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Molecular Phylogeny of the Genus *Paracanthonchus* (Nematoda: Chromadorida) with Description of *P. yeongjongensis* sp. nov. from Korea [†]

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Abstract: During an investigation of the intertidal zone at Yeongjongdo Island, Incheon, Korea, a new species belonging to the genus *Paracanthonchus* was found and is reported. *Paracanthonchus* Mikoletzky, 1924 is the largest genus within the family Cyatholaimidae, and the species identification of this genus has been difficult mainly due to overlapping characteristics and a lack of genus/species-defining apomorphic characters. The new species is characterized by the buccal cavity, armed with one large dorsal tooth and two subventral teeth, the presence of lateral differentiation, seventy-six tubular precloacal supplements, and proximally paired gubernaculum. Alongside the description, we are updating the review of the genus by providing an up-to-date list of valid species, as well as a comprehensive tabular key to the genus with measurements of species-discerning characteristics. We also provide partial sequences of mtCOI, 18S and 28S rRNA to verify the new species belongs to the genus *Paracanthonchus* and to discuss the phylogeny of the family Cyatholaimidae as well as the genus *Paracanthonchus*. Our phylogeny agrees with previous findings that, while Cyatholaimidae forms a monophyletic clade, many genera within, including *Paracanthonchus*, are paraphyletic. For now, it appears that subfamilies are not well-represented by molecular means, and much more molecular data along with species defining morphological traits will have to be accumulated to meaningfully organize this family and the genus within.

Keywords: free-living marine nematode; meiofauna; Cyatholaimidae

1. Introduction

The nematode family Cyatholaimidae Filipjev, 1918 is a relatively diverse group of marine free-living nematodes [1] comprising 26 genera and 257 species [2]. It consists of free-living marine nematodes which inhabit marine sediments, and their distribution and dispersal has been the subject of many studies [3–5]. The family was first erected as a subfamily (Cyatholaimini) by Filipjev in 1918 [6], to be reorganized as a family by De Coninck and Schuurmans-Stekhoven (1933) [7]. According to the latest revision of the family by Cunha et al. (2022), there are four subfamilies which make up the family Cyatholaimidae: Cyatholaiminae, Paracanthonchinae, Pomponematinae and Xenocyatholaiminae. NeMys [8], however, lists a total of five subfamilies, including *Nyctonema* Bussau, 1993 with a sole species, *Nyctonema portentosum* Bussau, 1993. On the other hand, the most recent classification of the phylum Nematoda by Hodda (2022) only lists two subfamilies, Cyatholaiminae and Paracanthonchiinae, and replaced Xenocyatholaiminae by placing

Xenocyatholaimus, a genus with lone species belonging to subfamily Xenocyatholiminae Gerlach & Riemann, 1973, in the subfamily Paracanthonchinae. Hodda (2022) does not include remarks on why he simplified the subfamilies, but it is clear he took gubernaculum structure as a key component in discerning the two subfamilies. Evidently, the validity of the subfamilies within Cyatholaimidae is not a fixed matter, but they are discerned by the structure of amphid, the structure of gubernaculum, the shape of the tail and the position of the vulva. The main problem, which means that this family does not have clear boundaries, is that these species, genera, and subfamilies' dividing morphological features are based on a combination of multiple non-phylogenetic informative characters [4]. To make matters more complicated, recent phylogenetic analyses suggest that while the family itself maybe monophyletic, most genera belonging to Cyatholaimidae lack synapomorphic characters and are therefore non-monophyletic. Cunha et al. (2022)'s review of the family Cyatholaimidae provides a comprehensive revision that clarifies the delimitation of genera within the family, but the core problem of mixed topology remains unresolved.

Paracanthonchus is a large genus comprised of cosmopolitan species [3,4] which makes up a fifth of the whole family Cyatholaimidae [2–4]. *Paracanthonchus* was first established by Micoletzky (1924) [9], when he described the type species *Paracanthonchus caecus*. Since then, over 70 species have been described and many revisions of the genus have taken place [10–14]. The genus *Paracanthonchus* can be characterized by the following characteristics: (1) cuticle with transverse rows of fine dots and lateral differentiation with 2–4 rows of larger dots, (2) buccal cavity with an especially big dorsal tooth, (3) distally expended and dentate gubernaculum, and (4) tubular precloacal supplements [10,15,16]. However, due to a lack of apomorphic characters, diagnostic characters often overlap across other genera, making the species delimitation of this group a difficult task [4]. While Cunha et al., 2022, did briefly provide updated diagnoses of the genus with an updated number of valid species, it was still missing critical elements for species delimitation, such as a tabular key. Prior to Cunha et al., 2022, the most recent and complete revision of *Paracanthonchus* was by Miljutina and Miljutin, 2015. While an in-depth taxonomic history of the genus and tabular key was provided, considerable changes have taken place since, and phylogenetic aspects were not discussed. Much like its parent family, the number of valid species varies among different sources for the genus *Paracanthonchus*. NeMys currently lists 59 valid species [8], with relevant papers all citing different numbers (Hodda, 2022, 80 species [2]; Miljutina and Miljutin, 2015, 72 species (including 20 species inquirenda) [3]; Tchesunov, 2014, 67 species [15]; Tchesunov, 2015, 63 species [17]; Lee et al., 2016, 72 valid species [18]).

With various papers citing contradicting number of valid species, we have taken on the role of providing a comprehensive, updated review of the genus including an updated list of valid species and a tabular key for species delimitation. Along with the description and depiction of the new species, we also provide molecular sequences (mtCOI, 18S, 28S rRNA) of our new species, supplemented with a phylogenetic tree, to discuss the topology of the family Cyatholaimidae and the genus *Paracanthonchus*. Geographically, with approximately 70 species of marine nematodes described in Korea [18–22], this new species is the third species of Cyatholaimidae (with *P. kamui* Kito, 1981 and *P. macrodon* (Ditlevsen, 1918) previously reported from the east [18]) to be reported in Korea and the tenth species of *Paracanthonchus* to be reported in East Asia (*P. brevicaudatus* Gagarin and Nguyen Vu Thanh, 2016, Vietnam [23]; *P. hawaiiensis* Allgén, 1951, Japan [24]; *P. heterocaudatus* Huang and Xu, 2013, China [25]; *P. kamui* Kito, 1981, Japan [26]; *P. macrodon* (Ditlevsen, 1918) Micoletzky, 1924, Korea [18]; *P. mamubiae* Miljutina and Miljutin, 2015, North West Pacific [3]; *P. multisupplementatus* Gagarin, 2012, South China Sea [27]; *P. perspicuus* Kito, 1981, Japan [26]; *P. securus* Nguyen and Gagarin, 2018, South China Sea [28]).

2. Materials and Methods

2.1. Morphological Analysis

Free-living marine nematodes were collected from the intertidal zone on Yeongjongdo Island, Incheon, Republic of Korea, (37°26′13″ N, 126°23′03″ E) on 26 August 2022 (Figure 1).

The sediments were collected using a spoon to scratch only the top layer, then fixed in 90% ethanol. Samples were brought back to the laboratory and stored in the freezer.
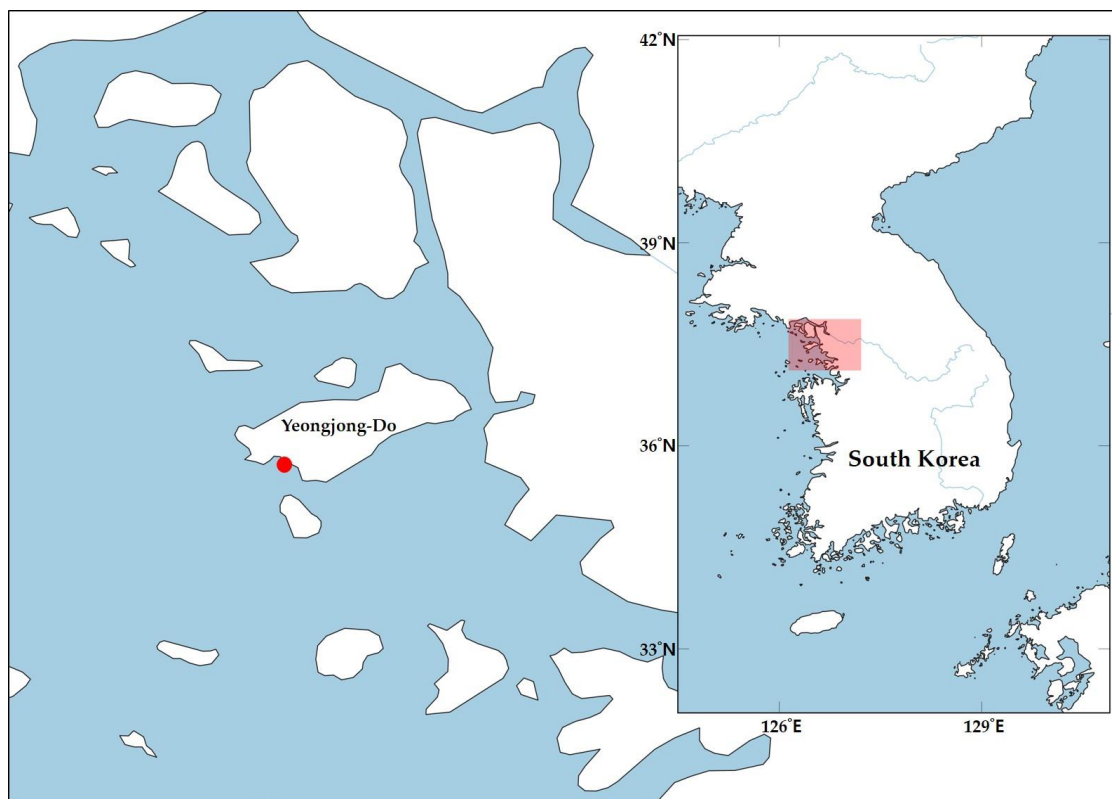

Figure 1. Map of the sample locality. The red box indicates region zoomed-in on the map; The red dot indicates the type locality of the new species. The map was made with QGIS v. 3.28.2 (downloaded from https://qgis.org/en/site/forusers/download.html, accessed on 3 February 2023).

Nematodes were sorted in a Petri dish under a dissecting microscope (Olympus SZX-10, Olympus, Tokyo, Japan) then placed in a pool containing a solution of 10% glycerol and distilled water. The dish was left out at room temperature for dehydration to take place, which lasted 1 to 2 days. After dehydration, the specimens were mounted with very small beads on anhydrous glycerin using a standard wax-ring method [29].

For species identification, each specimen was observed under a 100X objective lens with immersion oil using an optical microscope (Nikon ECLIPSE 80i, Nikon, Tokyo, Japan) with Nomarski Differential Interference Contrast (DIC) illumination. All pencil drawings were prepared using a drawing tube equipped with an optical microscope. Line drawings were digitally prepared with a Wacom Cintiq 16 tablet (Wacom, Saitama, Japan) and Adobe Illustrator (Adobe, San Jose, CA, USA). All morphometric measurements were measured with Fiji software [30].

The classification of marine nematodes reflects Hodda (2022) [2], and species identification referred to pictorial keys of Platt and Warwick (1988) [16], as well as all the original descriptions.

For scanning electron microscopy, the identified specimens were transferred to a well of distilled water to be washed, and gradually transferred to ethanol series for dehydration (20%, 30%, 40% 50%, 60%, 70%, 80%, 90%, 95%, and 100%, each 1 h). Following dehydration, specimens were mounted on a stub with gold in an ion sputter coater after being critical-point dried (CPD300, Leica, Wetzlar, German). Observations and photographs were taken using a JSM-6390LV (JEOL, Japan) scanning electron microscope.

2.2. gDNA Extraction and Amplification

For genomic DNA extraction, specimens of interest were handpicked from samples separately fixed with pure ethanol. Two specimens were found and swiftly observed with a temporary slide to confirm species identification, then transferred to a pool of distilled water on a Petri dish to wash and remove excess ethanol. For DNA extraction, DNAeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) was used following the manufacturer's instructions. DNA concentration of the DNA templates were measured using NanoDrop 2000 (ThermoScientific, Wilmington, NC, USA), and the concentrations ranged from 2.5 to 4.4 ng/μL. For DNA amplification, AccuPower® PCR Master Mix (Bioneer, Daejeon, Republic of Korea) was used, achieving 20 μL of total reaction volume (10 μL premix, 1 μL for each primer, 6 μL DNA template and 2 μL ultra-pure water). Amplification protocol and respective primer set information are provided below (Table 1). Amplification success was checked by visualizing PCR product via 1% agarose gel electrophoresis. PCR products of both strands were sent to Bioneer (Daejeon, Republic of Korea) to be purified and sequenced. All sequences newly obtained in this study were uploaded to GenBank (Accession numbers listed in Table S1).

Table 1. Primer-related information used in this study.

Marker	Primer (Direction)	Sequence 5′-3′	Amplification Condition	Sequence Length (bp)	Reference
mtCOI	JB3 (f)	TTTTTTGGGCATCCTG AGGTTTAT	94 °C for 5 min, 5 cycles of 94 °C for 30 s, 54 °C for 30 s and 72 °C for 30 s, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 30 s, followed by final step of 72 °C for 10 min	330	Derycke et al., 2010 [31]; Bowles et al., 1992 [32]
	JB5 (r)	AGCACCTAAACTTAAA ACATAATGAAAATG			
18s	18S-CL-F (f)	TCAAAGATTAAGCCA TGCAT	95 °C for 3 min followed by 36 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 45 s, and extension at 72 °C for 3 min, followed by final step at 72 °C for 7 min	494	Carta and Li, 2018; Carta and Li, 2019 [33,34]
	530R ®	GCGGCTGCTGGCACCA CACTT			
28S	D2A (f)	ACAAGTACCGTGAGG GAAAGTTG	95 °C for 5 min followed by 37 cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 1 min, and extension at 72 °C for 1 min 30 s min, followed by final step at 72 °C for 5 min	731–741 bp	De Ley et al., 2005 [35]
	D®(r)	TCGGAAGGAACCAGCT ACTA			

2.3. Phylogenetic Analysis

All sequences were visualized using FinchTV (v. 1.4.0, Geospiza, Inc.; Seattle, WA, USA; http://www.geospiza.com, accessed on 3 February 2023), and low-quality peaks were filtered by comparing both complementary strands. The two strands (forward and reverse) were aligned using ClustalW [36] implemented in MEGA (v. 11.0.13) [37]. Aligned sequences were compared against NCBI GenBank database using BLAST algorithm [38]. Pairwise distance between the two specimens of *Paracanthonchus* sp. nov. and other closely related species of mtCOI, 18S and 28S rRNA sequences were calculated with MEGA 11 using K2P model [39]. Phylogenetic trees were constructed using two nuclear genes (18S and 28S rRNA). Attempts were made to obtain longer 18S rRNA sequences, but with no success. Maximum likelihood (ML) and Bayesian Inference (BI) approaches were used when performing phylogenetic analyses. For ML analysis, IQ-TREE (multicore v. 2.0.3) [40] and the implemented ModelFinder [41] were used to determine the model of best-fit using Akaike information criterion (AIC). TIM3 + F + G4 and TIM3 + F + I + G4 models were determined to be the best-fit models for 18S and 28S datasets, respectively, and were selected for constructing ML tree with 1000 bootstrap replicates using IQ-Tree webservers using the ultrafast setting [42]. For BI analysis, jModelTest software (v. 2.1.7) [43] was used to find the best-fit model. BI tree was made utilizing MrBayes software (v. 3.2.6) [44] using the following model parameters for 18S rRNA dataset: Lset base = (0.2657, 0.2100,

0.2582, 0.2660) nst = 6 rmat = (1.0000, 1.7981, 1.0000, 1.0000, 3.2963, 1) rates = gamma shape = 0.4800 ncat = 4 pinvar = 0 with *Enoplus* sp. set as outgroup. For 28S rRNA dataset, the following model parameters were used: Lset base = (0.2115, 0.2252, 0.3175, 0.2459) nst = 6 rmat = (0.5077, 2.5356, 1.0000, 0.5077, 5.6541, 1) rates = gamma shape = 0.5890 ncat = 4 pinvar = 0.1870 with *Enoploides* sp. set as outgroup. Markov Chain Monte Carlo (MCMC) was run with ngen = 1,000,000, nchains = 4, samplefreq = 100, savebrlens = yes, printfreq = 1000, sump burnin = 250, and sumt burnin = 250. All trees were exported to FigTree (v. 1.4.4) [45], where visualizations and modifications were made.

3. Results

3.1. Systematics

　　Class: Chromadorea Inglis, 1983
　　Order: Chromadorida Chitwood, 1933
　　Family: Cyatholaimidae Filipjev, 1918 (De-Coninck and Schuurmans-Stekhoven, 1933)
　　Subfamily: Paracanthonchiinae De-Coninck, 1965
　　Genus: *Paracanthonchus* Micoletzky, 1923

3.2. Diagnosis (Followed Tchesunov, 2014)

Cyatholaimidae. Paracanthonchinae. Body cuticle composed of transverse rows of tiny fine dots, which laterally may be larger or irregularly arranged. Six outer labial and four cephalic seta jointed in one circle. Amphideal fovea multi-spiral. Cheilostom with twelve rugae. Buccal cavity with larger dorsal tooth and one or two pairs smaller subvetral teeth. Gubernaculum proximally paired, distally expanded and dentate. Tubular precloacal supplementary organs.

　　Type species: *Paracanthonchus caecus* (Bastian, 1865).

3.3. List of Valid Species

1. *Paracanthonchus austrospectabilis* Wieser, 1954 (Wieser, 1954: 19, Figure 100a–f; Chile, Islas Gueitecas, 3–5 m depth, small stones, sand and gravel; 5–7 m depth, from Mytilidae and echinids at stones with algae; from calcareous alga; Punta Arenas, algae, gravel and clay, mixed with mud; Talcahuano, rocks with small rock pools) [10].

2. *Paracanthonchus barka* Inglis, 1962 (Inglis, 1962: 271–272, Figure 66–67; France, Banyuls-sur-Mer, seaweeds with very little sediment on the rocks) [46].

3. *Paracanthonchus batidus* Gerlach, 1957 (Gerlach, 1957: 432, Figure 7e–i; Brazil, Säo Sebastiäo, fine sand) [47].

4. *Paracanthonchus bipapillatus* Kreis, 1928 (Kreis, 1928: 174–176, Taf. VI, Figure 24; Barents Sea, Kildin Island, few stones, a shell with algae) [48].

5. *Paracanthonchus bothnicus* Schiemer, Fritz, Jensen, Preben and Riemann, Franz, 1983 (Schiemer et al., 1983: 288, Figure 7; the Bothnian Bay, sand from 5 m depth, soft mud from 82 m) [49].

6. *Paracanthonchus brevicaudatus* Gagarin & Nguyen Vu Thanh, 2016 (Gagarin and Nguyen Vu Thanh, 2016: 9–14, Figure 1; Vietnam, artificial reservoirs) [23].

7. *Paracanthonchus breviseta* (Schuurmans Stekhoven, 1950) Hope & Murphy, 1972 (Schuurmans Stekhoven, 1950: 100–101, Figure 55a–c; (*Paraseuratiella breviseta*); Villefranche, 50m depth, grey mud. Hope and Murphy, 1972, transferred the species from the genus *Paraseuratiella* to *Paracanthonchus*) [11,12].

8. *Paracanthonchus bulbicola* Bussau, 1993 (Bussau, 1993: 160–165, abb. 50–52; Peru basin, a manganese lying on the seabed) [50].

9. *Paracanthonchus caecus* (Bastian, 1865) Micoletzky, 1924 (Bastian, 1865: 163, Plate VIII, fig, 213–214; (*Cyatholaimus caecus*); Falmouth, Marine surface-mud from estuary). Micoletzky, 1924, transferred the species from the genus *Cyatholaimus* to *Paracanthonchus*) [51].

10. *Paracanthonchus canadensis* Vincx, Sharma & Smol, 1982 (Vincx et al., 1982: 251, Figure 5A–G; Iona Island, Intertidal sand flat with medium-fine sand) [5].

11. *Paracanthonchus cheynei* Inglis, 1970 (Inglis, 1970: 6, Figure 6–11; Cheyne Beach, seaweed, and holdfasts with associated sand) [52].

12. *Paracanthonchus cochlearis* Gerlach, 1957 (Gerlach, 1957: 431–432, abb. 7a–d; Brazil. santos, fine sand) [47].

13. *Paracanthonchus cristatus* Wieser, 1954 (Wieser, 1954: 20, Figure 102a–c; Chile, Talcahuano, alga without detritus on rock) [10].

14. *Paracanthonchus elongatus* (de Man, 1907) Micoletzky, 1924 (de Man, 1907: 70–73, pl. IV, Figure 14; (*Cyatholaimus elongates*); Walcheren, tidal zone). Micoletzky, 1924, transferred the species from the genus *Cyatholaimus* to *Paracanthonchus*) [53].

15. *Paracanthonchus filipjevi* Micoletzky, 1924 (Filipjev, 1922: 113–114, Pl. 1, Figure 10a–b; (*Cyatholaimus caecus*); Black Sea, tidal zone) [54].

16. *Paracanthonchus gerlachi* Vincx, Sharma & Smol, 1982 (Vincx et al., 1982: 251–254, Figure 6A–G; (*Paracanthonchus caecus* sensu Gerlach 1965); Norway, Tromsø, tidal zone) [5].

17. *Paracanthonchus gynodiporata* Apolonio Silva De Oliveira, Decraemer, Moens, Dos Santos & Derycke, 2017 (Apolonio Silva De Oliveira et al., 2017: 1–17, Figure 5; Brazil, Cupe Beach, subtidal zone with brown seaweed) [55].

18. *Paracanthonchus hartogi* Inglis, 1970 (Inglis, 1970: 9–10, Figure 19–22; Australia, Shark Bay, 35 m depth, among mud and weed) [52].

19. *Paracanthonchus hawaiiensis* Allgén, 1951 (Allgén, 1951: 288–289, Figure 12; the coast of Honolulu, 10–40 m depth, tidal zone) [24].

20. *Paracanthonchus heterocaudatus* Huang & Xu, 2013 (Huang and Xu, 2013: 6–9, Figure 3; Yellow Sea, Yantai Coast, intertidal sandy sediment) [25].

21. *Paracanthonchus heterodontus* (Schulz, 1932) Vincx, Sharma & Smol, 1982 (Schulz 1932: 372–374, Figure 21a–d; (*Cyatholaimus heterodontus*) [56]; Bay of Kiel, tidal zone. Vincx et al., 1982: 254–256, Figure 7; Lake Grevelingen of the Netherlands, fine-medium sand) [5].

22. *Paracanthonchus kamui* Kito, 1981 (Kito, 1981: 273–275, Figure 12; Oshoro Bay of Japan, from *Sargassum confusum*) [26].

23. *Paracanthonchus kartanum* (Mawson, 1953) Wieser, 1959 (Mawson, 1953: 39–40, Figure 12–14; (*Harveyjohnstonia karanum*); Australia, Kangaroo Island, Pcnnington Bay, littoral rock. Wieser, 1959, transferred the species from the genus *Harveyjohnstonia* to *Paracanthonchus*) [13].

24. *Paracanthonchus latens* Gourbault, 1980 (Gourbault, 1980: 66–70, Figure 3; the South Atlantic Ocean, 2944–4180 m depth) [57].

25. *Paracanthonchus lissus* Gagarin & Klerman, 2008 (Gagarin and Klerman, 2008: 2–4, Figure 1; Mediterranean near Israel, 1–1.5 m depth, upper subtidal zone) [58].

26. *Paracanthonchus longicaudatus* Warwick, 1971 (Warwick, 1971: 99–100, Figure 2; England, the Northumberland coast, fine sand of 54m depth, silt of 80 m depth) [59].

27. *Paracanthonchus longispiculum* Pastor de Ward, 1985 (Pastor de Ward, 1985: 24–25, Figure 31; Argentina, Ria Deseado province, tidal and upper subtidal zones) [60].

28. *Paracanthonchus macrospiralis* Allgén, 1959 (Allgén, 1959: 228–229, abb. 4; Argentina, Fuegian Archipelago, Isla de los Estados, 36 m depth, gravel and shells) [61].

29. *Paracanthonchus major* (Kreis, 1928) Wieser, 1954 (Kreis, 1928: 176–177, Taf. II, III, VI, VII, Figure 25; (*Paracyatholaimus major*); Barents Sea, Kildin Island, few stones, a shell with algae. Wieser, 1954, transferred the species from the genus *Paracyatholaimus* to *Paracanthonchus*) [48].

30. *Paracanthonchus mamubiae* Miljutina & Miljutin, 2015 (Miljutina and Miljutin, 2015: North Pacific Ocean, Zenkevich Rise, ca. 5350 m depth) [3].

31. *Paracanthonchus margaretae* Inglis, 1970 (Inglis, 1970: 8–9, Figure 12–18; Australia, Cheyne Beach, weed and sand in 20 cm of silt) [52].

32. *Paracanthonchus medius* Galtsova, 1976 (Galtsova, 1976: 280–281, Figure 22; White Sea, slightly silted sand) [62].

33. *Paracanthonchus micoletzkyi* Schuurmans Stekhoven, 1943 (Schuurmans Stekhoven, 1943: 359–360, Abb. 28; Mediterranean, Alexandria) [63].

34. *Paracanthonchus micropapillatus* Wieser, 1954 (Wieser, 1954: 23, Figure 105a–f; Chile coast, littoral algae and sublittoral secondary substrate) [10].

35. *Paracanthonchus miltommatus* Leduc & Zhao, 2018 (Leduc and Zhao, 2018: New Zealand, Greta Point, low intertidal zone, from red seaweed on boulders) [1].

36. *Paracanthonchus multisupplementatus* Gagarin, 2012 (Gagarin, 2012: 60–68, Figure 1; Vietnam, Quang Ninh province, littoral zone, 2 m depth, sand) [27].

37. *Paracanthonchus multitubifer* Timm, 1961 (Timm, 1961: 56, Figure 42; Bay of Bengal, upper subtidal zone, bottom mud) [64].

38. *Paracanthonchus mutatus* Wieser, 1959 (Wieser, 1959: 40–41, Figure 39a–c; Seattle, patches of sand between the boulders) [14].

39. *Paracanthonchus nannodontus* (Schulz, 1932) Wieser, 1954 (Schulz, 1932: 370–372, Figure 20a–c; (*Cyatholaimus nannodontus*); Bay of Kiel, tidal zone, among seaweed. Wieser, 1954, transferred the species from the genus *Cyatholaimus* to *Paracanthonchus*) [56].

40. *Paracanthonchus olgae* Tchesunov, 2015 (Tchesunov, 2015: 356–361, Figure 10–13; Northern Mid-Atlantic Ridge, rainbow hydrothermal site, 2260–2350 m depth, washout from a druse of mussels *Bathymodiolus azoricus*) [17].

41. *Paracanthonchus parahartogi* Decraemer & Coomans, 1978 (Decraemer and Coomans, 1978: 531–535, Figure 7; Australia, Lizard Island, sand and algal mats from mangrove) [65].

42. *Paracanthonchus perspicuus* Kito, 1981 (Kito, 1981: 275–276, Figure 13; Oshoro Bay of Japan, from *Sargassum confusum*) [26].

43. *Paracanthonchus platti* Jayasree Vadhyar, 1980 (Jayasree Vadhyar, 1980: 376–378, Figure 1; Scotland, sandy beach) [66].

44. *Paracanthonchus platypus* Wieser & Hopper, 1967 (Wieser and Hopper, 1967: 267–268, Figure 31a–c; Florida, tidal zone) [67].

45. *Paracanthonchus quinquepapillatus* Wieser, 1959 (Wieser, 1959: 40, Figure 38a–b; Seattle, patches of sand between the boulders) [14].

46. *Paracanthonchus ruens* Wieser, 1954 (Wieser, 1954: 20, Figure 101a–d; Southern Chile, tidal belt, algae growing on boulders) [10].

47. *Paracanthonchus sabulicolus* Bouwman, 1981 (Bouwman, 1981: 52–53, Figure 20; Netherlands, the Ems estuary, low tidal zone) [68].

48. *Paracanthonchus sandspitensis* Nasira, Kamran & Shahina, 2007 (Nasira et al., 2007: 95–97, Figure 2; Pakistan, Sandspit beach, intertidal zone) [69].

49. *Paracanthonchus securus* Nguyen & Gagarin, 2018 (Nguyen and Gagarin, 2018: 90–94, Figure 3; Vietnam, Quang Ninh Province, artificial reservoirs, sand) [28].

50. *Paracanthonchus sonadiae* Timm, 1961 (Timm, 1961: 56–58, Figure 43; Bay of Bengal, upper subtidal zone, bottom mud) [64].

51. *Paracanthonchus stateni* Allgén, 1930 (Allgén, 1930: 27–28, Abb. 1; Fuegian Archipelago) [70].

52. *Paracanthonchus stekhoveni* Wieser, 1954 (Schuurmans Stekhoven, 1950: 101–103, Figure 56; (*Praeacanthonchus micoletzkyi*); France, Villefranche, coarse sand under *Posidonia*. Wieser, 1954, transferred the species from the genus *Praeacanthonchus* to *Paracanthonchus*) [11].

53. *Paracanthonchus steueri* (Micoletzky, 1922) Micoletzky, 1924 (Micoletzky, 1922: 86-88, Figure 4; (*Cyatholaimus steueri*); Egypt, coral reef, 1–2 m depth, sandy bottom with algae. Micoletzky, 1924, transferred the species from the genus *Cyatholaimus* to *Paracanthonchus*) [71].

54. *Paracanthonchus sunesoni* (Allgén, 1942) Wieser, 1954 (Allgén, 1942: 39–40, Abb. 9; (*Cyatholaimus sunesoni*); Mediterranean, Banyuls-sur-Mer, 0.5–1 m depth, under algae rock bottom. Wieser, 1954, transferred the species from the genus *Cyatholaimus* to *Paracanthonchus*) [72].

55. *Paracanthonchus thaumasius* (Schulz, 1932) Vincx, Sharma & Smol, 1982 (Schulz, 1932: 375–377, Figure 23a–c; (*Cyatholaimus thaumasius*); Bay of Kiel, tidal zone [56]. Vincx et al., 1982: 256–261, Figure 8–10; the Belgian and Netherlands coast, clean and fine-medium sand with some silt) [5].

56. *P.tumepapillatus* Timm 1957 (Timm, 1957: 133, Figure 1; Bay of Bengal, upper subtidal zone, bottom mud) [73].

57. *Paracanthonchus tyrrhenicus* (Brunetti, 1949) Gerlach, 1953 (Brunetti, 1949: 50–52, Figure 2B; (*Paracyatholaimus tyrrhenicus*) [74]; Mediterranean, tidal, or upper subtidal zone. Gerlach, 1953: 549, Abb. 15; Italy, Palermo, coastal waters) [75].

58. *Paracanthonchus uniformis* (Schuurmans Stekhoven, 1950) Wieser, 1954 (Schuurmans Stekhoven, 1950: 104, Figure 58A–E; (*Praeacanthonchus uniformis*); France, Villefranche, 5 m depth, sand. Wieser, 1954, transferred the species from the genus *Praeacanthonchus* to *Paracanthonchus*) [11].

59. *Paracanthonchus wellsi* Leduc & Zhao, 2023 (Leduc and Zhao, 2023: 92–97, Figure 52–55; Pāuatahanui Inlet, Wellington region, lower North Island, upper intertidal, gravelly sand) [76].

60. *Paracanthonchus yeongjongensis* Kim, Lee & Jeong, 2023 sp. nov. (Kim et al., 2023: Korea, Yeongjongdo Island, muddy-sand tidal zone) (This study).

3.4. Tabular Key to Valid Species (Table 2)

Based on the original list of Miljutina and Miljutin, 2015 [3], species newly reported since 2015 and re-evaluated species were added/removed, respectively. Species considered invalid (including species inquirenda) were omitted from the table, and characters considered detrimental for species delimitation within the genus were reviewed. Measurements were corrected (if erroneous) by reviewing original and related papers of all listed species.

Table 2. Tabular key of valid *Paracanthonchus* species with morphological characters (amended from Miljutina and Miljutin, 2015).

Species	Body Length (Male)	Body Length (Female)	Lateral Differentiation of Cuticle	Number of Subventral Teeth	Distance from Anterior to Amphid	Amphid Width	Turn Number of Amphid	Spicule Length as Arc	Gubernaculum Length	Number of Cusps on Distal End of Gubernaculum	Number of Supplements	Tail Shape
P. austrospectabilis	1440–2090	1370–2280	none/except on the tail	n/a	9	13	4.5	65	65	numerous	6	conical
P. barka	860		presence	n/a	5	7–8	4.5–5.5	19	18	2	4	conical
P. batidus	888		none	n/a	6	5.5	2.5	31	22	n/a	6	conical
P. bipapillatus	2046		none	none	10	13	2.5	70.2	59.8	2	5	conical
P. bothnicus	1568–1800	1715–1784	presence	4	9	10	6	50	45	4	5	conical
P. brevicaudatus	956–1230	992–1413	none	3	11	13–15	4.3–4.5	30	27	16–18	3	conical
P. breviseta	1092	1072	none	n/a	20	14	5.5	44	n/a	1	4	conical
P. bulbicola	1340	1230–1960	presence	4	11	15	6.25	38	23	numerous	5	conical
P. caecus	976–1470		presence	2–4	10	7–13	4.5–5.5	40–48	35–44	8–9	5	caecous
P. canadensis	960–1160	1089–1170	none	4	7	7	6	39.1–40.6	35.5–40.6	numerous	5	conical
P. cheynei	1240–1280	1090	none	4	14	9–10	4.25	46–48	39–42	numerous	6	conical
P. cochlearis	1123	1162	presence	2	10	17	6	36	30	2	5	conical–cylindrical
P. cristatus	1050–1360	1180–1300	presence	n/a	9–13	8	2.75–3	40	35	n/a	8–9	conical
P. elongatus	3025		presence	none	15	15	4–5	86 (as chord)	n/a	numerous	5	conical
P. filipjevi	1150	1300	n/a	n/a	7	9	4–5.5	46	41	2	3	conical
P. gerlachi	1194–1391	1045–1480	none	4	12	10	7.5	38–39	36	7	5	conical
P. gynodiporata	1001–1146	1075–1238	presence	4	11.3–12.9	7.4–10.7	4	38.9–42.1	34.5–40.8	numerous	4	conical
P. hartogi	1240–1420		none	none	15	12–13	3.25	44–49	39–44	4	6	conical
P. hawaiiensis	1625		presence	n/a	13	10	3	40–44	39–44	numerous	4	conical
P. heterocaudatus	1335–1570	1555–1750	presence	2	7	9–13	5–6	31–32	25–27	2	6	conical–cylindrical
P. heterodontus	1042–1668	1129–1842	none	4	13	13	4.5	56–67	46–54	numerous	5	conical
P. kamui	1658–1728	1761–1816	presence	4	6	10	4.25	53–60	45–55	numerous	6	conical
P. kartanum	870–1600	1450	none	4	15	9	3.5–4.5	23–38	26–36	numerous	6	conical
P. latens	1733–2458		none	4	8	10	6.5	84–87	43–50	numerous	4	conical
P. lissus	1165–1252	1334	none	n/a	10	10–12	4–4.5	39–43	45–49	4	4	conical
P. longicaudatus	1330–1510	1570–1790	none	4	12	9–10	5.3–6.25	43–51	40–42	numerous	5	conical–cylindrical
P. longispiculum	1780–1800	1900	n/a	2	11	6–10	2.5–3	70–76	70	none	8	conical
P. macrospiralis	3000		presence	n/a	12	20	3	65	32	n/a	n/a	conical
P. major	2608	2676	none	n/a	n/a	6.5–7.8	2.5	59.8	49.4	none	4	conical
P. mamubiae	1610–1831	1638–1971	none	4	3–7	7–14	4.15	60–65	46–51	numerous	3–5	conical–cylindrical
P. margaretae	1280–1480	1210	presence	4	10	10–11	4.75	47–48	44–45	numerous	6	conical
P. medius	913–1254	1232	none	n/a	n/a	n/a	n/a	48	44.4	numerous	4	conical
P. micoletzkyi	1020	1220	presence	n/a	12	12	4.5	41	25	none	4	conical

Table 2. *Cont.*

Species	Body Length (Male)	Body Length (Female)	Lateral Differentiation of Cuticle	Number of Subventral Teeth	Distance from Anterior to Amphid	Amphid Width	Turn Number of Amphid	Spicule Length as Arc	Gubernaculum Length	Number of Cusps on Distal End of Gubernaculum	Number of Supplements	Tail Shape
P. micropapillatus	960–1350	950–1400	none/except on the tail	n/a	5	8.5–10.5	3.25–2.5	34	25	2	7	conical
P. miltommatus	1827–2051	1885–2062	none/except on the tail	4	7–12	10–11	4.5–5	53–57	48–55	5–8	6	conical
P. multisupplementatus	1027–1308	1181–1365	presence	2(4?)	3.5–6.5	8–13	4–4.5	56–61	36–43	numerous	57–62	conical
P. multitubifer	1100–1200	1100–1240	none	n/a	8	7.5	3.5	36	28	3	21–22	conical–cylindrical
P. mutatus	930	1100	none/except anterior	1 or 2	10	9–10	5	30	24	none	5	conical
P. nannodontus	1600	1430	n/a	n/a	17	9	3.5–4	60	57	numerous	3?	conical
P. olgae	1300–1830	1445–2065	none	4	6–9.9	6–11	5	44.5–64	42–69	numerous	5 (rarely 2–4)	conical
P. parahartogi	1350–1440	1410–1560	presence	n/a	8	8–12	3.75	49–52	50–53	6	6	conical
P. perspicuus	1269–1287		presence	none	15	11	4.25	31	22–24	numerous	5	conical
P. platti	1500–1920	1820	none	2	12–14	12–13	5.5–6	40–45	35	9–11	5	conical
P. platypus	1180–1320		none	n/a	13–15	9–11	3.5	36	35	none	4	conical
P. quinquepapillatus	1360		presence	2?	13	13	4.5	38	28	none	5	conical
P. ruens	1610	1450–1530	none/except on the tail	n/a	8	9	3	75	72	none	7	conical
P. sabulicolus	1700–1800	1500–1700	none	2	9	12	5	40–45	37	12	4	conical
P. sandspitensis	1200–1400	1000–1600	none	4	10	8–10	5–6	32–41	25–30	numerous	5	conical
P. securus	931–1112	944–1205	none	2	15	11–13	4.3–4.5	39–43	24–26	7–10	3	conical–cylindrical
P. sonadiae	1150–1270	1110–1820	n/a	n/a	5	9	5.5	32	25	n/a	6	conical
P. stateni	920–1720		presence	n/a	5	9	5.5	33	22	2	5	conical
P. stekhoveni	1132		presence	n/a	12	10	4.5	45 (as chord)	36	numerous	5	conical
P. steueri	940	1070	presence	2–4?	10	7	3.5	32	25	n/a	6	conical
P. sunesoni	800–1390		presence	n/a	16	7	2.5–3	32	26	numerous	5–7	conical
P. thaumasius	1511–1902	1418–2001	none	4	14	10–15	5–6.5	43–65	36–49	9	5	conical–cylindrical
P. tumepapillatus	1200-1440		none	2	12	11	5	22	16	n/a	3	conical–cylindrical
P. tyrrhenicus	1700	1700–1800	n/a	4?	15	12–17	6	42	42	2	5	conical
P. uniformis	1272	1200	n/a	none?	11	7–12	4.5–5.5	21	18	n/a	4	conical
P. wellsi	1485–1512	1465–1595	presence	4	8–13	10	5	36–37	28–31	numerous	3	conical–cylindrical
P. yeongjongensis	1186–1295	1378–1401	presence	2	5	5.5–8	2.5	51–58	49–56	None (little denticle)	76	conical–cylindrical

3.5. Taxonomic Description

Paracanthonchus yeongjongensis sp. nov.
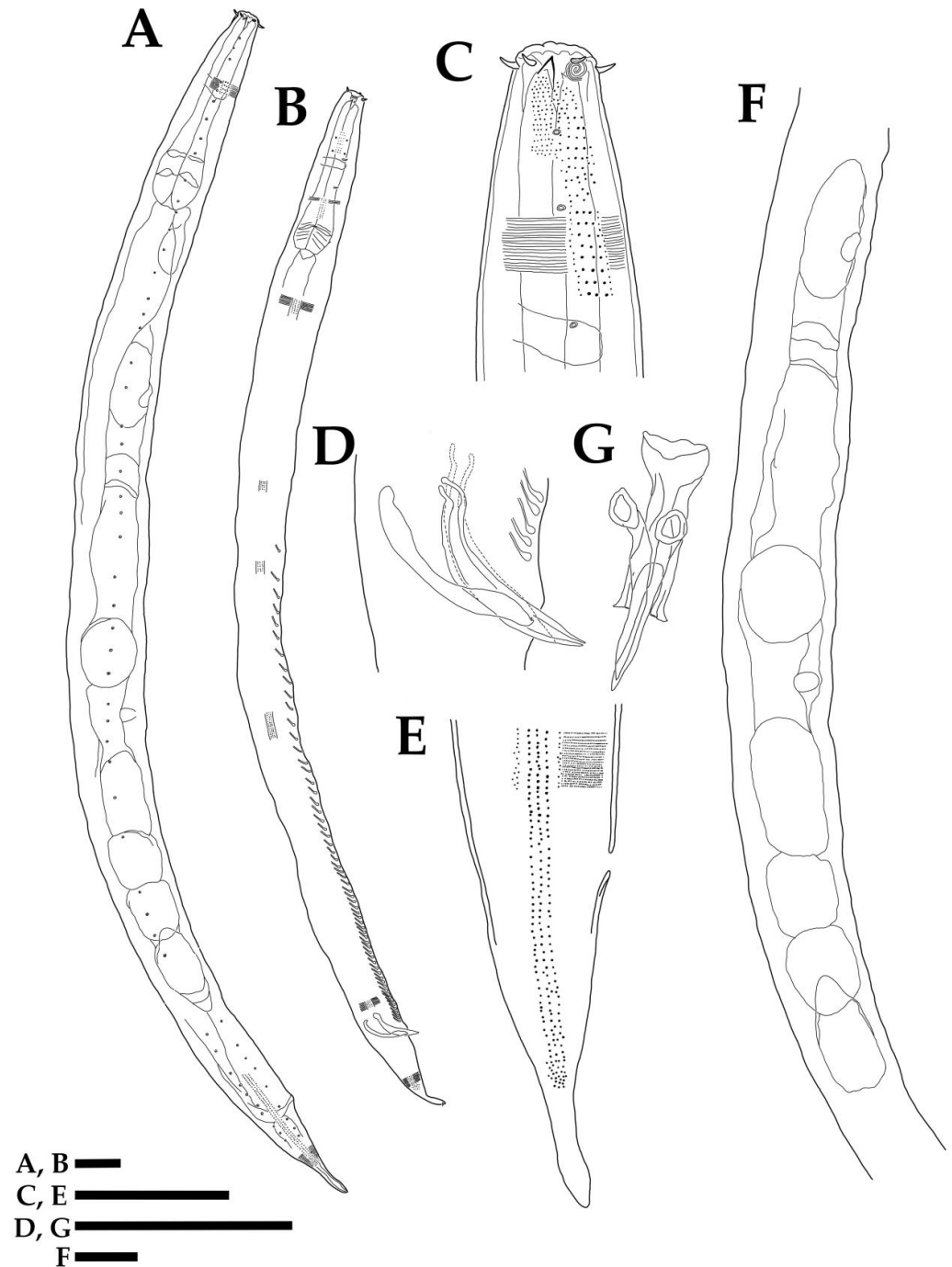(Figures 2 and 3, Table 3).

Figure 2. *Paracanthonchus yeongjongensis* sp. nov.: (**A**), female habitus (paratype 1); (**B**), male habitus (holotype); (**C**), female anterior part (paratype 2); (**D**), male cloacal region (holotype); (**E**), female tail region (paratype 2); (**F**), female reproductive system (paratype 1); (**G**), male spicule and gubernaculum, upper-side view. Scale bars: (**A–F**) = 50 μm.
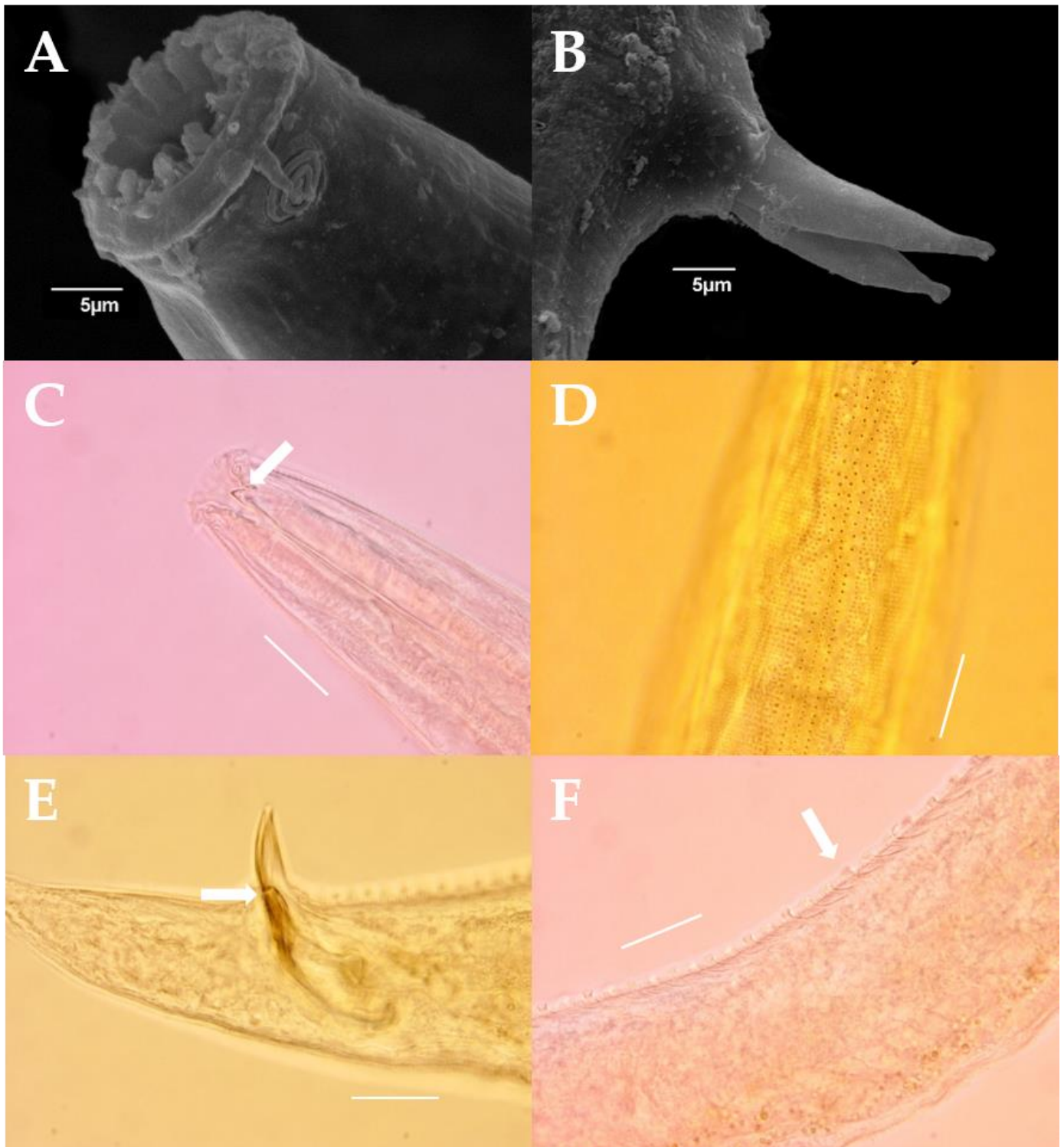
Figure 3. *Paracanthonchus yeongjongensis* sp. nov.: SEM micrographs—(**A**) anterior part with amphid, (**B**) cloacal part with distal part of spicule; light micrographs—(**C**) head with dorsal tooth, (**D**) lateral differentiation of punctations at mid, (**E**) spicule and gubernaculum (arrow, cuticularized with distal part of gubernaculum), (**F**) tubular supplements. Scale bars: (**A**,**B**) = 5 μm; (**C**–**F**) = 25 μm.

Table 3. Measurements of *Paracanthonchus yeongjongensis* sp. nov. (all measurements in μm, "-" indicates unavailable information, "n/a" indicates not applicable).

Characters	Holotype	Paratype (m1)	Paratype (m2)	Paratype (f1)	Paratype (f2)
L	1186	1253	1295	1401	1378
hd	20	19	20	25	18.5
LSL	4.5	5	5.5	7	5
CSL	4.5	5	5.5	7	5
bcl	22	17	14	18	21
amp	6	–	5.5	8	7
na	2.5	–	2.5	2.5	2.5
amp cbd	25	–	21	27	21.3
NL	95	80	76	91	104
ncbd	45	40	41	53	52
PL	183	194	198	232	212
pcbd	57	47	52.5	70	60
mbd	62	54	65	84	64.5
VL	n/a	n/a	n/a	800	776
ov	n/a	n/a	n/a	768/987	662/884
abd	41	40	41	50	44.5
spia	58	51	54	n/a	n/a
gub	56	49	52	n/a	n/a
ns	76	76	76	–	–
dps	11	16	13	–	–
das	558	611	599	–	–
TL	98	106	103	121	117
a	19.13	23.20	19.92	16.68	21.36
b	6.48	6.46	6.54	6.04	6.50
c	12.10	11.82	12.57	11.58	11.78
c′	2.39	2.65	2.51	2.42	2.63
V	–	–	–	0.57	0.56
amp′	0.24	–	0.26	0.30	0.33
s′	1.41	1.28	1.32	–	–

Zoobank registration:

urn:lsid:zoobank.org:act:A2502981-147C-4E5F-8276-92A8C88D7076

Type locality: Tidal zone on coast of Seonnyeobawi beach (37°26′13″ N, 126°23′03″ E), Inchoen, Republic of Korea, in fine muddy sediments.

Materials examined: Holotype 1♂(NIBRIV0000903934) on one slide from Seonnyeobawi beach (37°26′13″ N, 126°23′03″ E) on 26 August 2022. Paratypes 2♂♂and 2♀♀(NIBRIV0000903935–NIBRIV0000903938) on each slide, all from Seonnyeobawi beach (37°26′13″ N, 126°23′03″ E) on 26 August 2022.

Etymology: The species name *yeongjongensis* is given as the species was discovered on Yeongjongdo Island, Republic of Korea.

Description:

Male: Body roughly cylindrical, gradually growing from the anterior end to the nerve ring and then tapering from the anus to the tail (Figure 2A,B). Cuticle obviously ornamented with transverse rows of punctations at the whole body. Punctations aligned and laterally not differentiated until the posterior end of amphid. The lateral differentiation begins from under the amphid, with slightly larger punctations irregularly organized, three to four rows of punctations begin at nerve ring until tail region at the start point of cylindrical part (Figure 3D). Eight longitudinal rows of hypodermal pores present from anterior head end to the amphid body diameter depth until half of the tail (Figure 2C). Six inner labial setae short (only observed with SEM). Six outer labial setae and four cephalic setae present on the head and the same length, 4.5 μm long (head sensilla in the 6 + 10 pattern). Amphid located just below labial setae, multispiral with 2.5 turns, 6 μm wide (Figures 2C and 3A). Buccal cavity conical shape with twelve distinct rugae (Figure 3A), armed with one large conspicuous dorsal tooth and two small subventral

teeth (Figure 3C). Nerve ring situated slightly above the middle of the pharynx, 95 μm from anterior edge. Pharynx cylindrical, gradually expanding at two-thirds posterior region, 183 μm long. Excretory pore invisible. Ocelli absent. Reproduction system diorchic, opposed and outstretched. Spicule paired, curved, 58 μm long, slightly wider in the middle, shaped like a banana (Figure 3B,E). Gubernaculum paired, one on each side of the middle piece, 56 μm long (Figure 2D,G). The distal end of gubernaculum slightly extended from the upper-view, with a hook-like structure on the lateral distal part and some denticles on the inner half (Figure 2G). Seventy-six tubular precloacal supplemental organs, curved (Figures 2B and 3F), with distal end strongly cuticularized and tubular part weakly cuticularized. Distance between each supplement gradually increases toward the anterior part. The most anterior and the most posterior supplement at distances 11 μm and 558 μm from the anus. Tail, 98 μm long, conico-cylindrical with a little swollen tip, the cylindrical part very short (Figure 2E).

Female: Similar to males in basic forms such as cuticle patterns, part of buccal cavity, and body shape; however, body length longer than males. Body length 1378–1401 μm. Didelphic, two opposed, reflexed ovaries (Figure 2A,F). Vulva located at 56–57% of body length, 776–800 μm from anterior end.

3.6. Molecular Analysis

The seven specimens of the new species used for molecular analysis were near identical in terms of mtCOI, 18S and 28S rRNA sequences. The K2P distance between the supposed intraspecies showed no difference from 18S and 28S regions (Tables S2 and S3), with mtCOI showing a difference of 0.39% (Table S4). All available sequences of congeneric species, as well as closely related genera such as *Praecanthonchus*, *Acanthonchus* and *Cyatholaimus*, were retrieved and compared against. In terms of mtCOI congeneric K2P distance, the new species differed from other congeneric species such as *P. macrodon*, *P. caecus* and *P. gynodiporata* by a range of 29.9–40% (Table S4). For 18S rRNA, the new species were compared to congeners such as *P. macrodon*, *P. caecus* and *P. gynodiporata*, as well as specimens only identified at genus level, and their distance differed by a range of 3.9–8.1% (Table S2). For 28S rRNA, new species were compared to congeners such as *P. caecus*, *P. gynodiporata* and *P. miltommatus*, which differed by a range of 17.7–36.8%, respectively (Table S3). When compared to other genera belonging to the family Cyatholaimidae, mtCOI, 18S and 28S differed by ranges of 32.8–42%, 4.9–14.8% and 14.6–22.4%, respectively.

BI trees (Figures 4 and 5) and ML trees (Figures S1 and S2) based on the 18S and 28S rRNA region, respectively, both produced trees with identical topology, albeit with different support values. All major nodes leading up to the family Cyatholaimidae had high posterior probability or UFboot support. As BI trees are identical in topology to ML trees with better support, BI trees of 18S and 28S rRNA sequences were used for discussion, with ML trees of 18S and 28S rRNA sequences being included as supplementary figures for reference. The sequence availability was significantly higher for 18S rRNA sequences compared to 28S rRNA sequences. The 18S rRNA phylogenetic tree included various genera belonging to the family Cyatholaimidae, such as other congeneric *Paracanthonchus* species, *Longicyatholaimus*, *Marylynnia*, *Cyatholaimus* and *Praeacanthonchus*, whereas the 28S rRNA tree only had other congeneric *Paracanthoncus* species and a single *Metacyatholaimus* sequence. All species belonging to the family Cyatholaimidae formed a monophyletic clade in both 18S and 28S trees with high support (pp = 100 and 98%, respectively) (Figures 4 and 5). All seven sequences of the new species were included within this clade, and were seen grouped together with pp = 100% in both trees.
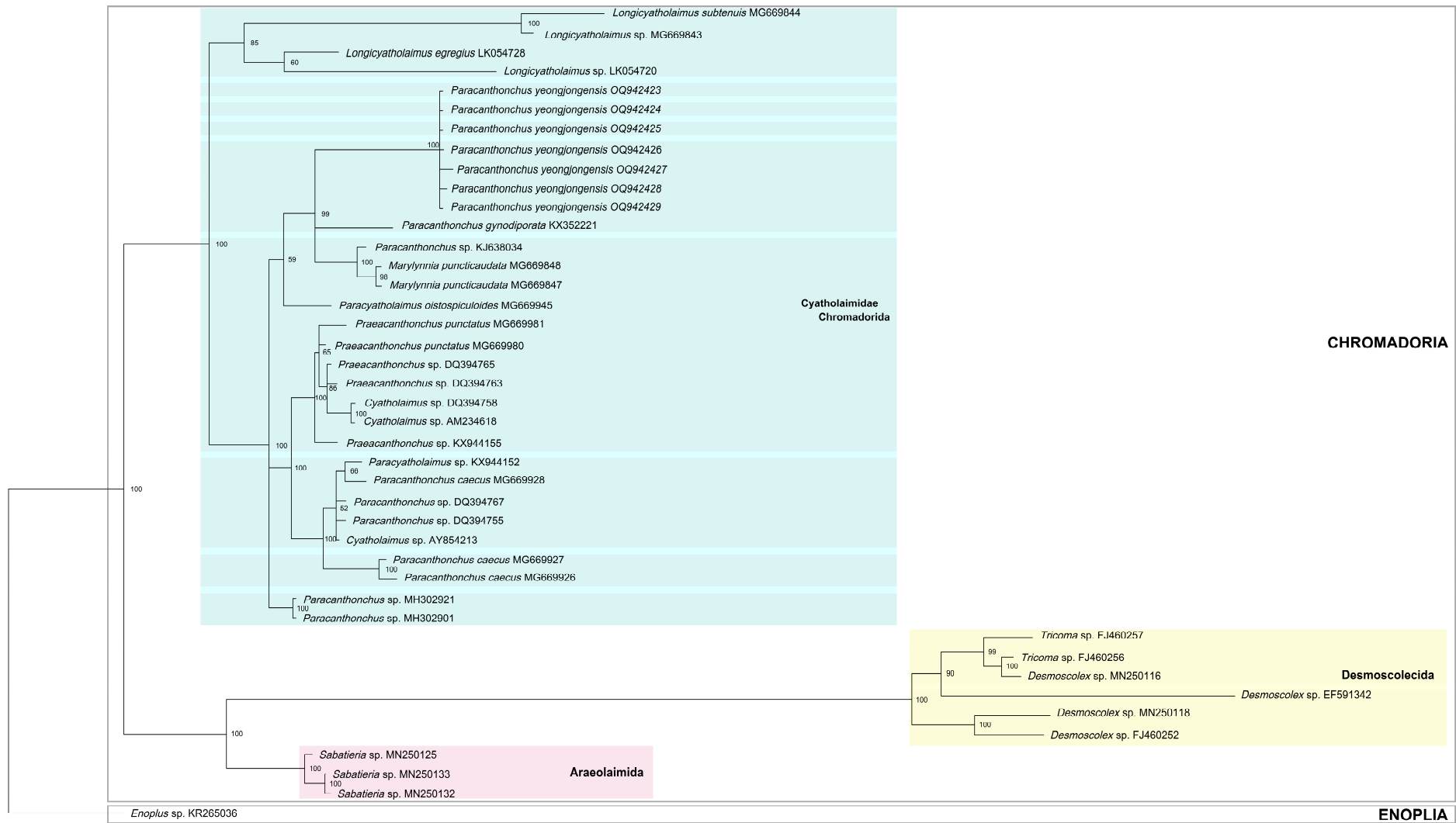
Figure 4. Rooted Bayesian Inference phylogenetic tree of 18S rRNA gene with *Enoplus* sp. set as an outgroup (with posterior probability support shown at each node).
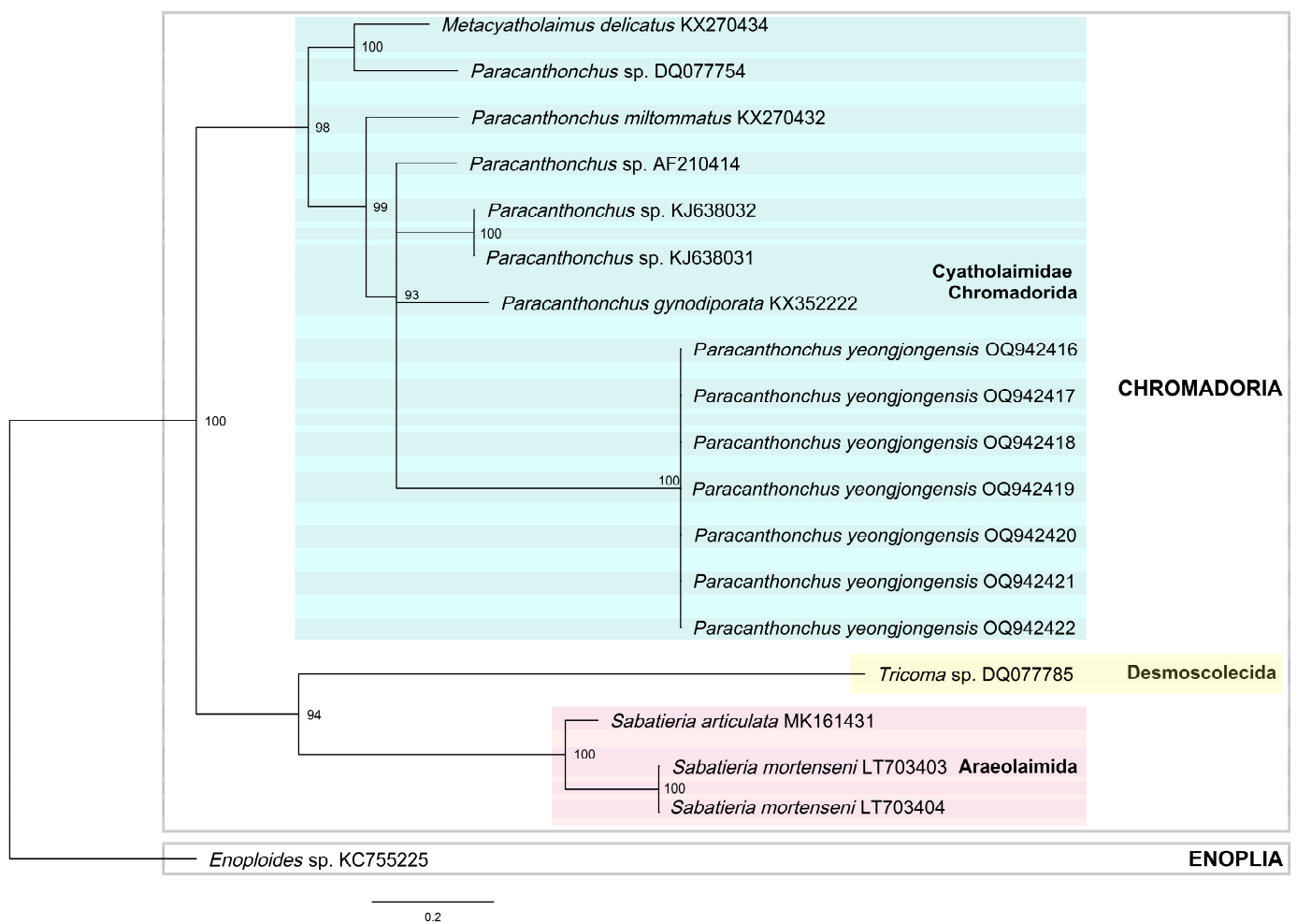
Figure 5. Rooted Bayesian Inference phylogenetic tree of 28S rRNA gene with *Enoploides* sp. set as an outgroup (with posterior probability as percentage shown at each node).

4. Discussion

4.1. List of Valid Species

Paracanthonchus is the most diverse and the largest genus within the family Cyatholaimidae [2]. The species identification of taxa belonging to the family Cyatholaimidae has been difficult, mainly due to their lack of apomorphic characters without clear interspecific/intergeneric boundaries. Many papers have discussed the morphological ambiguity of the genus *Paracanthonchus* [5,10], which stems from its type species, *P. caecus*. *Paracanthonchus caecus* has been mentioned in over a hundred papers, being reported in almost every marine habitat. Realizing this, Vincx et al., 1982, examined these highly variable species and determined that there are at least five different types of *P. caecus* and recognized four separate species from these types: *P. heterodontus*, *P. gerlachi*, *P. thaumasius* and *P. canadensis*. In addition to cases of species complex, many species have been transferred in and out of the genus [10–14]. To account for these frequent changes, many updated revisions of the genus exist. The most recent update to the genus was by Miljutina and Miljutin 2015, but the number of valid species has been inconsistent in most papers which followed [8,17,18]. Whether due to discrepancy or a lack of updated revisions, there is a clear need to re-evaluate the currently existing species to avoid any further convolution of an already complex genus. Accordingly, the species list was updated by referring to Miljutina and Miljutin 2015, NeMys, and original descriptions, as well as redescriptions. For the sake of clarity, any species already mentioned as being invalid in previous reviews were removed without remarks. Species which are ambiguous or require status updates are

discussed below. After careful re-evaluation, from Miljutina and Miljutin, 2015,'s 52 valid species (excluding species inquirenda), we list a total of 60 species to be valid, including the new species.

Since Miljutina and Miljutin, 2015, seven species (including the new species) have been added to the genus: *P. brevicaudatus*, *P. gynodiporata*, *P. miltommatus*, *P. olgae*, *P. secures*, *P. wellsi* and *P. yeongjongensis*. Aside from newly described species, there are several species which must be discussed in terms of validity. Firstly, *P. medius* and *P. sandspitensis* were missing from Miljutina and Miljutin, 2015,'s revision, for no particular reason. The original descriptions have been checked, but with no apparent conflicts, we are considering the two species as valid. *P. inglisi* was described by Coles, 1965; however, it was considered synonymous to *Praeacanthonchus* by Platt and Warrick, 1988, as it bears unpaired gubernaculum. Despite rightfully being transferred to *Praeacanthonchus*, the species was still considered valid in the recent revision by Miljutina and Miljutin, 2015. *P. steueri paracaecus* (Micoletzky, 1922) was classified as a subspecies of *P. steueri* by the dark and pale differences in the color of the ocelli. While it may have been fine to erect subspecies based on minor morphological variation in 1922, it certainly is not acceptable now to erect subspecies without molecular evidence. While subspecies may differ in minor morphological variation, it should be noted that the specimens have been fixed in ethanol for over fourteen years. Color variation can be very subjective, and ethanol samples are highly prone to discoloration. Until molecular evidence becomes available, it is our decision to consider this species synonymous with its parent species, *P. steueri*. For *P. tumepapillatus* Timm, 1957, which was synonymized as *Paracyatholaimus* in 1966 by Murphy based on the structure of precloacal supplements (by comparison of the depiction), we agree with Miljutina and Miljutin, 2015, in reinstating the species as a valid species of *Paracanthonchus*. We agree with previous claims that the shape of the supplements is a characteristic more suitable for *Paracanthonchus*.

The list of valid species up until this point mainly relies on morphological accounts, with the exception of some species (*P. caecus*, *P. gynodiporata*, *P. macrodon*, *P. mamubiae* and *P. miltommatus*) which have substantiating molecular data. With much uncertainty and ambiguity in discerning species of this group morphologically, corroborating molecular data on existing and new species will help to organize and understand the species delineation of Cyatholaimidae.

4.2. Differential Morphological Diagnoses

The new species, *Paracanthonchus yeongjongensis*, is identified by genus characteristics: it has a buccal cavity armed with one large dorsal tooth and subventral teeth, precloacal tubular supplements and the gubernaculum paired distally expanded. *Paracanthonchus yeongjongensis* differs from other species by considering the number of supplements, the arrangement form and median pieces of the gubernaculum. Tubular supplement is one of the major characteristics of the genus; with the exception of two species (*P. multisupplementatus* and *P. multitubifer*), the rest of the congeners bear fewer than ten supplements. Evidently, *P. yeongjongensis* has a lot of supplements (76). It is evident from the tabular key (Table 3) that there are seven congeneric species similar to the new species in lacking a cusp on the distal end of the gubernaculum (*P. longispiculum*, *P. major*, *P. micoletzkyi*, *P. mutantus*, *P. platypus*, *P. quinquepapillatus* and *P. ruens*), and two species with a similar number of supplements (*P. multisupplementatus* and *P. multitubifer*). The new species is most closely related to *P. multisupplementatus* in terms of the number of supplements (76 vs. 57–62), lateral differentiation, the size of amphid and body length. However, it differs in the shape of the gubernaculum and the presence of cusp (none vs. numerous), the length interval between supplements (gradually increasing vs. equal) and its tail shape. It is also closely related to *P. longispiculum* in size and turns of amphid, and the shape of the distal part of the gubernaculum, but differs in body length (1186–1295 vs. 1780–1800) and number of supplements (76 vs. 8). Only *P. elongatus* has a median piece at the gubernaculum like the new species; however, it differs in most other morphological traits.

4.3. Phylogeny and Topology

While all available congeneric sequences were retrieved and used for the K2P analysis, some sequences of 18S (such as *P. wellsi*) had to be omitted from the analysis due to them also being short partial sequences targeting different region of 18S, with no overlap. Only sequences that correctly aligned with the seven sequences were used for further analysis. All three markers showed very low intraspecific K2P distances (0–0.3%), indicating the seven specimens analyzed here belong to the same species (Tables S2–S4). The usual interspecific difference for the mtCOI gene for nematodes is 5%, which is often used as a threshold to detect cryptic species [31]. Our results showed that mtCOI K2P distances between other congeners ranged from 29.9 to 40%, and from 32.8 to 42% between species of other genera of the family Cyatholaimidae (Table S4). Similarly, little difference in range was observed between the congeneric distance and intergeneric distance (different genera belonging to the family Cyatholaimidae) among 18S and 28S rRNA sequences (3.9–8.1% vs. 4.9–14.8%; 17.7–36.8% vs. 14.6–22.4%, respectively) (Tables S2 and S3). This is indicative of the fact that while the family Cyatholaimidae may be a monophyletic clade, its constituting genera are clearly not. When considering the availability of molecular data, it seems that 18S rRNA sequences are most readily available compared to mtCOI and 28S rRNA. However, there is no definite guideline as to which marker is most suitable for each group. Additionally, there have been instances, such as *P. gynodiporata*, where species show high morphological variation and dispersion, all whilst showing low genetic differentiation [55]. This goes to show that there is no one single data type that completely reflects inter/intra species delineation. It is thus imperative to make it common practice to obtain as much datatype (to supplement morphological data with molecular data of variety of markers) as possible to be able to resolve a complex genus such as *Paracanthonchus*.

While there are not large amounts of molecular data available for species belonging to *Paracanthonchus*, there were enough to build phylogenetic trees to gain some insight on their supposed position within the phylum Nematoda. Much like previous documentations [1–3], the family Cyatholaimidae did form a monophyletic group within the class Chromadorea in the BI tree based on 18S and 28S rRNA sequences with a high posterior probability of 100% (Figures 4 and 5). However, its constituting genera formed mixed and low-support paraphyletic clades. This clearly questions the integrity of constituting genera within Cyatholaimidae and raises the need to re-evaluate highly ambiguous genera such as *Paracanthonchus* and *Praeacanthonchus*. In the latest classification of the phylum Nematoda by Hodda, 2022, the family Cyatholaimidae consisted of just two subfamilies: Cyatholaiminae and Paracanthonchinae. This was supported (although with low support) by a tree constructed by Leduc and Zhao, 2018, where the two subfamilies were seen forming two sister monophyletic clades. They did, however, mention the genus *Praecanthonchus* as being the sole exception. Their phylogenetic tree showed *Praecanthonchus* being included in the opposite subfamily, and based on this finding suggested that *Praecanthonchus* be placed in the subfamily Paracanthonchinae. Several years have passed since their publication, but species lists on NeMys as well as the classification of Hodda, 2022, still list *Praecanthonchus* as a constituent of the subfamily Cyatholaiminae. While Hodda, 2022, does acknowledge the suggestion of Leduc and Zhao, 2018, he argues that it is with a low level of support and with sparse taxon sampling. In the SSU phylogenetic tree built by Leduc and Zhao, 2018, it is evident that *Cyatholaimus* is also seen as an exception, like *Praecanthonchus*; however, they argued that *Cyatholaimus* itself is a very vague group which has been transferred in and out of different groups, such as *Acanthonchus*, *Metacyatholaimus*, *Paracanthonchus* and *Longicyatholaimus*. Based on this reason, they claimed that little credibility can be asserted to *Cyatholaimus* sequences. While this is true, to confirm the monophyletic nature of the two subfamilies we included more sequences of species belonging to the subfamily Cyatholaiminae, such as *Cyatholaimus*, *Marylynnia* and *Longicyatholaimus*, to our phylogenetic tree. However, many of the sequences retrieved from NCBI used in the phylogenetic tree were only identified to genus level, and erroneous species identification cannot be ruled out. Our BI 18S tree (Figure 4) showed that while *Longicyatholaimus* does form a

monophyletic sister group (as the subfamily Cyatholaiminae) with the subfamily Paracanthonchinae, it is with pp of 85% and there is more than one exception: *Cyatholaimus*, *Marylynnia* and *Praecanthonchus*, all of which are known to belong to Cyatholaiminae, are included within the Paracanthonchinae clade. We could suggest, based on our molecular results, that the three genera be placed in Paracanthonchinae, but as with the case of *Cyatholaimus*, the validity of the sequences can be argued. We instead acknowledge that the existing subfamilies of Cyatholaimidae cannot be represented well with a molecular phylogenetic tree, at least currently, with limited molecular data. Most species of this group still lack associated molecular sequences, and even those with available sequences are mostly partial without additional marker regions. As more molecular data accumulate over time, a more complete phylogeny may unearth insights that can be used to resolve these ambiguous genera/families. Granted, morphology and molecular phylogenies may not always match perfectly [77,78], but it is clear that no one datatype can resolve the issue alone. Both morphological and molecular results must be used to substantiate one another. The morphological distinction of the two subfamilies by the type of gubernaculum (unpaired/paired), may not be represented well phylogenetically, but it is still, nonetheless, currently the most distinctive group-defining character. It is uncertain whether complex topology of the group can be resolved by morphological or molecular means, as both are full of ambiguity and lack data. In all, a lack of apomorphic morphological traits and molecular data makes it extremely difficult to resolve this group, currently, but as more datatypes begin to accumulate, hopefully the topology of this group may become clearer in the future.

Abbreviations

a—body length divided by maximum body diameter; abd—anal body diameter, (μm); amp—transversal diameter of amphid, (μm); amp'—diameter of amphid divided by corresponding body diameter; amp cbd—corresponding body diameter at the level of amphid, (μm); b—body length divided by esophagus length; bcl—distance from anterior edge to base of buccal cavity; c—body length divided by tail length; c'—tail length divided by anal body diameter; cyln—length of cylindrical tail portion (μm); CSL—cephalic sensilla length, (μm); das—distance from anus to most anterior supplement; dps—distance from anus to most posterior supplement; EL—distance from anterior edge to excretory pore, (μm); hd—head diameter, (μm); L—total body length, (μm); LSL—outer labial sensilla length, (μm); mbd—maximum body diameter, (μm); NL—distance from anterior edge to nerve ring, (μm); na—number of turns in amphid; ns—number of supplements; ncbd—corresponding body diameter at the level of nerve ring, (μm); PL—pharynx length, (μm); pcbd—corresponding body diameter at base of pharynx, (μm); s'—spicule length as arc length divided by anal body diameter; spic—spicule length as arc, (μm); gub—gubernaculum length as arc, (μm); TL—tail length, (μm); V—vulva distance from anterior end divided by total body length; VL—distance from anterior end to vulva, (μm).

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