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Megadraconema cornutum, a New Genus and Species from Korea, with a Discussion of Its Classification and Relationships within the Family Draconematidae (Nematoda, Desmodorida) Based on Morphological and Molecular Characters

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A new genus and species of Draconematidae Filipjev, 1918, *Megadraconema cornutum* gen. nov., sp. nov., inhabiting subtidal sediments in Jejudo, Korea is described. *Megadraconema cornutum* gen. nov., sp. nov. is mainly characterized by a long body (1630-2220 μm), presence of a transverse circle of well-developed papillae-like cuticular protrusions at the base of the lip region, a head capsule with reticular structure of subcuticle, an amphid with a pore-like opening, and an internal, bar-shaped fovea. The diagnosis of the family Draconematidae is emended and a key to genus is provided based on their major differential diagnostic characteristics, summarized in a table. Phylogenetic relationships of all the genera within the Draconematidae are discussed for the first time, based on molecular analyses and morphological features. The phylogenetic position of the new genus and relationships within the family Draconematidae based on analysis of molecular sequence data are examined. Analysis of 18S rRNA gene sequences does not support the currently accepted classification, and indicates paraphyly of the subfamily Draconematinae.

Key words: morphology, morphometrics, key, SEM, DIC photomicrographs, 18S rRNA, taxonomy

INTRODUCTION

Draconematid nematodes are mainly characterized by the presence of specialized locomotory structures, such as cephalic adhesion tubes (CAT) in the head region and sublateral and subventral posterior adhesion tubes (PAT) in the posterior body region (Allen and Noffsinger, 1978; Decraemer, 1989; Decraemer et al., 1997). Table 3 gives an overview of the diagnostic features within the Draconematidae. Draconematid nematodes live in various marine habitats, from the shallow intertidal sandy beaches to deep-sea hydrothermal vents, and are found throughout the world. They have also been found frequently on the surface of sea grasses, and in coralline habitats (Allen and Noffsinger, 1978; Decraemer et al., 1997; Karssen and van Aelst, 2002).

Decraemer et al. (1997) revised the family Draconematidae Filipjev, 1918 and classified 14 genera of 70 valid species into two subfamilies: the Draconematinae Filipjev, 1918 including four genera of 41 species and Prochaetosomatinae Allen and Noffsinger, 1978 including 10 genera of 29 species. Since this revision, 14 new draconematid species have been described: *Dinetia decraemerae* Rho et al., 2006, *Dinetia orientalis* Rho and Kim, 2005c, *Dracogalerus koreanus* Rho and Kim, 2005d, *Paradraconema jejuense* Rho and Kim, 2005, *Prochaetosoma dokdoense* Rho et al., 2010 and *Tenuidraconema koreense* Rho and Kim, 2004a were discovered from Korea in the Northwest Pacific. *Tenuidraconema philippinense* Rho and Kim, 2005a was discovered from the Philippines in the West Pacific.

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Tenuidraconema tongaense Rho et al., 2007 was discovered from the Tonga Islands in the Southwest Pacific. *Cygnonema belgicae* Raes et al., 2009a, *Cygnonema verum* Raes et al., 2009a and *Tenuidraconema microsperma* Raes et al., 2009b were discovered from the Porcupine Seabight in the Northeast Atlantic. *Dracognomus americanus* Karssen and van Aelst, 2002 from Guadeloupe in the Caribbean Sea, and *Draconema brasiliense* Venekey, Lage and Fonséca-Genevois, 2005 and *Draconema fluminense* Venekey et al., 2005 were discovered from the Brazilian coast in the Southwest Atlantic. This brings the total number of draconematid species to 84 (Karssen and van Aelst, 2002; Raes et al., 2009a, b; Rho and Kim, 2004a, 2005a, b, c, d; Venekey et al., 2005; Rho et al., 2006, 2007, 2010).

Since the discovery of the first draconematid nematode, *Draconema cephalatum* Cobb, 1913 from the Yellow Sea (Steiner, 1921; originally as *Chaetosoma cephalatum*, 15 species of the Draconematidae have been hitherto recorded from various habitats in the Northwest Pacific Ocean (Table 1). In our continuous investigation of the free-living nematofauna in this region, which started in 2002, an unknown draconematid species was collected from the washings of shallow subtidal detritus and coarse sediments of Munseom Islet off Jejudo, Korea. Morphological studies under differential interference contrast (DIC) microscope and scanning electron microscope (SEM) revealed that the species is clearly different from all known species and genera of the family. The present paper describes this species as a new genus and species, *Megadraconema cornutum* gen. nov., sp. nov. and discusses its classification and phylogenetic relationships with other members of the family Draconematidae, based on both morphological and molecular characters. We also present an emended diagnosis of the Draconematidae and a key to all genera in the family.

Table 1. Draconematid nematodes discovered from the Northwest Pacific Ocean.

Species	Reference	Geography	Habitat and ecology
Family Draconematidae Filipjev, 1918			
Subfamily Draconematinae Filipjev, 1918			
Draconema cephalatum Cobb, 1913	Steiner (1921); Allen & Noffsinger (1978)	the Yellow sea, Japan, the Philippines	base of algae with sand
Draconema japonicum Kito, 1976	Kito (1976); Rho & Kim (2004b)	Japan, Korea	Sea weeds (*Sargassum* community); intertidal and subtidal sediments and various algae
Paradraconema floridense Allen & Noffsinger, 1978	Allen & Noffsinger (1978)	Japan	calcareous alga (*Halimeda* sp.)
Paradraconema singaporense Allen & Noffsinger, 1978	Allen & Noffsinger (1978)	Singapore	a alga (*Gracillaris* sp.)
Paradracoenema jejuense Rho & Kim, 2005b	Rho & Kim (2005b)	Korea	shallow subtidal zone (25–35 m), coarse sediments
Dracograllus filipjevi Allen & Noffsinger, 1978	Allen & Noffsinger (1978); Rho & Kim (2006)	Japan, Korea	holdfasts of kelp; shallow littoral zone (3–5 m deep), coralline algae
Dracograllus gerlachi Allen & Noffsiner, 1978	Allen & Noffsinger (1978)	Japan	brown algae growing on rocks
Tenuidraconema koreense Rho & Kim, 2004a	Rho & Kim (2004a)	Korea	subtidal zone (150–250 m), coarse sediments and various invertebrates (hermit crabs, sponges and bryozoans)
Tenuidraconema philippinense Rho & Kim, 2005a	Rho & Kim (2005a)	the Philippines	shallow subtidal zone (45 m), coarse sediments
Subfamily Prochaetosomatinae Allen & Noffsinger, 1978			
Cephalochaetosoma pacificum Kito, 1983	Kito (1983)	the Philippines	deep-sea (5507–5587 m), sediments and fragments of a broken coconut with fibrous coat
Bathychaetosoma uchidai Kito, 1983	Kito (1983)	the Philippines	deep-sea (5507–5587 m), sediments and fragments of a broken coconut with fibrous coat
Dracogalerus koreanus Rho & Kim, 2005d	Rho & Kim (2005d)	Korea	shallow subtidal zone (10–35 m), coarse sediments and various invertebrates (sponges and bryozoans)
Dinetia orientalis Rho & Kim, 2005c	Rho & Kim (2005c)	Korea	subtidal zone (250–300 m), sediments and small logs
Dinetia decraemerae Rho et al., 2006	Rho et al. (2006)	Korea	subtidal zone (200–250 m), coarse sediments and small logs infested with limnorid isopods
Prochaetosoma dokdoense Rho et al., 2010	Rho et al. (2010)	Korea	Coarse detritus and shell gravels

MATERIALS AND METHODS

Sampling of taxa

Draconematid nematodes studied in the present paper were collected by SCUBA diver in various intertidal and shallow subtidal habitats in Korean waters (Fig. 1); e.g., benthic detritus and coarse sediments; calcareous algae and various invertebrates, such as sponges (*Callyspongia elegans* and *Cliona celata*), polychaete tubes (*Pomatoleios krausii* and *Serpula vermocularis*), crustaceans (hermit crabs: *Dardanus arrosor* and *Pagurus pectinatus*), and dead and living bryozoans (*Adenonella platalea* and *Thalamoporella lioticha*). Deep-sea specimens of *Prochaetosoma* sp. 2 for molecular analyses were collected with a fishing net.

Sample processing and preparation of specimens

Meiofaunal organisms extracted from samples through freshwater shocking (Kristensen and Higgins, 1989) were filtered through a 63 μm mesh-size sieve, and fixed in 4% buffered formalin in sea water. They were kept in the formalin in seawater for morphological studies, or stored in 99% ethanol for molecular analyses. Coarse detritus and sediments were removed from the sample by decantation, and the meiobenthos was extracted subsequently by flotation in Ludox® (DuPont) HS 40 (Burgess, 2001). The draconematid nematodes were sorted out from the mixed meiobenthos under a high magnification of LEICA MZ 8 stereomicroscope equipped with DIC. Specimens for morphological studies were transferred to anhydrous glycerin following Seinhorst (1959) and mounted in anhydrous glycerin on H-S slides to examine both sides of the specimens (Shirayama et al., 1993). All specimens used in this study, except *Megadraconema cornutum* gen. nov., sp. nov., *Draconema japonicum* Kito, 1976 and *Paradraconema jejuense* Rho and Kim, 2005, were identified only to the generic level due to the small number of specimens.

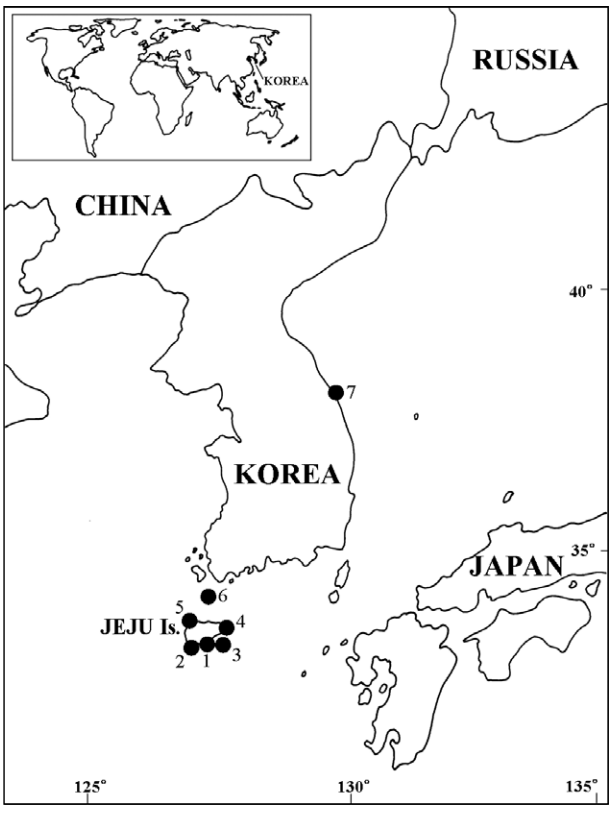

Fig. 1. Map showing sampling localities of the draconematid and epsilonematid nematodes studied. **(1)** Munseom Islet (*Megadraconema cornutum* gen. nov., sp. nov., *Dracograllus* sp. 1, *Prochaetosoma* sp. 1 and *Epsilonema* sp.). **(2)** Beomseom Islet (*Draconema japonicum* and *Prochaetosoma* sp. 4). **(3)** Supseom Islet (*Prochaetosoma* sp. 3). **(4)** Seongsanpo (*Paradraconema jejuense*). **(5)** Hyeobjae (*Paradraconema* sp.). **(6)** Geomundo (*Dracograllus* sp. 2). **(7)** Namae (*Prochaetosoma* sp. 2).

Light and scanning electron microscopy

The nematodes were observed with an Olympus BX51 microscope equipped with DIC. All drawings and measurements were made with a drawing tube. Photographs were taken with a Nikon Coolpix 990 digital camera, and the quality of the images was improved with Adobe Photoshop V7.0 software.

Two males, three females, and two fourth stage juveniles were examined using a scanning electron microscope (HITACHI S-4100). They were prefixed overnight at 4°C in a 2.5% phosphate buffered glutaraldehyde, followed by post fixation at 4°C with 1% phosphate buffered osmium tetraoxide. After dehydration through a graded series of ethanol (40–100%, with 10% intervals) for 30 min each, the specimens were critical-point dried and coated with gold-palladium in a high vacuum evaporator. The images were scanned with a CanoScan LIDE 60, digitally improved with Adobe Photoshop v.7.0, and mounted with the help of Microsoft Office PowerPoint 2003.

Type specimens are deposited in the nematode collections of the Royal Belgian Institute of Natural Sciences, Brussels, Belgium (RBINS), the National Institute of Biological Resources, Incheon, Korea (NIBR), the Invertebrate Resources Bank of Korea, Seoul National University (IRBK), and the specimen conservation room of the Marine Environment Research Department, Korea Ocean Research and Development Institute (KORDI).

Terminology and abbreviations

The terminology of the description and the measurements were conducted following Decraemer (1989). Abbreviations used in the text are as follows: L: total body length; CAT: cephalic adhesion tubes; CATn: number of cephalic adhesion tubes; mbd: maximum body diameter at mid body level; (mbd): minimum body diameter; mbd ph: maximum body diameter in pharyngeal region; ph: length of pharynx; PAT: posterior adhesion tubes; 1SlATl: length of anteriormost sublateral posterior adhesion tubes; SlATn: number of sublateral posterior adhesion tubes; 1SvATl: length of anteriormost subventral posterior adhesion tubes; SvATn: number of subventral posterior adhesion tubes; VAT: ventral adhesion tubes; t: tail length; tmr: length of non-annulated tail terminus; t/tmr: proportion of tail length to non-annulated tail terminus; abd: anal body diameter; spic: spicule or length of spicule measured along the median line; gub: gubernaculum or length of gubernaculums; V: position of the vulva as a percentage of the total body length from anterior; a, b, c, c′: proportions *sensu* de Man (1880).

DNA extraction, amplification, purification and sequencing

To infer the molecular phylogenetic position and relationships of *Megadraconema cornutum* gen. nov., sp. nov. within the family Draconematidae, nearly complete 18S rRNA gene sequences of 10 draconematid nematodes (*Draconema japonicum*, *Megadraconema cornutum* gen. nov., sp. nov., *Paradraconema* sp., *Paradraconema jejuense*, *Dracograllus* sp. 1, *Dracograllus* sp. 2, *Prochaetosoma* sp. 1, *Prochaetosoma* sp. 2, *Prochaetosoma* sp. 3 and *Prochaetosoma* sp. 4) and one epsilonematid nematode (*Epsilonema* sp.) were amplified and sequenced (Table 2). A single animal for each of the species was rinsed several times with distilled water to remove detritus particles. Samples were then subjected to DNA extraction. Total genomic DNA used as a template for the polymerase chain reaction (PCR) was extracted using a QIAamp tissue kit (QIAGEN Inc.), following the manufacturer's instructions.

The 18S rRNA gene of the draconematid nematodes was

Table 2. Species used in molecular analyses, with GenBank accession numbers for sequences. Sequences reported in this paper are marked with an asterisk (*).

Taxonomy/taxa	Locality/habitat/reference	Accession number
Ingroup		
Family Draconematidae		
Subfamily Draconematinae		
**Draconema japonicum*	Beomseom, Jejudo, Korea (33°13′21″N, 126°30′34″E); subtidal sediments (5 m deep)	FJ182217
**Megadraconema cornutum* gen. nov., sp. nov.	Munseom Is., Jejudo, Korea (33°13′87″N, 126°34′04″E); subtidal detritus and coarse sediments with mollusk shell gravels and bryozoan particles (38.5 m deep)	FJ182219
**Paradraconema* sp.	Hyeobjae, Jejudo, Korea (33°23′16″N, 126°14′42″E); intertidal brown algae	FJ182221
**Paradraconema jejuense*	Seongsanpo, Jejudo, Korea (33°27′40″N, 126°56′33″E); subtidal sediments (30 m deep)	FJ182220
**Dracograllus* sp. 1	Munseom Is., Jejudo, Korea. (33°13′66″N, 126°34′18″E); subtidal detritus and shell gravels (37 m deep)	FJ182215
**Dracograllus* sp. 2	Geomundo Is., Jeonranamdo, Korea (34°05′57″N, 127°14′84″E); intertidal invertebrates	FJ182216
Subfamily Prochaetosomatinae		
**Prochaetosoma* sp. 1	Munseom Is., Jejudo, Korea (33°13′65″N, 126°34′61″E); subtidal detritus and shell gravels (34 m deep)	FJ182222
**Prochaetosoma* sp. 2	Namae, Gangwondo, Korea (37°57′07″N, 128°46′41″E); subtidal sediments and various invertebrates (200 m deep)	FJ182223
**Prochaetosoma* sp. 3	Supseom Is., Jejudo, Korea (33°13′71″N, 126°35′59″E); subtidal detritus and shell gravels (15–30 m deep)	FJ182224
**Prochaetosoma* sp. 4	Beomseom Is., Jejudo, Korea (33°13′95″N, 126°30′95″E); subtidal detritus and sands (37 m deep)	FJ182225
Outgroup		
Family Epsilonematidae		
**Epsilonema* sp.	Munseom Is., Jejudo, Korea (33°13′87″N, 126°34′04″E); subtidal detritus and shell gravels (38.5 m deep)	FJ182218
Epsilonematidae sp.	Holterman et al. (2008)	EF591340

amplified by PCR through the following procedure: an initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 1 min, primer annealing at 54.5°C for 1 min 30 sec and an extension at 72°C for 2 min. Each reaction was terminated by a final extension step at 72°C for 10 min. All species were amplified with the primer set 328/329 designed on basis of sequences of the conserved regions at the 5′ and 3′ ends of eukaryotic homologs (Nelles et al., 1984). Detailed information for PCR and the sequencing primer sets used in the study is shown elsewhere (e.g., Table 3 in Hwang et al., 2009). For each sample, 2.5 μl of genomic DNA was amplified in a total reaction volume of 50 μl by using Taq DNA polymerase (Promega, Madison, USA), according to the manufacturer's recommendation, and then PCR products were isolated from 1% agarose gel and purified with a GeneClean (Bio 101) Nal/glass-powder Kit. The purified PCR products of the 18S rRNA gene were electrophoresed on an ABI PRISM® 3100 automated DNA Analyzer using a Big Dye Terminator Cycle Sequencing Ready Kit (Applied Biosystems) according to the manufacturer's instructions. Base correction was carried out using Sequence Navigator v.1.0.1 program. All corrected sequences have been submitted to GenBank (Accession numbers FJ182215 to FJ182225).

Sequence alignment and phylogenetic analysis of 18S rRNA gene sequences

GenBank accession numbers of the twelve nematode species sequenced for this study are listed in Table 2. Molecular sequences have not previously been produced from any of the ingroup taxa, comprising species of the family Draconematidae. The outgroup taxa, *Epsilonema* sp. sequenced for this study and an unidentified Epsilonematidae species gathered from NCBI, were chosen from the closely related family Epsilonematidae. Both forward and reverse strands were sequenced, and twelve nearly complete 18S rRNA gene sequences were initially aligned using the Muscle ver.3.7 multiple alignment program (Edgar, 2004). The alignment was further adjusted manually by eye and hand using Se-Al ver.2.0a11 (Rambaut, 1996). To evaluate the genetic distance of the marine draconematid and epsilonematid nematodes, uncorrected *p*-values of the 18S rRNA gene sequences were calculated using MEGA ver.4.0 (Tamura et al., 2007) (Tables 6 and 7).

Maximum parsimony (MP), maximum-likelihood (ML), and Bayesian inference (BI) methods were used to reconstruct the phylogeny from the aligned sequence data. The optimal sequence evolution model was selected using MODELTEST 3.7 (Posada and Crandall, 1998), and the HKY+I+G model was selected as the optimal model for the aligned data set under the likelihood ratio test. The following are parameters related to the selected model: base frequencies of A, C, G and T were 0.2552, 0.2151, 0.2630 and 0.2667, respectively; transition-transversion ratio (Ti/Tv) was 1.9162; proportion of invariable sites was 0.5419; shape parameter of Gamma distribution was 0.7598. The selected model and parameters were applied to the analyses of ML and BI. The MP and ML analyses were performed with PAUP* 4.0b10 (Phylogenetic Analysis Using Parsimony) computer software (Swofford, 2002), and BI was conducted using MrBAYES 3.1.2 (Ronquist and Huelsenbeck, 2003). Reliability of the nodes of the MP and ML tree was assessed by the bootstrap procedures with 1,000 and 100 replications, respectively. The following settings for BI were applied: number of generations 100,000; sample frequency 100; number of chains 4; burnin 10,000.

Table 3. Diagnostic features of *Megadraconema* gen. nov., with other draconematid genera of the family (after Decraemer et al., 1997).

Taxa	Characters								
	Head capsule	Body cuticle	CAT	Position of CAT	PAT	Amphid	Buccal cavity	Pharynx	Lumen wall of end bulb
Apenodraconema	present	presence of spine	typical	posterior to helmet	typical	spiral	with a small dorsal tooth	cylindrical, with posteriorly slightly swollen	not thickened
Bathychaetosoma	present	presence of spine	modified	posterior to helmet	typical	spiral	with a dorsal tooth and two subventral teeth	cylindrical, with well developed endbulb	moderately thickened
Cephalochaetosoma	present	smooth or presence of spine	modified	posterior to helmet	typical	spiral	with a dorsal tooth and two subventral teeth	cylindrical, with well developed endbulb	moderately thickened
Cygnonema	present	smooth	typical	posterior to helmet	typical	spiral	with a small dorsal tooth	cylindrical, posteriorly slightly swollen	not thickened
Dinetia	absent	lateral field present	modified	posterior to helmet	typical	spiral	unarmed or with a small dorsal tooth	cylindrical, with well developed endbulb	moderately thickened
Dracogalerus	present	smooth	modified	on helmet	typical	spiral	narrow, unarmed	cylindrical, with posteriorly slightly swollen	not thickened
Dracognomus	present	smooth	modified	on helmet	modified	reduced inverted U shape	with a small dorsal tooth	with mid-corpus swelling and endbulb	moderately thickened
Dracograllus	present	smooth or lateral field present	typical	on helmet	typical	loop shaped	narrow, unarmed	dumbbell-shaped	not thickened
Draconactus	present	smooth	typical	posterior to helmet	typical	spiral	with a small dorsal tooth	cylindrical, with posteriorly slightly swollen	not thickened
Draconema	present	higher size of the first (7–14) annules	typical	on helmet	typical	loop shaped	narrow, unarmed	dumbbell-shaped	not thickened
Megadraconema gen. nov.	present	reticular subcuticle on helmet	typical	on helmet	typical	Pore-opening with a longitudinal tubular bar	narrow, unarmed	dumbbell-shaped	not thickened
Notochaetosoma	present	smooth	typical	posterior to helmet	typical	spiral	narrow, unarmed	cylindrical, with posteriorly slightly swollen	not thickened
Paradraconema	present	presence of spines	typical	on helmet	typical	loop shaped	narrow, unarmed	dumbbell-shaped	not thickened
Prochaetosoma	present	presence of spines	typical	posterior to helmet	typical	spiral	with a dorsal tooth and two subventral teeth	cylindrical, with well developed endbulb	strongly thickened
Tenuidraconema	present	lateral field present	typical	on helmet or posterior to helmet	typical	spiral	narrow, unarmed	cylindrical, with well developed endbulb	not thickened

RESULTS

Systematics based on morphological observations

Draconematidae Filipjev, 1918

Diagnosis (emended after Decraemer et al., 1997). Desmodoroidea. Body short, S-shaped, usually with more or less pronounced, enlarged pharyngeal and mid-body region. Body cuticle annulated except for head capsule and tail terminus, often with minute vacuoles. Annules usually smooth,

rarely with spines, minute vacuoles/granules or a longitudinal lateral field in mid-body region or tail region in adults and juvenile stages. Cephalic sensory organs in three circles: six anterior labial papillae, six short posterior labial setae and four cephalic setae, and with or without a transverse circle of well-developed papillae-like cuticular protrusions near anterior border of head capsule. Head capsule present, except in *Dinetia*. Amphidial fovea spiralized to elongate loop-shaped, rarely reduced with inverted U-shaped or an internal longitudinal bar, located sublateral to dorsolateral on head capsule, when present, or near anterior head end. Cephalic adhesion tubes (CAT or cephalic ambulatory setae) present, located dorsally on head capsule, just posterior to it, or more posterior in cervical region, varying in number but absent in the first juvenile stage. Somatic setae arranged in eight longitudinal rows; four rows in tail region. Buccal cavity narrow to well-developed, usually armed with a dorsal tooth, with or without two smaller ventrosublateral

Table 4. Morphometrics of *Megadraconema cornutum* gen. nov., sp. nov. All measurements are in μm and in the form: mean ± standard deviation (range). (Abbreviations are explained in the section of materials and methods)

	Male		Female		J4	J3	J2
	Holotype	Paratypes	Allotype	Paratypes	Paratypes	Paratypes	Paratypes
n	–	6	–	5	5	4	3
L	1800	1919 ± 216 (1630–2220)	1840	1847 ± 51.7 (1815–1910)	1412 ± 178.4 (1140–1550)	957.5 ± 148.6 (850–1170)	770 ± 60 (710–830)
a	28.1	30 ± 2.2 (28.1–33.9)	20.1	21.8 ± 2.2 (18.5–23.7)	24.3 ± 4.6 (16.7–28.9)	28.4 ± 2.8 (25–31.9)	25.9 ± 2.8 (22.7–27.7)
b	14.9	14.5 ± 1.4 (13–16.7)	13.1	13 ± 1.1 (11.1–13.9)	11.7 ± 0.6 (11–12.6)	10.1 ± 0.9 (9.3–11)	9 ± 0.3 (8.8–9.4)
c	15.1	15.7 ± 1.9 (13.5–18)	18.9	19.5 ± 1.2 (17.8–20.7)	15.2 ± 1.9 (12.5–16.8)	11.3 ± 1.1 (10.7–12.9)	9.9 ± 0.7 (9.1–10.4)
c′	4.4	4.1 ± 0.2 (3.7–4.7)	4	4.3 ± 0.1 (4.1–4.5)	4.7 ± 0.4 (4.0–5.1)	4.9 ± 0.2 (4.6–5.1)	4.5 ± 0.4 (4.2–4.9)
V	–	–	62	62.8 ± 1.8 (60–65)	–	–	–
mbd	64	64.3 ± 9.8 (48–77)	91	85.4 ± 10.7 (78–103)	60.6 ± 18.4 (45–91)	34 ± 6.6 (27–41)	30 ± 4 (26–34)
(mbd)	31	34.5 ± 3.1 (32–38)	34	37 ± 2.7 (33–40)	31.4 ± 3.9 (26–36)	23.8 ± 5.6 (19–30)	22.3 ± 1.5 (21–24)
mbd ph	75	81.7 ± 3.4 (78–88)	79	81.8 ± 5.4 (75–90)	78.8 ± 3.4 (74–83)	59.0 ± 10.9 (49–73)	52.3 ± 2.5 (50–55)
ph	121	132.7 ± 16.1 (105–152)	140	142.8 ± 11.0 (133–161)	121 ± 20.1 (94–138)	95.8 ± 20.1 (80–125)	85.3 ± 4.6 (80–88)
abd	27	29.8 ± 2.1 (27–33)	24	22 ± 0.7 (21–23)	20 ± 1.9 (18–23)	17.5 ± 1.3 (16–19)	17.3 ± 1.2 (16–18)
t	119	122.5 ± 5.6 (113–128)	97	95 ± 4.1 (91–102)	92.6 ± 1.8 (91–95)	84.5 ± 6.5 (78–91)	77.7 ± 2.5 (75–80)
tmr	52	55.8 ± 1.7 (54–59)	54	55.6 ± 3.6 (52–60)	47 ± 1.6 (45–49)	44 ± 2.4 (42–47)	41.7 ± 1.2 (41–43)
t/tmr	2.3	2.2 ± 0.1 (2–2.3)	1.8	1.7 ± 0.1 (1.6–1.8)	2.0 ± 0.1 (1.9–2.1)	1.9 ± 0.1 (1.9–2)	1.9 ± 0.1 (1.8–2)
spic	102	112.5 ± 9.9 (109–130)	–	–	–	–	–
gub	24	27.2 ± 2.1 (23–29)	–	–	–	–	–
CATn	12	12 ± 0 (12–12)	12	12 ± 0 (12–12)	4 ± 0 (4–4)	3 ± 0 (3–3)	1 ± 0 (1–1)
Amphid fovea length	13	13.3 ± 1.4 (11–15)	13	10.8 ± 0.6 (10.3–11.5)	8.4 ± 0.5 (8–9)	7.5 ± 0.6 (7–8)	7.3 ± 0.6 (7–8)
1SlATl	76	85.3 ± 6.7 (78–96)	76	79.2 ± 2 (77–81)	64.2 ± 3 (59–66)	56 ± 5.2 (52–63)	51.3 ± 8.6 (42–59)
SlATn	13	14.8 ± 0.8 (14–16)	18	18 ± 0.7 (17–19)	6 ± 0 (6–6)	4 ± 0 (4–4)	2 ± 0 (2–2)
1SvATl	67	72.5 ± 3.5 (69–78)	71	72 ± 4.6 (69–80)	63.4 ± 1.8 (61–66)	–	–
SvATn	18	18.8 ± 0.4 (18–19)	19	19.8 ± 0.4 (19–20)	10 ± 0 (10–10)	–	–

teeth. Pharynx either dumbbell-shaped or largely cylindrical, with or without well-defined terminal bulb (with medium swelling in *Dracognomus*). Secretory-excretory system absent. At least the anteriormost posterior adhesion tubes (PAT or posterior ambulatory setae) arranged in four longitudinal rows: two sublateral and two subventral rows (except in *Prochaetosoma vitielloi* Allen and Noffsinger, 1978 with posteriorly a single ventral row) located on posterior third of body; PAT with or without differentiated (= bell-shaped) tip, usually straight, rarely long and flexible. Female reproductive system didelphic-amphidelphic, located anterior to PAT region. Male monorchic, single testis outstretched; copulatory apparatus with two spicules and a trough-shaped gubernaculum. Three caudal glands usually extending beyond anal region.

Draconematinae Filipjev, 1918
Genus *Megadraconema* gen. nov.

Diagnosis. Draconematidae. Body typical *Draconema*-shaped, with strongly swollen pharyngeal region. Head capsule with thin-walled anterior collar-like portion and thickened main part with reticular subcuticle. Twelve CAT located dorsally on head capsule and arranged in two transverse rows composed of six longitudinal rows each. Amphid without sexual dimorphism in shape: a pore-like opening and an inner longitudinal tubular fovea located laterodorsally on head capsule. Amphid in fourth- and third-stage juveniles similar to adults; second-stage juveniles with pore-like opening connected with an inner elongated spiral fovea. A transverse circle of well-developed papillae-like cuticular protrusions present near anterior end of head capsule in all stages; number of protrusions variable among stages. All PAT with well marked bell-shaped end and tongue-like inner structure; situated precloacally. PAT arranged in four longitudinal rows: two sublateral and two subventral rows with almost equally large number (13–20) of adhesion tubes. PAT becoming slightly shorter caudally.

Etymology. The generic name *Megadraconema*, neuter in gender, is a compound of "*mega*" (Gr., meaning large) and the name of the type genus *Draconema* of the family Draconematidae.

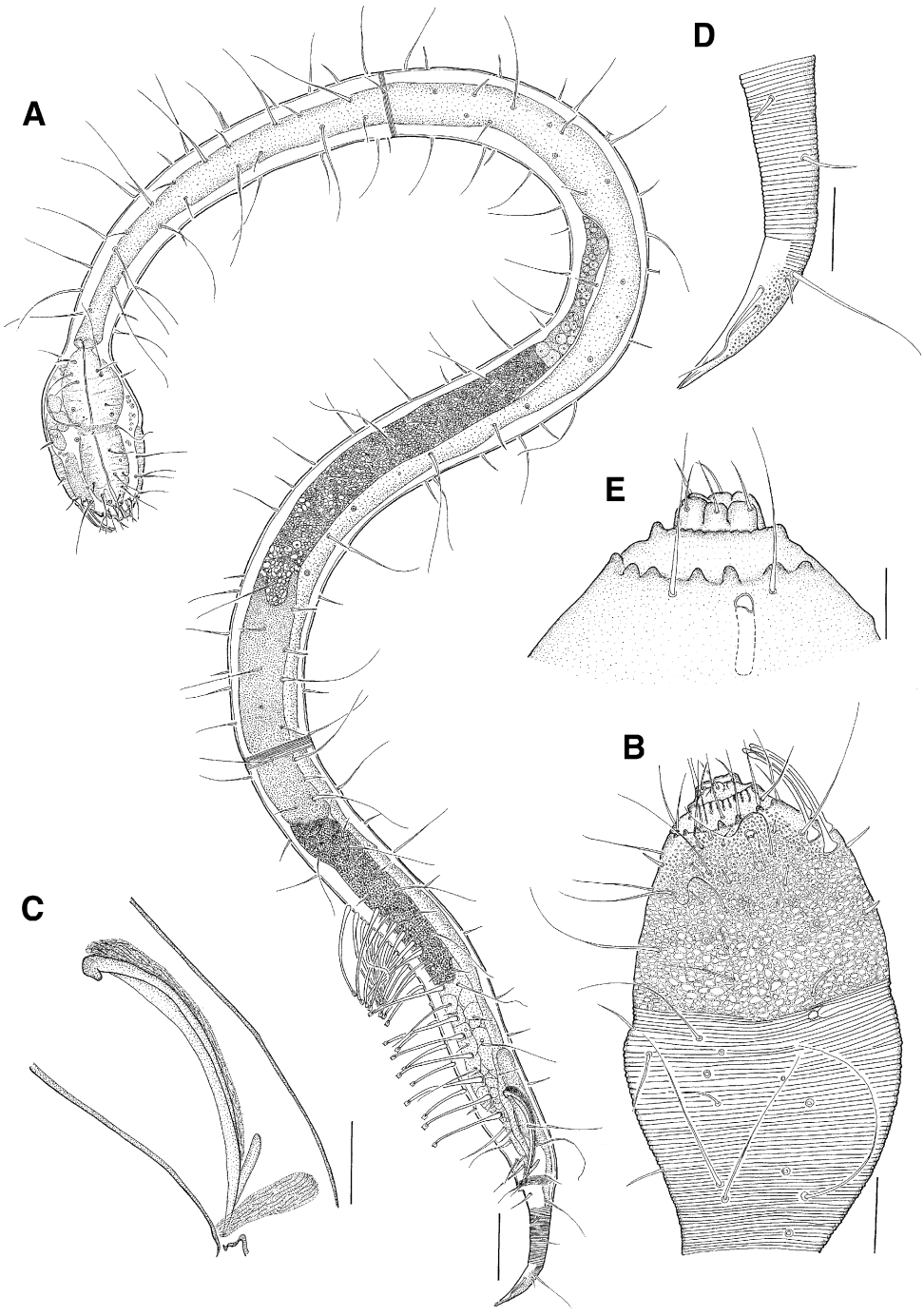

Fig. 2. *Megadraconema cornutum* gen. nov., sp. nov., male (holotype RIT751, A–D; paratype, RIT753, E). **(A)** Habitus, lateral view. **(B)** Surface view of lateral head and pharyngeal region showing amphidial fovea and cephalic adhesion tubes. **(C)** Copulatory apparatus, lateral view. **(D)** Posterior tail region, lateral view. **(E)** Detail of head capsule showing lip region and amphidial fovea, lateral view. Scale bars: 50 μm (A), 20 μm (B–D), 10 μm (E).

Type species. *Megadraconema cornutum* sp. nov.

Differential diagnosis. As shown in "Relationships within the family based on morphological features" of the Discussion and Table 3, *Megadraconema* gen., nov. differs from all other genera of the Draconematidae by the presence of a transverse circle of well-developed papillae-like cuticular protrusions at the base of the lip region, a head capsule with reticular structure of subcuticle, a pore-like amphid opening, and an internal, bar-shaped amphidial fovea.

Megadraconema cornutum gen. nov., sp. nov. (Figs. 2–9)

Type material. Holotype: adult male (RIT751). Allotype: adult female (RIT752). Paratypes: each set of five specimens (one male, one female, one fourth stage juvenile, one third stage juvenile and one second stage juvenile: RIT753–757; NIBRIV0000105001, 0000105061–0000105064; IRBK102–106) and 11 paratypes (four male, three female and two fourth stage juvenile, one third stage juvenile and one second stage juvenile: KORDI015–025).

Etymology. The proposed specific name, *cornutum*, refers to the transverse circle of well-developed papillae-like cuticular protrusions near the anterior border of the head capsule.

Diagnosis. Body 1630–2220 μm long, with swollen pharyngeal region, 6–9% of total body length. Twelve CAT located anterodorsally on head capsule and arranged in two transverse rows composed of six longitudinal rows each. Amphid with pore-like opening, connected with short narrow inner longitudinal tubular fovea in both sexes and fourth and third stage juveniles, located laterodorsally near anterior edge of head capsule. Second stage-juvenile with pore-like opening connected with an inner elongate spiral fovea. All PAT precloacal, arranged in four longitudinal rows: two sublateral rows with 13–16 adhesion tubes in males and 17–19 adhesion tubes in females, and two subventral rows with 18–19 adhesion tubes in male and 19–20 adhesion tubes in female. Spicule length up to 5.7% of total body length (102–114 μm), slightly arcuate with well-developed velum; capitulum of retracted spicule reaching to twelfth sublateral adhesion tube. Tail short, gradually tapering to non-annulated conical tip (ratio c: 15.6 $\pm$ 1.7 in male and 19.4 $\pm$ 1.1 in female). PAT becoming slightly shorter caudally.

Type locality and habitat. A subtidal zone of Munseom Islet off Jejudo, Korea (33°13′87″N, 126°34′04″E), collected from detritus and coarse sediments containing dead mollusk

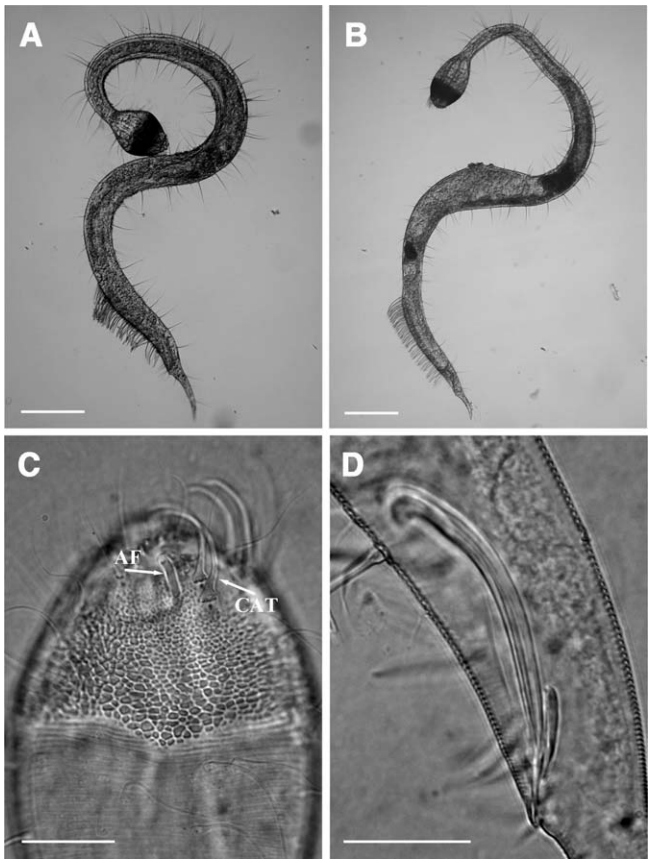

Fig. 3. *Megadraconema cornutum* gen. nov., sp. nov., DIC photomicrographs of male (paratype specimens KORDI015, A, C–D) and female (paratype KORDI018, B). **(A)** Habitus, male, lateral view. **(B)** Habitus, female, lateral view. **(C)** Head region, lateral view, with indication of cephalic adhesion tubes (CAT) and amphidial fovea (AF). **(D)** Copulatory apparatus, lateral view. Scale bars: 100 μm (A, B), 30 μm (C, D).

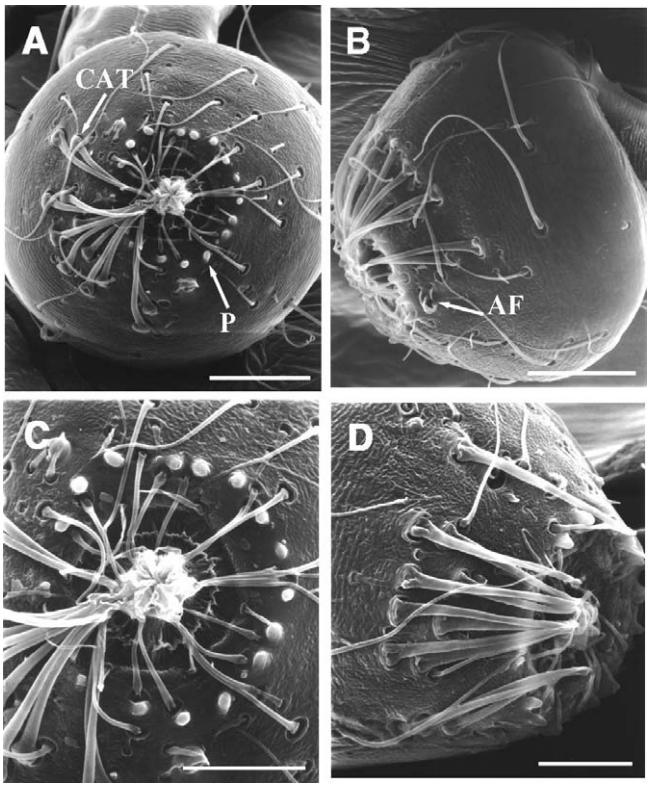

Fig. 4. *Megadraconema cornutum* gen. nov., sp. nov., SEM photomicrographs of male (paratype, KORDI023). **(A)** Head region, en face view, with indication of cephalic adhesion tubes (CAT) and a transverse circle of papillae-like cuticular protrusions (P). **(B)** Head region, lateral view showing CAT, arrow points to amphidial opening (AF). **(C)** En face view of head with protruding lips and a transverse circle of papillae-like cuticular protrusions. **(D)** Head region showing widened base of CAT, dorsal view. Scale bars: 25 μm (A, B), 15 μm (C, D).

shell gravel and bryozoan particles (25–38.5 m deep) on 7 Nov 2000, 13 Oct 2002, 23 Jun 2003 and 28 Oct 2005 by HS Rho and JW Choi.

Measurements. See Table 4.

Description. *Males (mainly based on the holotype and partly paratypes RIT753, KORDI015, KORDI023).* Body with swollen pharyngeal region (6–8% of total body length), narrow and slightly widening part along anterior intestine, wider cylindrical part with reproductive system, and tail tapered posteriorly and ending in conical tip; anterior body often strongly ventrally curved in fixed specimens (Figs. 2A, 3A). Cuticle finely annulated except for head capsule and tail terminus, with no lateral differentiation.

Head with well-cuticularized capsule. Head capsule truncated, dome-shaped (Fig. 2B, E) with thickened cuticle except for thinner anterior cuticular collar with fringed border; anterior border of capsule with a transverse circle of papillae-like cuticular protrusions (Figs. 2B, E, 4A, C). Surface of thick head capsule appearing smooth but with reticulated subcuticle (Figs. 2B, 3C); surface of head capsule slightly wrinkled in SEM (Fig. 4A–D). Lip region extruded, 5 μm in height, in holotype; lip region partially or completely retracted in most fixed specimens. Six lips well-developed: each lip with a short labial seta (8 μm long in holotype) (Fig. 2B, E). Anterior thin collar-like part of head capsule with about 18 setae in holotype, more or less arranged in two transverse rows; posterior row (about 12 setae in holotype) just in front of papillae-like cuticular protrusions; cephalic setae probably four, but indistinguishable from many subcephalic setae of differing size. Amphidial opening pore-like (2.4 μm in diameter, holotype) with protruding corpus gelatum in SEM (Fig. 4B), connected with an inner longitudinal tubular fovea (11 μm long or 19.6% of head capsule length in holotype) (Fig. 2E). Twelve CAT located on anterior-third of thickened head capsule and arranged in two transverse rows of six longitudinal pairs of tubular setae with enlarged insertion base and slightly swollen tip (Fig. 4D); CAT bent ventrally, 36–40 μm in length, posterior tubes longer than anterior ones in holotype. Four pairs of CAT located almost dorsally, and two pairs of outer ones located laterodorsally (Fig. 4A, D).

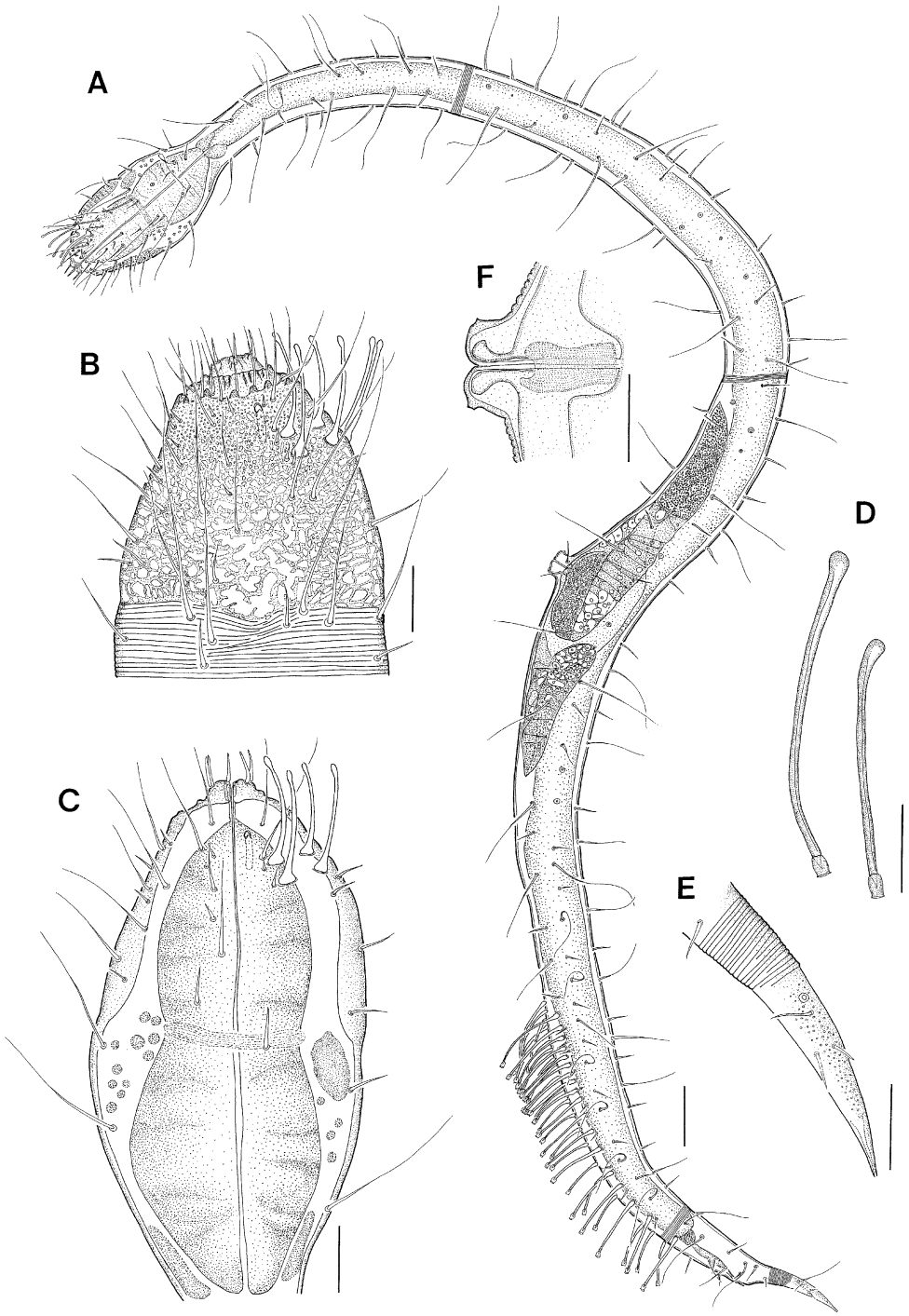

Fig. 5. *Megadraconema cornutum* gen. nov., sp. nov., female (allotype RIT752). **(A)** Habitus, lateral view. **(B)** Surface view of lateral head region. **(C)** Pharyngeal region, optical section. **(D)** Posterior sublateral (left side) and subventral adhesion tube (right side), lateral view. **(E)** Posterior tail region, lateral view. **(F)** Detail of vulva and vagina, lateral view. Scale bars: 50 μm (A), 20 μm (B–F).

Stoma narrow, unarmed. Pharynx dumbbell-shaped, lumen wall not thickened (Fig. 2A). Nerve ring at level of indentation of mid pharynx. Cardia well-developed (7.3 μm long in holotype). Intestine broad, more or less cylindrical. Secretory-excretory system not observed.

Somatic setae hair-like with broadened base, 5–71 μm long in holotype; longest setae (mean length 56 μm) occurred at swollen anterior part. Somatic setae densely distributed and arranged in 14 longitudinal rows in pharyngeal region: two rows each in mediodorsal, subdorsal, mediolateral, lateroventral, subventral, and medioventral positions (Fig. 2B). Slender region posterior to pharyngeal bulb and region anterior to PAT with eight longitudinal rows: two mediodorsal rows, two laterodorsal rows, two lateroventral rows, and two medioventral rows of somatic setae (Fig. 2A). Six pairs of pericloacal setae, three pre-cloacal pairs, two cloacal pairs and one post-cloacal pair, all uniformly tapered. Three pre-cloacal setae at each side composed of one short (10 μm long in holotype) and two long setae (anterior seta 21 μm long and posterior one 31 μm long, respectively, in holotype). Two cloacal setae at each side: anterior seta (30 μm long) longer than posterior one (16 μm long); single post-anal seta at each side 20 μm long, situated just posterior to cloacal opening in holotype. All PAT with well-marked bell-shaped tip containing tongue-like extension of inner canal, widened at insertion (Fig. 5D). PAT located anterior to cloacal opening and arranged in four longitudinal rows: two sublateral rows each with 13 adhesion tubes intermingled with four somatic setae, and two subventral rows consisting of 18 (left side) and 17 (right side) adhesion tubes, respectively, without intermingling somatic setae in holotype. PAT becoming slightly shorter caudally. In holotype, first SlAT and SvAT located at 77% and 78.4% of body length, respectively. Distance between anteriormost and posteriormost SlAT 10.6% of total body length in holotype.

Reproductive system typical for Draconematidae: a single, outstretched anterior testis (monorchic) extending far anteriorly up to 42.2% of total body length from anterior end and located ventrally to intestine in holotype (Fig. 2A). Spicules slightly arcuate, 102–114 μm long, up to 5.7% of total body length in holotype; gradually narrower posteriorly and ventrally with well-developed velum; capitulum offset, usually blunt beak-shaped (Figs. 2C, 3D). Capitulum of retracted spicule reaching up to twelfth sublateral adhesion tube. Gubernaculum thin, parallel to spicules. Short anal flap present and smooth.

Tail gradually tapering up to non-annulated conical tip, 43.7% of tail length; three pairs of somatic setae, two subdorsal and one subventral, on annulated part, and five pairs of setae, three subdorsal (long anterior and two short posterior pairs) and two subventral (anterior 37 μm and posterior 11 μm long) (Figs. 2D, 6D). Cuticle of non-annulated tail terminus finely vacuolated, mainly in dorsal region. Caudal gland reaching to posterior fourth sublateral adhesion tube and ending on well-developed spinneret.

Females (allotype RIT752 and paratypes KORDI018 and KORDI024). Similar to male in most respects (Figs. 3B, 5A–D). Body with strongly enlarged pharyngeal region, 7–9% of total body length; greatest body width at level of vulva region; anterior body region slightly curved ventrally. Head region largely similar to that in male, but pattern of reticulation of subcuticule somewhat different (Fig. 5B). Amphidial pore-like opening 2 μm in diameter, inner tubular fovea 13 μm long and 15.8% of head capsule length (Fig. 6A, B). Twelve CAT located on anterior third of head capsule, as in male (Fig. 5B, C). All PAT located anterior to anus, arranged in four longitudinal rows; two sublateral rows each consisting of 18 adhesion tubes, and two subventral rows consisting of 19 (left side) and 17 (right side) adhesion tubes, respectively, in allotype; PAT without intermingling somatic setae. Anteriormost SlAT and SvAT located at 79.5% and 80.4% of body length, respectively. Distance between anterior and posterior SlAT 11.9% of total body length.

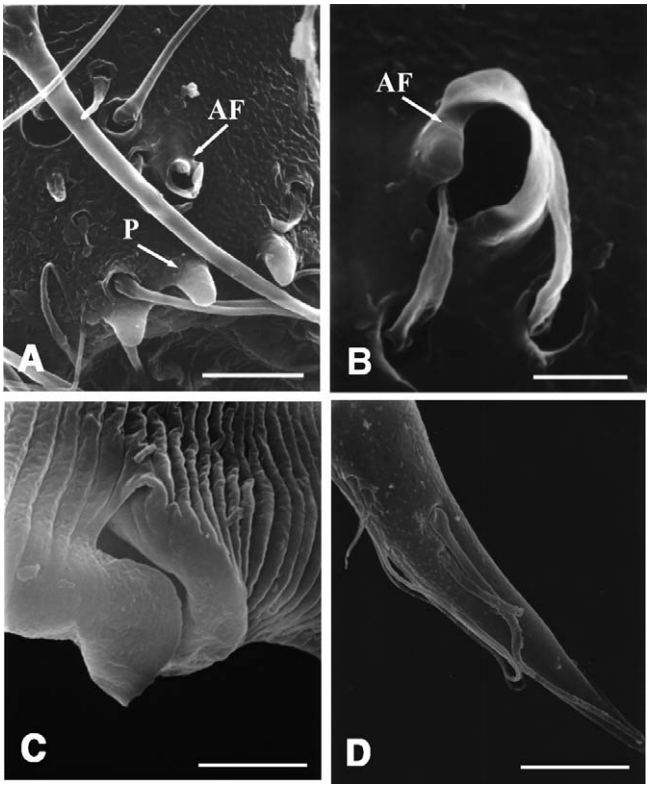

Fig. 6. *Megadraconema cornutum* gen. nov., sp. nov., SEM photomicrographs of female paratype KORDI024, A–C) and male (paratype KORDI023, D). **(A)** Detail of head region showing amphidial fovea and some papillae-like cuticular protrusions. **(B)** Amphid opening, lateral view. **(C)** Anal flap and cloacal region, oblique view. **(D)** Non-annulated tail terminus. AF = Amphidial fovea; P = Papillae-like cuticular protrusions. Scale bars: 5 μm (A, C), 2 μm (B), 10 μm (D).

Reproductive system typical for Draconematidae, didelphic-amphidelphic with ovaries reflexed to left side. Spermathecae not observed. Vagina long, *pars vaginae distalis* surrounded by constrictor muscle. Vulval lips protruding (Fig. 5F). Two pairs of paravulval setae present (19 μm long). Short anal flap present, not crenate (Fig. 6C).

Tail very short, gradually tapering up to conical, smooth terminus; terminus non-annulated but finely vacuolated dorsally, with well-developed spinneret, 55.7% of total tail length (Fig. 5E). Tail with two pairs of somatic setae: one pair subdorsally and one pair subventrally on annulated part, and four pairs of somatic setae on smooth terminal part, i.e. three subdorsal pairs and one subventral pair. Cuticle of

non-annulated tail end finely vacuolated dorsally; well-developed spinneret present.

Juveniles. Three juvenile stages (J2, J3, J4) were observed. Developmental stages were mainly differentiated by the number of CAT and the arrangement (number of longitudinal rows) and number of PAT.

Fourth-stage juvenile (RIT755). Habitus similar to adult (Figs. 7A, 8A). Body with strongly swollen pharyngeal region, 8.6% of total body length. Head capsule with a transverse circle of papillae-like protrusions as in adult; number of protrusions lower than in adults (Figs. 7B, C, 8D). Amphidial opening 1 μm in diameter; inner longitudinal fovea 8 μm long and 24% of head capsule length. Four CAT located about medially of thickened head capsule and arranged in one transverse row of tubular setae with enlarged insertion base and slightly swollen tip: inner two CAT situated dorsally, outer two laterodorsally. PAT arranged in three longitudinal rows anterior to anal opening, two sublateral rows, each consisting of six adhesion tubes and one row of VAT with 10 tubes. No intermingling somatic setae. Anteriormost SlAT and SvAT located at 76.5% of body length. Distance between anteriormost and posteriormost SlAT 11.3% of total body length. Genital primordium, 106 μm in length. Short anal flap present, not crenate. Tail gradually tapering up to conical, non-annulated but finely punctated end, 48.4% of total tail length (Fig. 7D). Annulated part of tail with one pair of subdorsal and one pair of subventral somatic setae; smooth part of tail with three pairs of somatic setae. Spinneret well-developed.

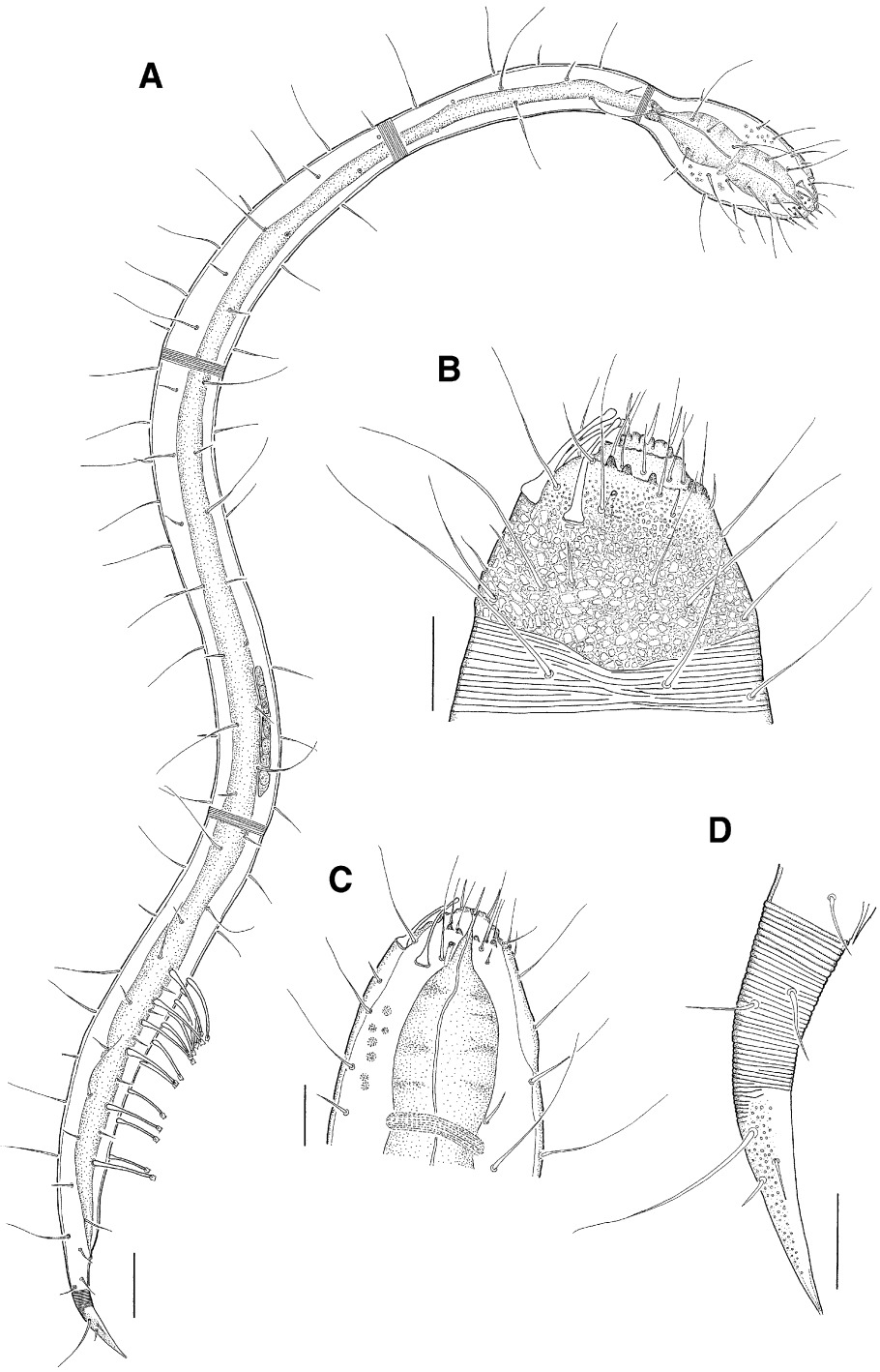

Fig. 7. *Megadraconema cornutum* gen. nov., sp. nov., fourth-stage juvenile (paratype RIT755). **(A)** Habitus, lateral view. **(B)** Head region, external view. **(C)** Anterior pharyngeal region, optical section. **(D)** Posterior tail region, lateral view. Scale bars: 50 μm (A), 20 μm (B–D).

Moulting third-stage juvenile (paratype RIT756). Habitus similar to adults (Figs. 8B, 9A). Pharyngeal region strong swelling, 10.6% of total body length. Head capsule with slightly wrinkled surface and a transverse circles of papillae-like protrusions as in adults (Fig. 9B); number of protrusions smaller than in adults and fourth stage juveniles. Amphid similar to fourth stage juveniles, longitudinal tubular bar 23.8% of head capsule. Three CAT arranged in a transverse row about mid-way head capsule, one mediodorsal and two laterodorsal. PAT arranged in two sublateral longitudinal rows anterior to anal opening, each row consisting of four adhesion tubes and no intermingling somatic setae. PAT becoming slightly shorter caudally. Anteriormost SlAT located at 75.5% of body length. Distance between anteriormost and posteriormost SlAT 9.4% of total body length. Genital primordium 41 μm in length. Tail with numerous fine transverse striae and well-developed spinneret; terminal region

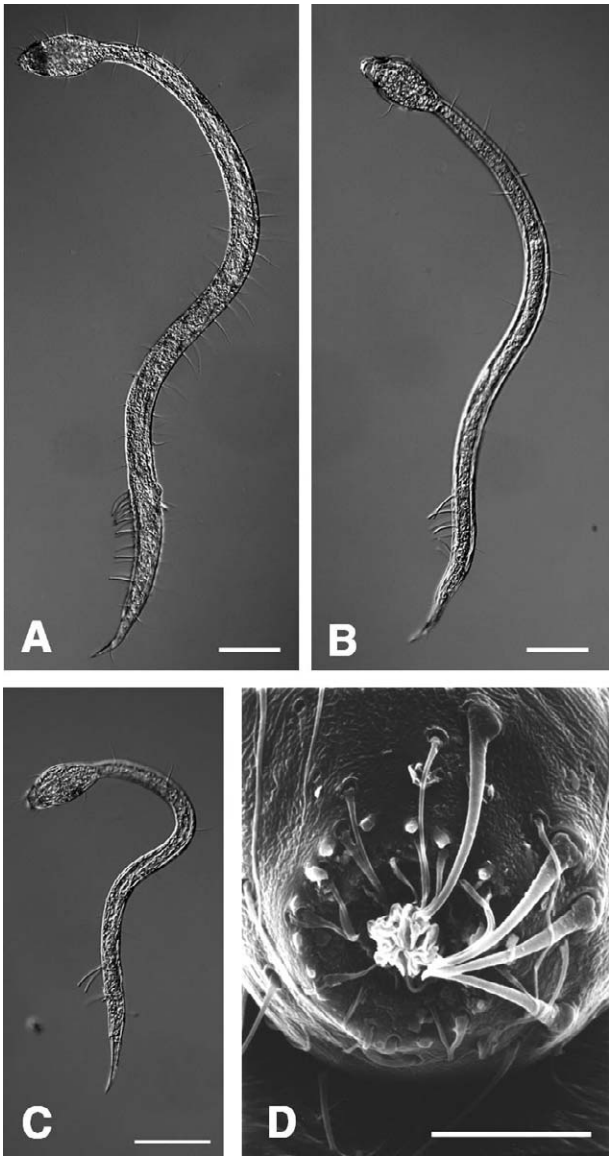

Fig. 8. *Megadraconema cornutum* gen. nov., sp. nov., DIC photomicrographs of juveniles (A–C, lateral view) and SEM photomicrograph of juvenile (D, en face view). **(A)** Habitus, fourth-stage juvenile (paratype RIT755). **(B)** Habitus, third-stage juvenile (paratype RIT756). **(C)** Habitus, second-stage juvenile (paratype RIT757). **(D)** Head region, fourth-stage juvenile (paratype KORDI025), showing protruding lips, a transverse circle of cuticular papillae-like protrusions and four cephalic adhesion tubes. Scale bars: 100 μm (A–C), 15 μm (D).

non-annulated but finely punctated, 49.5% of total tail length. Anal flap short, not crenate.

Second-stage juvenile (paratype RIT757). Habitus similar to adults (Figs. 8C, 9C). Body with strongly swollen pharyngeal region, 11.3% of total body length. Head capsule with a transverse circle of papillae-like protrusions (Fig. 9E); protrusions absent in molting second-stage juvenile (Fig. 9D). Shape of amphidial fovea differing from adults, third and fourth stage juveniles: a pore-like opening connected to an elongated spiral fovea, 21.1% of head capsule length. One CAT located dorsally about mid head capsule. PAT arranged in two sublateral longitudinal rows; each row consisting of two adhesion tubes and no intermingling somatic setae. Anterior SIAT located at 74.9% of body length from anterior end, slightly longer than posterior SIAT, 51 and 45 μm long, respectively; distance of between two SIAT 6.2% of body length. Genital primordium 31 μm in length and consisting of a few cells. Tail with non-annulated but finely punctuated terminus, 55.1% of total tail length. Short anal flap present; not crenate.

Sexual dimorphism. Males and females are very similar in most respects, other than the shape of the mid-body region (body diameter larger in female because of well-developed female reproductive system), and the ratio of the tail length to the non-annulated tail terminus (1.7 in female *vs* 2.2 in male). Females have a much shorter tail, but an equally long non-annulated terminal part.

Phylogenetic analysis of molecular data and genetic distances

Nearly complete 18S ribosomal RNA gene sequences were determined for 10 draconematid nematodes and one epsilonematid nematode for the first time in the present study: *Draconema japonicum* (1,749 nucleotides), *Megadraconema cornutum* gen. nov., sp. nov. (1,691 nucleotides), *Paradraconema* sp. (1,691 nucleotides), *Paradraconema jejuense* (1,690 nucleotides), *Dracograllus* sp. 1 (1,690 nucleotides), *Dracograllus* sp. 2 (1,752 nucleotides), *Prochaetosoma* sp. 1 (1,689 nucleotides), *Prochaetosoma* sp. 2 (1,690 nucleotides), *Prochaetosoma* sp. 3 (1,689 nucleotides), *Prochaetosoma* sp. 4 (1,689 nucleotides) and *Epsilonema* sp. (1,690 nucleotides). A multiple alignment analysis of sequences from 12 nematodes including two outgroup taxa showed 1,768 positions including indels (insertions and/or deletions). Of these, our phylogenetic analyses were carried out based on the unambiguously aligned regions including a total of 1,661 nucleotide positions with gaps included. The aligned sequences included 213 (12.8%) variable characters and 102 (6.1%) parsimony-informative characters. The A + T content of *Megadraconema cornutum* gen. nov., sp. nov. was 52%.

Tree topologies generated by the MP, ML and BI were exactly congruent with each other, and are represented by the maximum likelihood tree in Fig. 10. The Bayesian inference showed a higher degree of supporting values than ML and MP analyses, except for the clade consisting of *Prochaetosoma* sp. 3 and *P.* sp. 4. The clade representing the subfamily Prochaetosomatinae, including only four species of the genus *Prochaetosoma*, always formed a single monophyletic group with high support values (> 82%). The monophyletic *Prochaetosoma* was divided into two clades with relatively low support values: ((*P.* sp. 1 + *P*. sp. 2) + (*P*. sp. 3 + *P*. sp. 4)). The subfamily Draconematinae is shown to be paraphyletic, because the genus *Dracograllus* (*D*. sp. 1 and *D*. sp. 2) did not group with the other members of the subfamily (*Draconema*, *Megadraconema* and *Paradraconema*) in any of our analyses. The genus *Dracograllus* diverged first at the basal line of all analyzed draconematid nematodes, with very high supporting values in all analyses (100%). In all analyses *Megadraconema cornutum* gen. nov., sp. nov. clustered with a group including *Paradraconema* + *Draconema*, but the supporting values

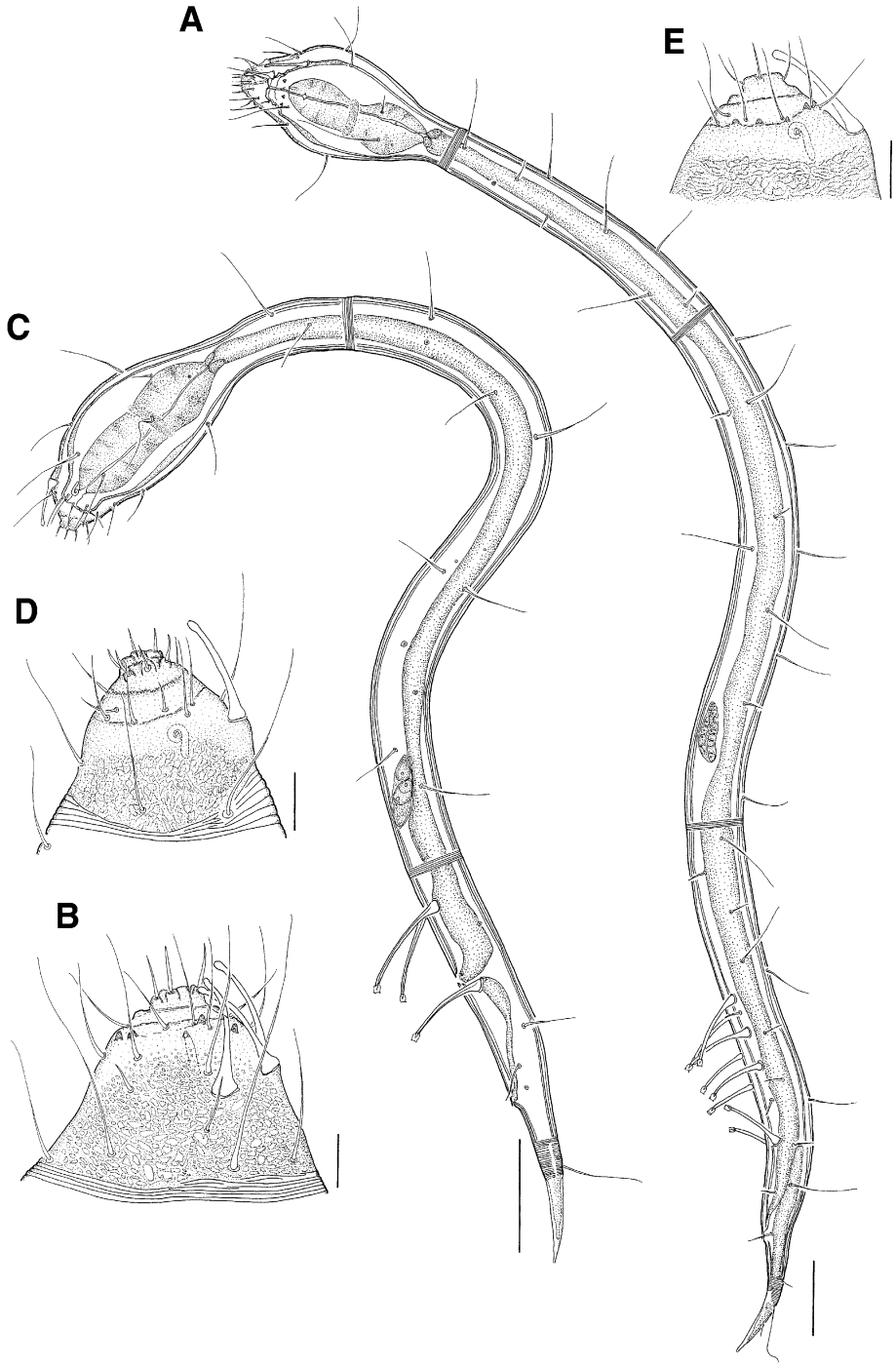

Fig. 9. *Megadraconema cornutum* gen. nov., sp. nov., third-stage juvenile (paratype RIT756, A, B) and second-stage juvenile (paratype RIT757, C, D; KORDI022, E). **(A)** Habitus, lateral view. **(B)** Head region, external view. **(C)** Habitus, lateral view. **(D)** Head region, external view. **(E)** Head capsule with papillae-like protrusions. Scale bars: 50 μm (A, C), 10 μm (B, D, E).

were very low and variable (20–67%). The two *Paradraconema* species, *P*. sp. and *P. jejuense* clustered together, with 53–98% as supporting values, and then also grouped with *D. japonicum* with relatively low supporting values (26–63%).

In this study, we also analyzed the genetic distance of 18S rRNA gene sequences for 12 nematode species, including 10 draconematid and two epsilonematid nematodes, based on the *p*-distance method using MEGA ver.4.0 to test the potential of 18S rRNA gene sequences as a molecular marker for species recognition and discrimination (Tables 5 and 6). The results of this analysis showed different rates of evolutionary substitution among taxa. Representatives of the family Epsilonematidae, used as outgroup taxa, showed relatively higher genetic distances than the species of the family Draconematidae as shown in Table 5 and 6. In the genus *Paradraconema*, the percentage of sequence distance and total nucleotide difference between two congeneric species, *P.* sp. and *P*. *jejuense*, were 2% and 31 bp. In the genus *Prochaetosoma*, the percentage of sequence distance and the total nucleotide difference among four congeneric species were very low and ranged from 0.3 to 0.7% and from 4 to 11 bp, respectively. The percentage of sequence distance and total nucleotide difference between two congeneric species of the genus *Dracograllus*, *D*. sp. 1 and *D*. sp. 2, were 2.8% and 44 bp, which is the highest value of genetic distance among species of Draconematidae examined in this study (Table 5). The mean distance of 18S rRNA gene sequences among genera within the family Draconematidae ranged from 1.4 (between *Megadraconema* and *Prochaetosoma*) to 3.6% (*Paradraconema* and *Dracograllus*), and congeneric species differed by from 0.5 (*Prochaetosoma*) to 2.7% (*Dracograllus*) (Table 6).

DISCUSSION

Relationships within the family based on morphological features

The first phylogenetic analysis of the family Draconematidae was based on 13 apomorphic morphological characters for 14 genera (Decraemer et al., 1997). The strict consensus tree based on maximum parsimony in this study suggested two monophyletic groups and six genera of the Prochaetosomatinae with unresolved position (Decraemer et al., 1997). Of the two monophyletic groups, one group was formed by the genera of the subfamily Draconematinae (*Dracograllus* Allen and Noffsinger, 1978, *Draconema* Cobb, 1913, *Paradraconema* Allen and Noffsinger, 1978), including *Tenuidraconema* Decraemer, 1989 (previously

classified in Prochaetosomatinae) and one group formed by the solely deep-sea genera (*Bathychaetosoma* Decraemer et al., 1997, *Cephalochaetosoma* Kito, 1983 and *Dinetia* Decraemer and Gourbault, 1997). The interrelationships within these clades remained unresolved. The Draconematinae is characterized by three synapomorphies: (1) general body shape with pronounced enlarged pharyngeal region, (2) dumbbell-shaped pharynx and (3) narrow and unarmed buccal cavity. *Tenuidraconema* are considered as closely related with the three genera of the Draconematinae since its differing structure, cylindrical pharynx with well-developed end bulb, is considered to be due to a secondary loss of the dumbbell-shaped pharynx.

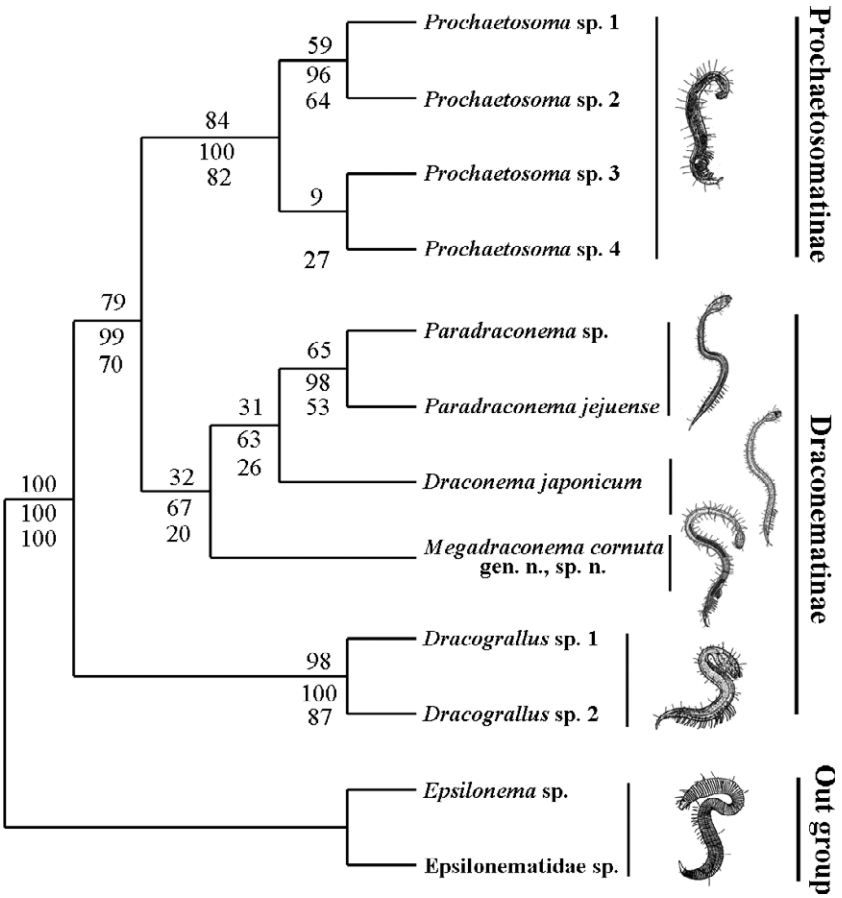

Fig. 10. Phylogenetic tree of the family Draconematidae based on an alignment of 18S rRNA gene sequences recovered for maximum likelihood. Congruent topologies were recovered using Bayesian inference, maximum likelihood, and maximum parsimony. Two epsilonematid nematodes, *Epsilonema* sp. and Epsilonematidae sp., were designated as outgroup taxa. Numbers indicated above each internode are the bootstrap values for the maximum likelihood; numbers below are supporting values (posterior probabilities) for Bayesian inference (top), and bootstrap values for the maximum parsimony (below).

The new genus *Megadraconema* gen. nov. possesses the three putative synapomorphies of the subfamily Draconematinae and is hereby assigned to it. *Megadraconema cornutum* gen. nov., sp. nov. differs from the other genera of the subfamily by the presence of a pore-like amphidial opening and an inner tubular fovea (not visible with SEM), the presence of a transverse circle of well-developed papilla-like cuticular protrusions and reticulate appearance of the cephalic subcuticle. The structure of the amphid with pore-like opening and inner tubular fovea as well as the dimorphism in shape of the amphidial fovea in second stage juveniles, differs from what was found in all other taxa of the family Draconematidae. The small, bar-shaped fovea observed in *Dracognomus* is different, as it is formed by an excavation of the cuticle and thus visible in SEM (Karssen and van Aelst, 2002).

Megadraconema cornutum gen. nov., sp. nov. is considered most closely related to species of the genus *Draconema* in the general habitus and in head structure, but can be easily distinguished from that genus by the character states mentioned above. SEM photos of *Draconema japonicum* (see Rho and Kim, 2004b: 243, Fig. 8A) also show six protruded lips, each with a setiform sen-

Table 5. Pairwise genetic distance based on *p*-distance method of 18S rRNA gene sequence of the family Draconematidae and Epsilonematidae used in this study (above diagonal: number of differences; below diagonal: pairwise distance).

Taxa	1	2	3	4	5	6	7	8	9	10	11	12
1. *Draconema japonicum*	–	29	36	33	61	49	27	25	30	25	207	120
2. *Megadraconema cornutum* gen. nov., sp. nov.	0.019	–	36	31	59	52	22	20	27	22	204	120
3. *Paradraconema* sp.	0.023	0.023	–	31	65	60	32	32	33	30	213	126
4. *Paradraconema jejuense*	0.021	0.02	0.02	–	59	51	25	25	32	27	209	113
5. *Dracograllus* sp. 1	0.039	0.037	0.042	0.038	–	44	51	49	56	51	209	134
6. *Dracograllus* sp. 2	0.031	0.033	0.038	0.033	0.028	–	46	46	51	46	210	123
7. *Prochaetosoma* sp. 1	0.017	0.014	0.02	0.016	0.033	0.029	–	4	11	6	212	116
8. *Prochaetosoma* sp. 2	0.016	0.013	0.02	0.016	0.031	0.029	0.003	–	11	6	210	116
9. *Prochaetosoma* sp. 3	0.019	0.017	0.021	0.02	0.036	0.033	0.007	0.007	–	9	214	120
10. *Prochaetosoma* sp. 4	0.016	0.014	0.019	0.017	0.033	0.029	0.004	0.004	0.006	–	210	116
11. *Epsilonema* sp.	0.132	0.13	0.136	0.133	0.133	0.134	0.135	0.134	0.137	0.134	–	201
12. Epsilonematidae sp.	0.077	0.077	0.08	0.072	0.086	0.079	0.074	0.074	0.077	0.074	0.128	–

Table 6. Mean % sequence distances within and between different nematode taxa.

Taxa	% sequence distances
Within Draconematidae	2.2
Within Epsilonematidae	12.9
Within *Dracograllus*	2.7
Within *Paradraconema*	2
Within *Prochaetosoma*	0.5
Draconematidae-Epsilonematidae	10.5
Megadraconema-Draconema	2
Megadraconema-Paradarconema	2.1
Megadraconema-Dracograllus	3.4
Megadraconema-Prochaetosoma	1.4
Draconema-Paradraconema	2.3
Draconema-Dracograllus	3.4
Draconema-Prochaetosoma	1.8
Paradraconema-Dracograllus	3.6
Paradraconema-Prochaetosoma	1.8
Dracograllus-Prochaetosoma	3

sillum and a thin-walled collar-like part between the lips and the thickened head capsule.

Phylogenetic relationships within the family based on molecular analyses

Our molecular phylogenetic analysis is largely in concordance with the results of the previous phylogenetic analysis of Decraemer et al. (1997) based on morphological synapomorphies except for the phylogenetic position of the genus *Dracograllus*. The cluster of the two species of *Dracograllus* was not consistently grouped with the other members of the subfamily Draconematinae: it diverged earlier, at the root of all draconematids used in this study; the node is supported by high bootstrap support values (100%) in all analyses (Fig. 10). The branching order of the draconematid nematodes was as follows: (*Dracograllus* ((*Megadraconema* (*Draconema, Paradraconema*)) *Prochaetosoma*)). In the previous morphological analyses of Decraemer et al. (1997), *Dracograllus* was grouped with the other members of the subfamily Draconematinae, and the clade was supported by the presence of a dumbbell-shaped pharyngeal apparatus and narrow, unarmed buccal cavity. This hypothesis is not in concordance with present results based on 18S rRNA gene sequences, and indicates that the synapomorphic morphological characters uniting the members of the subfamily Draconematinae need to be reconsidered and checked for possible convergent evolution especially with relation to *Dracograllus*. According to the putative synapomorphic morphological conditions proposed by Decraemer et al.'s (1997) phylogenetic analysis, *Megadraconema cornutum* gen. nov., sp. nov. appears to be closely related to the subfamily Draconematinae. Our results, generated from the 18S rRNA gene sequences, support a similar relationship of *Megadraconema cornutum* gen. nov., sp. nov. with its basal position within the subfamily Draconematinae compared to the constituents of *Paradraconema* and *Draconema*, but the supporting values were very low and variable depending on the analyses (20–67%) (Fig. 10). We also have to take into account that in the present study only four species of the genus *Prochaetosoma* represent the Prochaetosomatinae. A study of the other genera of the Prochaetosomatinae as well as a more highly evolving gene sampling are necessary for a more reliable investigation of the draconematid relationships.

Several attempts to develop molecular diagnostic techniques using 18S rRNA gene sequences have been achieved among various nematode taxa, such as plant parasitic nematodes, soil nematodes, and free-living marine nematodes (Floyd et al., 2002, 2005; Foucher and Wilson, 2002; Waite et al., 2003; Power, 2004; Cook et al., 2005; Bhadury et al., 2006a, b, 2007, 2008). Holterman et al. (2006) also mentioned that although a defined number of nucleotide differences cannot always be linked unequivocally, nematodes with relatively high substitution rates can mostly be recognized using a DNA barcode-based identification. The genetic distance of 18S rRNA gene sequences for marine draconematid and epsilonematid nematodes are so far unknown. According to our findings, based on the mean genetic distances of 18S rRNA sequences shown in Table 6, the draconematid nematodes have relatively lower genetic distances than the epsilonematid nematodes (2.2% *vs* 12.9%). *Dracograllus* showed the fastest substitution rates (2.7%), and *Prochaetosoma* showed the slowest substitution rates (0.5%) within the Draconematidae. Moreover, the mean genetic distance of 18S rRNA gene sequence among genera within the family Draconematidae ranged from 1.4 to 3.6% and congeneric species differed by from 0.5 to 2.7%. On the basis of the above results, we recognized that the genetic distance of 18S rRNA gene sequence has a possibility for the recognition and discrimination among the marine draconematid and epsilonematid nematodes.

DICHOTOMOUS KEY TO GENERA OF THE FAMILY DRACONEMATIDAE

The following dichotomous key to genera of the family Draconematidae was developed based on the key characters of the previous literatures by Allen and Noffsinger (1978) and Decraemer et al. (1997), and we just added the new apomorphic character of the new genus, *Megadraconema* gen. nov., such as the structure of the subcuticle of head region and a transverse circle of well-developed papillae-like protrusions near anterior end of head capsule.

1. Head capsule present 2
– Head capsule absent .... *Dinetia* Decraemer and Gourbault, 1997
2(1). Pharynx with swollen corpus and swollen posterior bulb (dumbbell-shaped) 3
– Pharynx with cylindrical corpus, or with minor swelling, and terminal posterior bulb 6
3(2). Cephalic sublateral acanthiform setae present on head capsule .....*Paradraconema* Allen and Noffsinger, 1978
– Cephalic sublateral acanthiform setae absent on head capsule (except in *Dracograllus steckhoveni*) 4
4(3). Swollen anterior body region less than 13% of total body length 5
– Swollen anterior body region more than 18% of total body length (18–25%) ... *Dracograllus* Allen and Noffsinger, 1978
5(4). Anteriormost annules (7–16) larger with subcuticular granulation ..... *Draconema* Cobb, 1913

– Head with reticular subcuticle; a transverse circle of well-developed papillae-like protrusions present near anterior end of head capsule..... *Megadraconema* gen. nov.
6(2). Lumen wall of end bulb of pharynx not thickened..... 7
– Lumen wall of end bulb of pharynx moderately or strongly thickened 12
7(6). Buccal cavity with a dorsal tooth..... 8
– Buccal cavity unarmed..... 10
8(7). Number of cephalic adhesion tubes less than 8; body less than 900 μm in length 9
– Number of cephalic adhesion tubes higher than 10; slender body more than 1000 μm in length .....
..... *Cygnonema* Allen and Noffsinger, 1978
9(8). Head capsule conical; pharyngeal region swollen dorsally and ventral side almost straight; cuticle annulations smooth; tail cylindrical-conoid .....
..... *Draconactus* Allen and Noffsinger, 1978
– Head capsule broadly rounded; pharyngeal region without dorsal swelling, nearly cylindrical; cuticle with spiny ornamentation; tail elongate cylindrical-conoid .....
.....*Apenodraconema* Allen and Noffsinger, 1978
10(7). Buccal cavity collapsed or weakly developed; pharynx with inconspicuous terminal swelling; cuticle very thick... 11
– Buccal cavity not collapsed, narrow; pharynx with muscular terminal bulb; cuticle thin or smooth with lateral alae.....
.....*Tenuidraconema* Decraemer, 1989
11(10). Cephalic adhesion tubes located anteriorly on head capsule ..... *Dracogalerus* Allen and Noffsinger, 1978
– Cephalic adhesion tubes located at border of head capsule and anterior body annules.....
..... *Notochaetosoma* Irwin-Smith, 1918
12(6). Number of cephalic adhesion tubes higher than 16..
..... 13
– Number of cephalic adhesion tubes less than 14..... 14
13(12). Slightly swollen anterior body region more than 23% of total body length; head capsule differentiated without a thickened cuticle; number of CAT 16–18 .....
..... *Bathychaetosoma* Decraemer et al., 1997
– Slightly swollen anterior body region less than 19% of total body length; head capsule present; number of CAT 18–27
..... *Cephalochaetosoma* Kito, 1983
14(12). Amphidial fovea reduced, inverted U-shaped or tubular; pharynx with moderately thickened lumen wall in bulb; PAT without differentiated tip.....
..... *Dracognomus* Allen and Noffsinger, 1978
– Amphidial fovea C-shaped; pharynx with a well thickened lumen wall in bulb; PAT with bell-shaped tip.....
..... *Prochaetosoma* Micoletzky, 1922

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