Timi & Sardella, 2002; Alarcos et al., 2006; Timi et al., 2009; Paschoal et al., 2014) or Pacific Ocean (e.g. Moravec & Justine, 2011; Li et al., 2014) (Table 2).

Dichelyne (C.) szidati Timi & Sardella, 2002, *D. (C.) mariajuliae* Alarcos, Timi, Etchegoin & Sardella, 2006, *D. (C.) sciaenidicola* Timi, Lanfranchi, Tavares & Luque, 2009 and *D. (C.) tornquisti* Paschoal, Vieira, Cezar & Luque, 2014, reported from the Southwestern Atlantic (Argentinean waters) (Table 2), can be clearly differentiated from *D. (C.) romani* n. sp. in the position of the excretory pore and deirids near to or at posterior end of oesophagus, and the greater spicule to body length ratio (8–22 vs 5%). Similarly, *D. (C.) pleuronectidis* (Yamaguti, 1935) from the East China Sea has deirids at the posterior third of oesophagus or anterior to the oesophago-intestinal junction, excretory pore anterior to deirids, and greater spicule to body length ratio (15%) (Li et al., 2014).

In the Mediterranean, three species have been reported, i.e. *D. (C.) adriaticus* (Törnquist, 1931), *D. (C.) minutus* (Rudolphi, 1819), and *D. (C.) tripapillatus* (Gendre, 1927), though the latter two were originally described from other geographical areas (see Table 2) (Törnquist, 1931; Gibson, 1972; Moravec, 1994; Ternengo et al., 2009). The new species differs from *D. (C.) minutus* in having larger body size (males: 3.53–21.98 vs 2.62–4.60 mm; females:

3.96–25.21 vs 2.53–4.90 mm), excretory pore located distinctly anterior to the posterior end of oesophagus, smaller spicule to body length ratio (2.6–7.8 vs 22%) and in parasitising species of a different fish family (Notacanthidae vs Pleuronectidae) (Moravec, 1994). *Dichelyne* (C.) *adriaticus* originally described from a sparid fish has a smaller body size (males 1.90–2.68 mm; females 2.43–3.70 mm) (Törnquist, 1931) than the new species described here. Although some specimens of *D. (C.) romani* n. sp. have a comparable body size as *D. (C.) adriaticus*, they exhibit a larger distance from the nerve-ring to the anterior extremity and a longer oesophagus and tail. *Dichelyne* (C.) *tripapillatus* differs from the new species in having two intestinal caeca (Li et al., 2014).

There are three species of *Dichelyne* from deep-sea or cold-water fishes. *Dichelyne* (D.) *etelidis* Moravec & Justine, 2011, described from the deep water red snapper *Etelis carbunculus* Cuvier (Lutjanidae) in the South Pacific Ocean (Moravec & Justine, 2011), belongs to a different subgenus. *Dichelyne* (C.) *dichelyneformis* described from fishes off Tierra del Fuego, Argentina (Szidat, 1950), differs from *D. (C.) romani* n. sp. in having deirids and excretory pore allegedly situated anterior to nerve-ring, smaller body length (males and females: 4.1 mm), and greater spicule to body length ratio (26%). *Dichelyne* (C.) *fraseri* collected from sub-Antarctic and Antarctic fishes (Baylis, 1929; Zdzitowiecki & Cielecka, 1996) has a similar position of the excretory pore, deirids, and distribution of the caudal papillae as the new species, but smaller body size (males: 3.06–5.55, females: 3.34–7.34 mm) and larger spicule to body length ratio (22%).

In the present study the number of the NE Atlantic fish samples was smaller in comparison to those collected in the Mediterranean, though the better condition of these samples from the former area facilitated the morphological analysis and the selection of the type- and paratype specimens. Although the number of nematodes measured from the Atlantic and Mediterranean was different, it is noteworthy to indicate that there exist differences in their body size from both regions and hosts. Specimens from the Mediterranean were distinctly smaller than those from the Atlantic, where male nematodes from *N. bonaparte* were also smaller than those from *N. chemnitzii* (14.83–15.71 vs 18.46–21.98 mm) (see Table 1 for ranges).

Despite the morphometric differences, nematode specimens from both hosts and areas were considered to belong to the same species because most body ratios and other values were identical among them (see Table 1). Differences in the development of nematodes related to host species have also been observed in other cucullanid nematodes, such as *D. (C.) sciaenidicola*, which attained a larger body size in one of the sciaenid host species studied (*Micropogonias furnieri* [Desmarest]) than in *Umbrina canosai* Berg (Timi et al., 2009). Parasite body size can be affected by biological-environmental parameters, such as temperature, host species, host size and condition (Poulin, 1998; Sasal et al., 2000; Timi et al., 2009), as well as biochemical factors (e.g. lipid content in host tissue) or the infection site (Strømnes & Andersen, 2003). Moreover, larger hosts offer more resources and feeding places, thus allowing parasites to grow better (Sasal et al., 2000). The larger size of Atlantic notacanthids in comparison to Mediterranean fish is a common phenomenon occurring in deep-sea fish of the two regions (Stefanescu et al., 1992; Massuti et al., 2004). Apparently, latitudinal gradients and ecological factors (e.g. temperature, limited resources) might affect the body size of deep-sea fish (Stefanescu et al., 1992), thus affecting host physiology and producing cascading effects on the nematodes (Poulin, 1998).

A broad intraspecific variability was observed in the new species, especially in the distribution of the caudal papillae and in relation to the presence of intestinal caecum. This variation was not only observed among specimens from different hosts and geographical regions, but also within the specimens from the same host specimen. De & Maity (1995) observed similar variations in *D. (D.) alatae* De & Maity, 1995 parasitising a percoid fish (Sillaginidae) from West Bengal, India and detected an additional pre- and postcloacal papilla on one side of the body of two different specimens, respectively. More recently, Li et al. (2014) showed strong morphological differences in *D. (C.) pleuronectidis* and, on the basis of molecular analysis, confirmed that observed differences in the number of intestinal caeca (one, two or absent) and the position of deirids (at the level of posterior third of oesophagus or oesophago-intestinal junction) can be considered as intraspecific variability. Sometimes, such differences could be related to the fixation procedure or the examination of material from fresh or frozen hosts.

This is the first report of a species of the subgenus *Cucullanellus* in a notacanthid fish and the fourth species recorded in the Mediterranean.

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