



# Article **Two New Species of Free-Living Marine Nematode of the Genus** Anticyathus Cobb, 1920 (Linhomoeidae) from Mangroves Sediment of Shenzhen and Shantou, China<sup>+</sup>

Yuzhen Chen<sup>1</sup>, Rengui Zhou<sup>1</sup>, Huilan Zhu<sup>1</sup> and Yuqing Guo<sup>1,2,\*</sup>

- <sup>1</sup> Fisheries College, Jimei University, Xiamen 361021, China
- <sup>2</sup> Fujian Provincal Key Laboratory of Marine Fishery Resources and Eco-Environment, Jimei University, Xiamen 361021, China
- \* Correspondence: guoyuqing@jmu.edu.cn
- + urn:lsid:zoobank.org:act:166AFE51-8361-41E2-8927-F70EB5175075 and urn:lsid:zoobank.org:act:6899EB5B-E486-49CE-A4DF-EE5696D6A4FE.

Abstract: Two new species belonging to the genus Anticyathus were discovered during an ecological investigation of mangrove sediment in China. Anticyathus shenzhensis sp. nov. and Anticyathus communis sp. nov. are both characterized by: annulated cuticles; ten cephalic setae in a circle; buccal cavity lined with six longitudinal reinforcements; amphids in broken circles or cryptic spirals, located at the very anterior of the head; pharynx with anterior cuticularized, gradually enlarging in posterior portion but without bulb; conical tail; arcuate spicules, the distal ends pointed, gradually broadening towards the proximal; gubernaculum has a dorsal-caudal apophysis; female with two opposed, outstretched ovaries. Anticyathus shenzhensis sp. nov. can be distinguished by ten cephalic setae of length about 2–4 µm; amphids diameter about 20–29% of corresponding body diameter; pharynx occupy 0.07–0.10 times of body length; cardia length about 25 µm; tail length 2.5-3.4 times of anal body diameter (abd) in males and 3.4-4.7 abd in females; spicules length  $51-59 \mu m$  as arc and 0.71-1.04 abd; apophysis length 18–19 μm; female vulva at 52–56% of body length. Anticyathus communis sp. nov. is identified by ten cephalic setae length about 3-5 µm; amphids diameter about 20-32% of corresponding body diameter; cardia 17-27 µm long; tail length 3.6-4.5 abd in male, 5.3-5.5 abd in female; spicules proximal with distinctly cephalated, 0.74-0.96 abd; gubernaculum shape of "L", consisting of sleeves around spicule and dorsal-caudal apophysis; five to seven pre-anal and seven to ten post-anal almost invisible papilla or papillae-like setae occur on each ventrally submedian line on the posterior body part of male; the tip of male tail with three slight elevation; female vulva at 52% of body length. A dichotomous key for the genus Anticyathus is given and 39 sequences of 18S rRNA gene from ten individuals of Anticyathus shenzhensis sp. nov. are provided in Genbank database.

**Keywords:** taxonomy; marine nematode; *Anticyathus shenzhensis* sp. nov.; *Anticyathus communis* sp. nov.; 18S r DNA; mangrove; Guangdong Province

# 1. Introduction

Free-living marine nematodes comprise the most diverse taxa and the largest amount of biomass and production within the meiofauna [1–3]. It is estimated that there are about 50,000 or more free-living marine nematode species [4]. However, most of the marine nematode species are yet unidentified or undiscovered [3].

Free-living nematodes of the family Linhomoeidae Filipjev, 1922 are common in seas and frequently settle in silty substrates in huge numbers [5]. Filipjev [6] and Chitwood [7] referred several genera to Linhomoeidae. However, the features of this family are not well characterized. Wieser [8] gave a key to the genera of what can be considered the family Linhomoeidae sensu latissimo, following Filipjev and Chitwood, but leaving out some of



Citation: Chen, Y.; Zhou, R.; Zhu, H.; Guo, Y. Two New Species of Free-Living Marine Nematode of the Genus *Anticyathus* Cobb, 1920 (Linhomoeidae) from Mangroves Sediment of Shenzhen and Shantou, China. *J. Mar. Sci. Eng.* **2022**, *10*, 1107. https://doi.org/10.3390/ jmse10081107

Academic Editor: Azizur Rahman

Received: 26 June 2022 Accepted: 3 August 2022 Published: 12 August 2022

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**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). the most doubtful genera. The main grouping is based on the number of cephalic setae. Gerlach [9] revised the Linhomoeidae based on Wieser's key.

The genus *Anticyathus* was established by Cobb in 1920 with the species *Anticyathus tenuicaudatus* [10]. It is characterized by: cuticle finely striated; buccal cavity simple and not very cuticularized; amphid circular in outline, but clearly showing the interruption at the rear edge and thus representing spirals with a turn; two opposed outstretched ovaries; tail conical [9,11].

So far, only six species have been described in the genus *Anticyathus* (Electronic database: http://www.marinespecies.org/index.php; http://nemys.ugent.be [accessed on 15 June 2022]). However, no gene sequences of genus *Anticyathus* can be found in the Genbank database up to the current date. It seems that molecular data of the genus *Anticyathus* is still lacking (Electronic database: https://www.ncbi.nlm.nih.gov/ [accessed on 15 June 2022]). Two new species of the genus of *Anticyathus* were found during a preliminary survey of the meiofauna in mangrove mudflats of China and are described in the present study. In addition the 18S rRNA gene of *Anticyathus shenzhensis* sp. nov. were sequenced.

## 2. Materials and Methods

#### 2.1. Sample Collection, Meiofauna Exraction, and Nematode Identification

Meiofaunal samples were collected from Futian mangrove mudflats in Shenzhen (22.52° N, 114.01° E) and Shantou (23.46° N, 116.87° E), China. Part of the samples were fixed with 5% formalin in filtered seawater and then stained with 0.1% rose Bengal for more than 24 h for species identification. Some samples were fixed with DESS for DNA extraction. Sediment sampling and nematode extraction were performed as described in our previous studies [12,13].

#### 2.2. DNA Extraction, PCR Amplification, and Sequencing

Ten individual nematodes were prepared for total genomic DNA extraction using the Tiangen Dp324-03 DNA Isolation Kit. The 18S rDNA was selected for genetic analysis. The primers used in this study are as follows: (1) MN18F (5'-CGC GAA TRG CTC ATT ACA ACA GC-3') and Nem\_18S\_R (5'-GGG CGG TAT CTG ATC GCC-3') [14]; (2) MN18F (5'-CGC GAA TRG CTC ATT ACA ACAGC-3') and 22R (5'-GCC TGC TGC CTT CCT TGG A-3') [15]; (3) 1813F (5'-CTG CGT GAG AGG TGA AAT-3') and 2646R (5'-GCT ACC TTG TTA CGA CTT TT-3') [16]; (4) 988F (5'-CTC AAA GAT TAA GCC ATG C-3') and 1912R (5'-TTT ACG GTC AGA ACT AGG G-3') [16]. Each reaction was performed in a 25  $\mu$ L solution, including 4  $\mu$ L DNA template, 12.5  $\mu$ L 2X Pro Taq Master Mix, 0.5  $\mu$ L for each forward and reverse primer, and 7.5  $\mu$ L distillation H<sub>2</sub>O. PCR amplifications were performed as described in corresponding reference of each pair of primers. All positive PCR amplicons were sequenced by Sangon Biotech, Ltd. (Shanghai, China).

#### 2.3. Terminology and Abbreviations

Measurements are in micrometers. Abbreviations are as follows: a = body length/maximum body diameter; b = body length/pharynx length; c = body length/tail length; abd = anal body diameter; cbd = corresponding body diameter; vbd = vulval body diameter; c' = tail length/abd; V = Vulva from the anterior end; V% = position of vulva as % of body length from anterior end, that is, V/body length. All holotypes and paratypes are deposited in Jimei University, Xiamen, China.

#### 3. Results

#### 3.1. Description of Anticyathus Shenzhensis sp. nov.

#### 3.1.1. Type Material

Four males and three females were collected from Station SZFT in July 2020. Holotype: ° 1 on slide number SZFT202007 2L104. Paratypes: ° 2 on slide number SZFT202007 2M115, ° 3 on slide number SZFT202007 2L104, ° 4 on slide number SZFT202007 3M116, ° 1 on slide number SZFT202007 2L108, ° 2 on slide number SZFT202007 3L115, ° 3 on slide number SZFT202007 2M106.

#### 3.1.2. Etymology

The species is named after the seashore city, Shenzhen City, Guangdong Province, China, from where it was collected.

#### 3.1.3. Type Locality and Habitat

All specimens were collected from a mangrove forest in the mangrove reservation of Shenzhen (22.52° N, 114.01° E), China. Characteristics of surface sediments of sampling stations are shown in Table 1.

Station	Temperature (°C)	pН	Salinity (‰)	Total Nitrogen (mg·g <sup>-1</sup> )	Total Organic Carbon (%)
SZFT 2L	27.0	6.1	2.5	2.14	13.93
SZFT 3L	28.5	6.3	3.1	1.50	14.90
SZFT 2M	26.5	6.2	4.8	4.34	11.69
SZFT 3M	27.5	6.3	3.3	1.75	16.66

Table 1. Characteristics of surface sediments of sampling stations.

## 3.1.4. Morphological Description

Body cylindrical, maximum body diameter 72–99  $\mu$ m. Cuticle annulated, the annule begins a little posterior to the level of the base of the buccal cavity, about 13  $\mu$ m from head end. Cuticle annule about 2.3  $\mu$ m broad at anterior body part and 1.5  $\mu$ m at midbody part. Ten cephalic setae in a circle, length about 2–4  $\mu$ m. The setae appear to be at one level in submedian pairs. Head truncate, 17–18  $\mu$ m in diameter. Buccal cavity length 5–10  $\mu$ m and width 5–8  $\mu$ m, lined with 6 thick, noncuticularized, longitudinal reinforcements. Amphids broken circles or cryptic spiral, length 6–7  $\mu$ m, width 5–7  $\mu$ m or 20–29% of corresponding body diameter. Amphids located at very anterior of head, anterior margin 2–3  $\mu$ m from anterior end. Pharynx cylindrical, with anterior cuticularized, 0.07–0.10 times body length, gradually enlarging in posterior portion but without bulb. Nerve ring located at 51–59% of pharynx length. The excretory pore is anterior to the nerve ring, 69–98  $\mu$ m from the anterior end. Renette cell extends posterior to the base of pharynx. Long clear cardia, about 25  $\mu$ m long. Tail conical, tapering to bluntly rounded tip. Tail 2.5–3.4 anal body diameters long in male, 3.4–4.7 in female. The caudal glands are poorly visible, but they probably have an efferent duct-spinneret.

Male with two testes arranged in tandem, located at the ventral side of the body. Spicules small, arcuate,  $41.0-48.5 \mu m$  long as chord and  $51-59 \mu m$  long as arc. The distal ends pointed and gradually broaden to proximal, proximal slightly cepahlated. The gubernaculum has a dorsal–caudal apophysis, length  $18-19 \mu m$ .

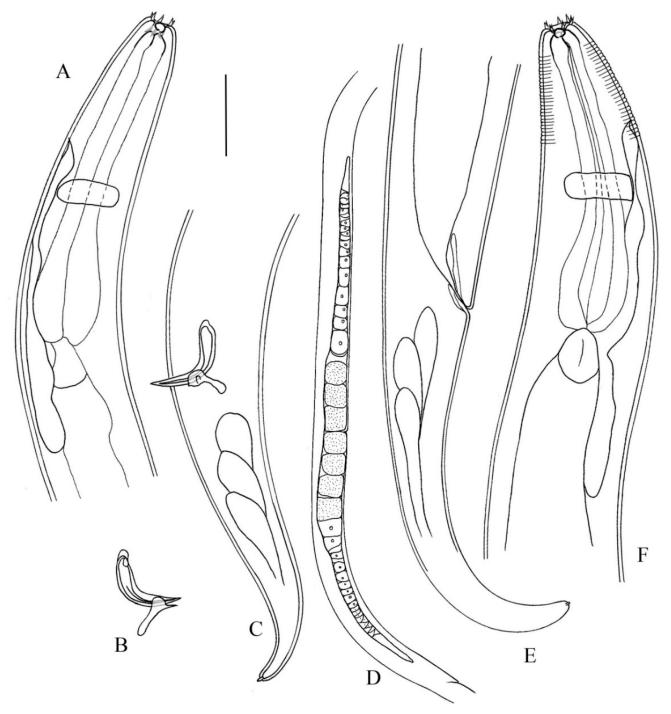
Female with two opposed, outstretched ovaries, the anterior is left of the intestine, the posterior shifted ventrally or towards the right. Vulva located at 52–56% of body length (Figures 1 and 2, Table 2).

#### 3.1.5. Differential Diagnosis

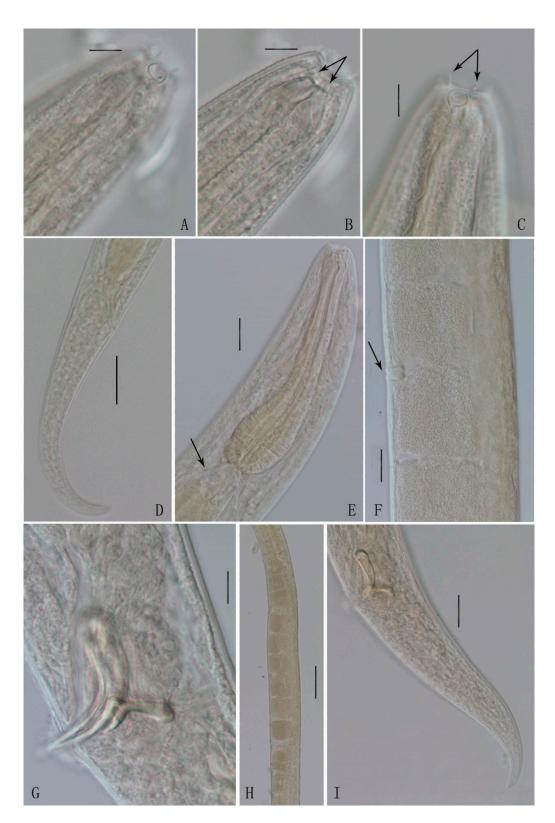
Anticyathus shenzhensis sp. nov. is characterized by ten cephalic setae length in a circle, about 2–4  $\mu$ m; amphids located at very anterior of head, 2–3  $\mu$ m from anterior end; tail conical, 2.5–3.4 abd in male and 3.4–4.7 abd in female; spicules arcuate, 0.71–1.04 abd, the distal ends pointed, gradually broaden towards proximal; gubernaculum has a dorsal–caudal apophysis, length 18–19  $\mu$ m; female with two opposed, outstretched ovaries; vulva at 52–56% of body length.

From all valid species, this new species can be easily distinguished from *A. tenuicaudatus* Cobb, 1920 and *A. septentrionalis* (Cobb, 1914) Timm, 1978 by the tail (tail 8–11 abd, cylindroid in the posterior fourth in *A. tenuicaudatus* Cobb, 1920) [10] and the shape of spicule (description from Cobb [17]: spicule slender and uniform; description from Timm [18]: highly angular, distinctly cephalated, with head turned ventrally), respectively. It differs from *A. boreicaspius, A. plicibucca*, and *A. trochus* by male without pre-anal supplement (a

pre-anal supplement present in *A. boreicaspius* Tchesunov 1978, *A. plicibucca* Tchesunov & Yushin, 1991 and *A. trochus* (Gerlach, 1957) Gerlach, 1963) [5,19,20]. Compared with *A. primitivus*, it can be distinguished by ten cephalic setae arranged in a circle (arranged in two circles in *A. primitivus*) and amphid and vulva located relative anterior of body (in *A. primitivus*: amphid at 6–7 µm from anterior end and vulva at 71% of body length) [9,21].



**Figure 1.** *Anticyathus shenzhensis* sp. nov.—holotype male and paratype female in lateral view. (**A**) lateral view of male head end; (**B**) lateral view of male copulatory apparatus; (**C**) lateral view of male tail region; (**D**) lateral view of female body part, showing ovaries; (**E**) lateral view of female tail region; (**F**) lateral view of female head end. Scale bar:  $(A-C,E,F) = 50 \mu m$ ; (**D**) = 250  $\mu m$ .



**Figure 2.** *Anticyathus shenzhensis* sp. nov.—DIC photomicrographs. (**A**) lateral view of male head end, showing amphid; (**B**) lateral view of female head end, showing buccal cavity and longitudinal reinforcements; (**C**) lateral view of female head end, showing amphid and cephalic setae; (**D**) lateral view of female tail region; (**E**) lateral view of female body part, showing pharynx and cardia; (**F**) lateral view of female body part, showing vulva; (**G**) lateral view of male body part, showing ovary and eggs; (**I**) lateral view of male tail region. Scale bar: (**A**–**C**,**G**) = 10 µm; (**E**,**F**,**I**) = 25 µm; (**D**) = 50 µm; (**H**) = 100 µm.

Characters	Holotype	types					
Characters	 ♂1	<b>₫</b> 2	്3	₫4	<b>♀1</b>	<b>₽2</b>	♀ <b>3</b>
Body length	2232	2422	2172	2399	2486	2042	2255
Head diameter	17	18	19	18	18	17	18
Length of cephalic setae	2–3	2–3	2–3	4	2–3	2–3	2-3
Buccal cavity length	8	10	6	8	6	7	5
Buccal cavity diameter	5	7	8	7	9	6	7
Amphids from the anterior end	2	3	3	2	2	3	2
Amphid length	6	5	6	7	5	5	5
Amphid width	5	7	5	7	5	6	6
Amphid cbd	23	23	23	24	25	25	22
Amphid width/Amphid cbd	0.20	0.29	0.22	0.28	0.22	0.22	0.27
Excretory pore from the anterior end	84	89	74	98	69	79	84
Excretory pore cbd	52	63	54	69	58	51	63
Nerve ring from the anterior end	108	117	98	120	94	106	99
Nerve ring cbd	57	69	59	74	67	55	66
Pharynx length	198	211	191	203	184	205	191
Pharynx cbd	65	77	72	88	78	63	74
Maximum body diameter	73	84	79	98	99	72	88
abd	57	65	62	76	54	52	61
Tail length	193	189	156	196	253	209	208
c′	3.4	2.9	2.5	2.6	4.7	4.0	3.4
Spicule length as chord	48	41	42	49	-	-	-
Spicule length as arc	59	54	51	54	-	-	-
Spicule length as arc/abd	1.04	0.83	0.82	0.71	-	-	-
Gubernaculum	14	11	10	13	-	-	-
Length of apophysis	19	18	19	19	-	-	-
V'	-	-	-	-	1296	1118	1263
vbd	-	-	-	-	99	72	88
V%	-	-	-	-	52	55	56
a	30.58	28.83	27.49	24.48	25.11	28.36	25.63
b	11.27	11.48	11.37	11.83	13.51	9.96	11.81
c	11.56	12.81	13.92	12.22	9.83	9.77	10.84

Table 2. Morphometrics of Anticyathus shenzhensis sp.nov. (in µm).

#### 3.1.6. DNA Sequence Results

A total of 39 sequences of 18S rRNA gene were obtained from ten individuals of *Anticyathus shenzhensis* sp. nov. in this study. All sequences were deposited in GenBank. GenBank accession numbers are as follows: ON694124-ON694132, ON763151-ON763180.

#### 3.2. Description of Anticyathus communis sp. nov.

#### 3.2.1. Type Material

Five males and two females were collected from Station ST 13L2, ST 13L3, ST 11H2, and ST 11H3 in September 2020. Holotype:  $\sigma$ 1 on slide number ST20200902 13L2 103. Paratypes:  $\sigma$ 2 on slide number ST20200902 13L2 103,  $\sigma$ 3 on slide number ST20200902 11H3 106,  $\sigma$ 4 and  $\sigma$ 5 on slide number ST20200902 13L2 108,  $\varphi$ 1 on slide number ST20200902 11H2 109, and  $\varphi$ 2 on slide number ST20200902 13L3 108.

## 3.2.2. Etymology

The specific name *communis* ("ordinary" in Latin) is derived from the usual body form of the new species.

## 3.2.3. Type Locality and Habitat

All specimens were collected from a mangrove forest in the mangrove reservation of Shantou (23.46 $^{\circ}$  N, 116.87 $^{\circ}$  E), China.

## 3.2.4. Morphological Description

Body cylindrical, maximum body diameter 53–66  $\mu$ m. Cuticle annulated, the annule begins a little posterior to the level of the base of the buccal cavity, annule about 2.2  $\mu$ m broad at anterior body part, and 1.3  $\mu$ m at midbody part. Ten cephalic setae, length about 3–5  $\mu$ m. The setae appear to be at one level in submedian pairs. Head truncate, 17–21  $\mu$ m in diameter. Buccal cavity length 9–11  $\mu$ m and width 7–9  $\mu$ m, lined with 6 thick, non-cuticularized, longitudinal reinforcements. Amphids broken circles or cryptic spiral, width 5–6  $\mu$ m or 20–32% of corresponding body diameter, length 6–7  $\mu$ m. Amphids located at very anterior of head, anterior margin 1–2  $\mu$ m from anterior end. Pharynx cylindrical, with anterior cuticularized, 0.09–0.11 times of body length, gradually enlarging in posterior portion but without bulb. Nerve ring located at 49–60% of pharynx length. The excretory pore is anterior to the nerve ring, 78–96  $\mu$ m from the anterior end. Renette cell extends posterior to the base of pharynx. Long clear cardia, 17–27  $\mu$ m long. Tail conical, tapering to bluntly rounded tip. Tail 3.6–4.5 anal body diameters long in male, 5.3–5.5 in female. The caudal glands are poorly visible, but they probably have an efferent duct-spinneret.

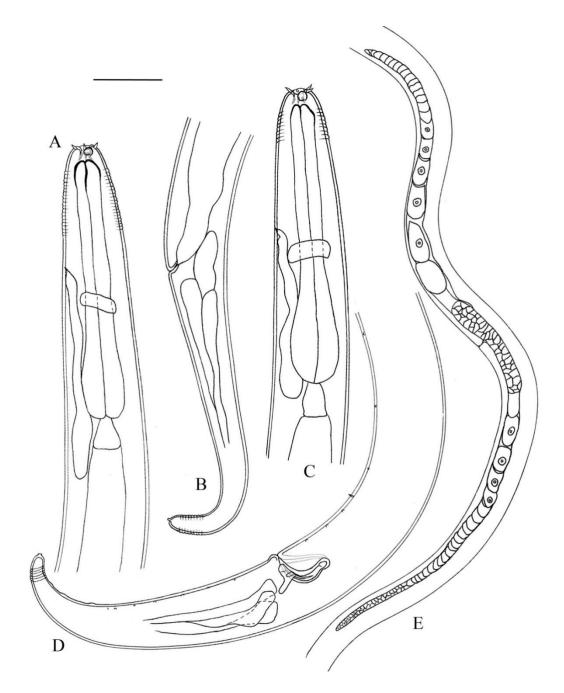
Male with two testes arranged in tandem, located at the ventral side of the body. Spicules arcuate, the distal ends pointed, gradually broaden to proximal, proximal distinctly cephalated, 31–37  $\mu$ m long as chord and 43–49  $\mu$ m long as arc. Gubernaculum "L"-shaped, consisting of sleeves around spicule and dorsal–caudal apophysis. Apophysis length 13–21  $\mu$ m. Five to seven pre-anal and seven to ten post-anal almost invisible papilla or papillae-like setae, if the cuticle is thick, occur on each ventrally submedian line on the posterior body part of male. The tip of the tail with three slight elevations.

Female with two opposed, outstretched ovaries, the anterior is right to the intestine, and the posterior shifts to the left. Vulva at 52% of body length (Figures 3 and 4, Table 3).

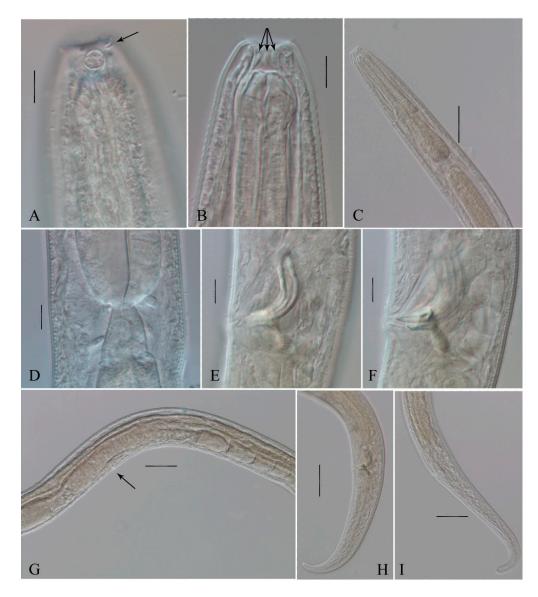
Characters	Holotype						
Characters	1്	<b>₫</b> 2	്3	₫4	ಿ5	<b>♀1</b>	<b>2</b>
Body length	2462	2447	2373	2266	1892	2176	2242
Head diameter	18	19	21	19	17	18	19
Length of cephalic setae	4	4	4	4	3	5	4
Buccal cavity length	11	11	11	12	9	10	11
Buccal cavity diameter	7	8	8	8	8	9	7
Amphids from the anterior end	1	2	2	1	2	2	2
Amphid length	6	7	6	6	6	6	7
Amphid width	6	6	6	6	6	5	6
Amphid cbd	22	20	25	25	22	25	23
Amphidwidth/Amphid cbd	0.26	0.32	0.25	0.23	0.27	0.20	0.25
Excretory pore from the anterior end	96	96	93	78	88	89	86
Excretory pore cbd	49	51	53	50	43	49	46
Nerve ring from the anterior end	112	111	113	101	105	117	113
Nerve ring cbd	50	51	54	53	49	53	49
Pharynx length	221	210	217	205	201	194	209
Pharynx cbd	53	57	60	60	51	57	56
Maximum body diameter	55	58	65	65	53	66	59
abd	49	52	60	54	46	42	40
Tail length	220	220	219	205	176	227	215
c′ Ü	4.5	4.3	3.6	3.8	3.9	5.5	5.3
Spicule length as chord	36	34	34	37	31	-	-
Spicule length as arc	46	43	44	49	44	-	-
Spicule length as arc/abd	0.95	0.84	0.74	0.90	0.96	-	-
Gubernaculum	12	12	11	12	12	-	-
Length of apophysis	21	13	13	17	17	-	-
V' V'	-	-	-	-	-	1142	1177
vbd	-	-	-	-	-	65	57
V%	-	-	-	-	-	52	52

Table 3. Individual measurements of Anticyathus communis sp. nov. (in µm).

Characters	Holotype	Paratypes							
xmxmxmxmxm	്1	<b>₫</b> 2	്3	₫4	ೆ5	<b>♀1</b>	<b>₽2</b>		
a	44.99	42.54	36.47	34.95	35.64	33.04	38.30		
b	11.16	11.65	10.93	11.03	9.42	11.21	10.73		
с	11.22	11.12	10.86	11.03	10.78	9.57	10.43		



**Figure 3.** *Anticyathus communis* sp. nov.—holotype male and paratype female in lateral view. (A) lateral view of female head end; (B) lateral view of female tail; (C) lateral view of male head end; (D) lateral view of male tail region; (E) lateral view of female body part, showing ovaries. Scale bar: (A–D) =  $50 \ \mu m$ ; (E) =  $125 \ \mu m$ .



**Figure 4.** *Anticyathus communis* sp. nov.—DIC photomicrographs. (**A**) lateral view of male head end, showing amphid and cephalic setae; (**B**) lateral view of female head end, showing buccal cavity and longitudinal reinforcements; (**C**) lateral view of female anterior part, showing pharynx; (**D**) lateral view of male body part, showing cardia; (**E**) lateral view of male body part, showing spicule; (**F**) lateral view of male body part, showing gubernaculum and apophysis; (**G**) lateral view of female body part, showing ovary and vulva; (**H**): lateral view of male tail region; (**I**) lateral view of female tail region. Scale bar: (**A**,**B**,**D**–**F**) = 10 µm; (**C**,**G**–**I**) = 50 µm.

## 3.2.5. Differential and Diagnosis

*Anticyathus communis* sp. nov. most closely resembles *A. shenzhensis* sp. nov. by the similar body size, the location of amphid and vulva, the shape of the tail, the arrangement of the cephalic setae, and the absence of pre-anal supplement. However, it can be separated from *A. shenzhensis* sp. nov. by a more slender body (maximum body diameter 53–66, a = 33–45 vs. maximum body diameter 72–99, a = 24.5–30.6 in *A. shenzhensis*); a relatively shorter tail (3.6–4.5 abd in male and 5.3–5.5 abd in female vs. 2.5–3.4 in male and 3.4–4.7 in female in *A. shenzhensis*); a proximal spicule, significantly cephalated (proximal of spicule slightly cephalated in *A. shenzhensis*); posterior body part of male with five to seven pre-anal and seven to ten post-anal almost invisible papilla or papillae-like setae (*A. shenzhensis* sp. nov. without pre-anal or post-anal papilla).

#### 4. Discussion

There are six species in this genus have been described up to now (Electronic database: http://nemys.ugent.be; http://www.marinespecies.org/index.php (accessed on 15 June 2022)). All valid species are listed below. *A. primitivus* was originally described as *Linhomoeus (Eulinhomoeus) primitiva* based on a single female [20]; Gerlach [19] added the description of male, and considered it to be synonymous *Paralinhomoeus primitivus* Allgén, 1934; *A. trochus* was first described as *Prospharolaimus trochus* by Gerlach in 1957. Gerlach [9] gave a dichotomous key to the genus *Anticyathus*, transferred *Linhomoeus (Eulinhomoeus) primitiva* and *Prospharolaimus trochus* to *Anticyathus*. *A. septentrionalis* was originally described as *Laxus septentrionalis* Cobb, 1914 [17], Timm (1978) [18] redescribed and illustrated it, and transferred it to *Anticyathus*.

#### 4.1. List of Valid Anticyathus Species

Anticyathus boreicaspius Tchesunov 1978

Anticyathus plicibucca Tchesunov & Yushin, 1991

Anticyathus primitivus (Allgén, 1933) Gerlach, 1963 syn Linhomoeus primitiva Allgén, 1933; Paralinhomoeus primitivus Allgén, 1934

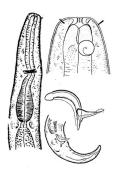
Anticyathus septentrionalis (Cobb, 1914) Timm, 1978

Anticyathus tenuicaudatus Cobb, 1920

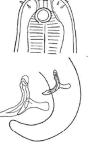
*Anticyathus trochus* (Gerlach, 1957) Gerlach, 1963 syn *Prospharolaimus trochus* Gerlach, 1957 Figure 5 shows the pictorial key to 6 valid species as well as the two newly described species of the genus *Anticyathus*. Table 4 shows the differentiating characters of all known *Anticyathus* species.



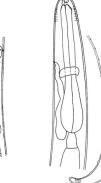
*A. boreicaspius* (*apud* Tchesunov, 1978)

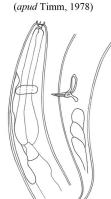


A. plicibucca (apud Tchesunov & Yushin, 1991)



A. primitivus (anud Gerlach, 1963)





A. septentrionalis

A. shenzhensis sp. nov. Original





Figure 5. Pictorial key for genus Anticyathus.

A. trochus (apud Gerlach, 1957)

A. communis sp. nov. Original

Species	A. boreic	caspius	A. plicib	исса	A. prin	nitivus		A. septen	trionali	5		uicau- tus	A. tr	ochus		A. communis sp. A. nov.		hensis sp. ov.	
References	[19	]	[5]		[9]	[21]	[1	17]	[1	8]	[1	0]	[2	20]	This A	Article	This A	This Article	
Sex	₫	ę	്	ę	്	Ŷ	o™	ę	ď	ę	്	ę	്	ę	്	ę	്	Ŷ	
Body length	2480	2160	2140-3050	2550	2172	1725	2000	1800	2150– 2870	2040– 2990	5200	6500	875	1044	1892– 2462	2176– 2242	2172– 2422	2042– 2486	
Amphids from the anterior end	5	6.3	11.9	15	7	6	-	-	-	-	-	-	-	-	1–2	2	2–3	2–3	
Amphid width	6.9–7		8.5	6.8	9	6	-	-	-	-	-	-	-	-	6	5–6	5-7	5–6	
Maximum body diameter	75	50	61	67.5	55	45	38 *	37.8 *	-	-	-	-	30	-	53–65	59–66	73–98	72–88	
abd	50	43.7	51	41	52	30 *	34 *	23.4 *	-	-	88.4 *	84.5 *	26	24	46-60	40-42	57-76	52-61	
Tail length	150 *	140 *	-	-	147	130	108 *	84.6 *	-	-	728 *	975 *	-	-	176–220	215-227	156–196	208–253	
c′	3	3.2	3.8-4.1	4	3–4	4.3 *	3.18 *	3.62 *	3.1–4	2.6–4	11.5 *	8.24 *	3	4	3.6-4.5	5.3-5.5	2.5 - 3.4	3.4–4.7	
Spicule length as arc	43.7/47.5	5 -	40	-	45	-	51 *	-	40	-	88 *	-	23	-	43–49	-	51–59	-	
Spicule length as arc/abd	0.87/0.95 *	; -	0.78	-	0.87	-	1.50	-			1.00	-	0.88	-	0.74– 0.96	-	0.71– 1.04	-	
Length of apophysis	-	-	15	-	-	-	17 *	-	23	-	22 *	-	-	-	13–21	-	18–19	-	
V%	-	-	-	62.6%	-	71.01%	) -	64.00%	-	51.4– 58.6%	-	50% *	-	54%	-	52%	-	52–56%	
а	33	43.2	50	37.7	40	38.3	53 *	48 *	50– 64	41– 49	47 *	37 *	29	29	35.0– 45.0	33.0– 38.3	24.5– 30.6	25.6– 28.4	
b	6.5	5.9	15.4–18.2	15.6	8.5	7.36	12.8 *	13.2 *	10.5– 18.8	10.2– 13.0	13.3 *	20 *	5.4	6	9.4–11.7	10.7– 11.2	11.3– 11.8	10.0– 13.5	
с	15.5	15.9	18.2–23.3	22.6	15	13.27	18.5 *	21.3 *	13.7– 19.4	15.7– 26.8	7.14 *	6.67 *	11.1	9.2	10.8– 11.2	9.6–10.4	11.6– 13.9	9.8–10.8	

Table 4. Differentiating characters of all known	Anticyathus species (in $\mu$ m).
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Abbreviations: - data absent; \* data calculated from descriptive formula.

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4.2. Identification Key to Species of the Genus Anticyathus (emended after Gerlach, 1963) [9]
1. Tail 8–11 abd, cylindroid in the posterior fourth
- Tail shorter, conical
2. Spicule relatively longer, 1.5 abd
- Spicule relatively shorter, about 1 abd or less than 1 abd
3. Male with a pre-anal supplement
- Male without pre-anal supplement
4. Cephalic sensilla setaceous-like, length 5.1 μm; amphid located posterior to the buccal
cavityA. plicibucca Tchesunov & Yushin, 1991
- Cephalic sensilla reduced to blunt cone papillae or very short setae; amphid located at the
base of buccal cavity
5. Body size smaller (body length 875–1044 $\mu$ m, spicule length 23 $\mu$ m), cuticle without
annuleA. trochus (Gerlach, 1957)
Body size bigger (body length 2160–2480 µm, spicule length 43.7 and 47.5 µm), cuticle with
annules A. boreicaspius Tchesunov 1978
6. 10 cephalic sensilla arranged in one circle
- 10 cephalic sensilla arranged in two circle
7. Tail relatively longer, 2.5–3.4 abd in male, 3.4–4.7 abd in female, without papilla of
seatae
- Tail relatively shorter, 3.6-4.5 abd in male, 5.3-5.5 abd in female, with papilla or
seatae

#### 4.3. Discussion and Notes on the Molecular Research of Anticyathus

The nucleotide divergence of genes could be used as a diagnostic tool for the discrimination of nematode species [13]. The combination of molecular with morphological approaches can help in the identification and resolution of evolutionary relationships within marine nematodes [22–24]. However, the number of marine nematode species with nucleotide sequences in databases is very low. By searching the Genbank database (Electronic database: https://www.ncbi.nlm.nih.gov/ [accessed on 15 June 2022]), a total of 207 nucleotide sequences belong of family Linhomoeidae have been deposited. No gene sequences of genus *Anticyathus* can be found in Genbank database to date. It seems that the molecular data of genus *Anticyathus* is still lacking, which limits the further resolution of evolutionary relationships between *Anticyathus* and other marine nematodes. A comprehensive comparative molecular database is needed. In the present work, 39 sequences of 18S rRNA gene were obtained from ten individuals of *Anticyathus shenzhensis* sp. nov. and provided to the Genbank database, serving as a foundation for further study.

**Author Contributions:** Conceptualization, data curation, writing—original draft preparation, and writing—review and editing, Y.C. and Y.G.; methodology, R.Z.; investigation, H.Z.; funding acquisition, Y.G. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the National Natural Science Foundation of China, grant number 31772416.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors thank Yi-Jia Shih for providing valuable comments on the manuscript and thank Pan Chao and Futian-CityU Mangrove Research & Development Centre for for great assistance in the sampling.

Conflicts of Interest: The authors declare no conflict of interest.

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