Journal of the Marine Biological Association of the United Kingdom, 2015, 95(6), 1127–1132.
doi:10.1017/S0025315414002069

Review of *Onyx* Cobb (Nematoda: Desmodoridae) with description of two new species from the Yellow Sea, China

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Genus Onyx *Cobb, 1891 has been reviewed and 19 valid species including two new species have been reported in the world. Two new species,* Onyx rizhaoensis *sp. nov. and* Onyx minor *sp. nov. from the Yellow Sea coast are described and illustrated. In addition to the genus characters* Onyx rizhaoensis *sp. nov. has relatively long cephalic setae; a single loop-shaped amphidial fovea; 12 S-shaped tubular precloacal supplements in 10 + 2 arrangement, posterior 10 closely spaced, anterior two set apart; female vulva situated at almost midpoint of the body.* Onyx minor *sp. nov. is characterized by a relatively small body for the genus (body length usually shorter than 800 μm); gubernaculum slender parallel to spicules and with a hooked dorsal apophysis; 12 S-shaped tubular precloacal supplements fairly evenly spaced; female vulva situated at about midbody. At the same time, a dichotomous key for* Onyx *males is proposed.*

Keywords: free-living marine nematode, *Onyx rizhaoensis* sp. nov., *Onyx minor* sp. nov., taxonomy

Submitted 28 March 2014; accepted 18 December 2014; first published online 27 April 2015

INTRODUCTION

To study the biodiversity of free-living marine nematodes in the Yellow Sea, China, sediment samples were collected in many sites from the intertidal to the sublittoral region of the Yellow Sea in the past few years. More than 260 species have been recorded from these habitats (Huang, 2008; Huang & Cheng, 2012; Huang & Xu, 2013; Huang & Zhang, 2014). Two new *Onyx* species were found in the sediment samples from Rizhao coast. The present paper describes two new species and reviews the species of *Onyx*.

The genus *Onyx* was established by Cobb in 1891 with the type species *Onyx perfectus*. This genus differs from other related genera of Family Desmodoridae in the presence of fine cuticle striation, amphidial fovea not surrounded by cuticle striations, buccal cavity with a long-spear-like dorsal tooth, posterior pharyngeal bulb elongated and with a slight constriction in the middle, spicules short and arcuate, precloacal supplements normally S-shaped tubes (Platt & Warwick, 1988). Up to now, 18 species have been recorded in the world (Gerlach & Riemann, 1973; Blome & Riemann, 1994; Hourston & Warwick, 2010; Nasira, Rehmat & Shahina, 2011; Tu *et al*., 2011). Out of them, *Onyx ferox* (Ditlevsen, 1921) Gerlach, 1951 had been described by a single female specimen. Males provide the main diagnostic features to differentiate species of *Onyx*. In the absence of males we consider *Onyx ferox* as invalid species. The list of 17 valid species of the genus *Onyx* Cobb, 1891 is as follows.

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Onyx adenophorus Blome & Riemann, 1994
Onyx balochiensis Nasira *et al*., 2011
Onyx blomei Tu *et al*., 2011
Onyx cangionensis Tu *et al*., 2011
Onyx cannoni Blome & Riemann, 1994
Onyx cephalispiculus Hourston & Warwick, 2010
Onyx cobbi Tu *et al*., 2011
Onyx dimorphus Gerlach, 1963
Onyx macramphis Blome & Riemann, 1994
Onyx mangrovi Tu *et al*., 2011
Onyx orientalis Tu *et al*., 2011
Onyx paradimorphus Tu *et al*., 2011
Onyx perfectus Cobb, 1891
Onyx potteri Hourston & Warwick, 2010
Onyx rugatus Wieser, 1959
Onyx sagittarius Gerlach, 1950
Onyx septempapillatus Wieser, 1954

MATERIALS AND METHODS

Undisturbed sediment samples were collected using a syringe with a 2.6 cm inner diameter, pushed into the sediment down to 8 cm depth at Rizhao intertidal zone (119°34′E 35°26′N) of the Yellow Sea coast in July 2008. Samples were stratified by 0–2, 2–8 cm and fixed with 5% formalin in seawater respectively for long-term preservation. In the laboratory, samples were stained with 0.1% rose bengal for 24 h (Higgins & Thiel, 1988). The samples were washed to remove the formalin and sieved over two mesh sizes (500 and 42 μm) in order to separate the macrofauna (500 μm) from the meiofauna (42 μm). Heavier sediment particles were removed using centrifugation in Ludox-^{TM} with a specific gravity adjusted to

1.15 (Jonge & Bouwman, 1977). Each sample was washed into a lined Petri dish and the meiofauna was sorted under a stereoscopic microscope up to higher taxonomic levels. Nematodes were transferred into a 10 ml 9:1 (V:V) solution of 50% ethanol : pure glycerin in block cavity to slowly evaporate ethanol and then mounted in glycerin on permanent slides. The descriptions were made from glycerol mounts using interference contrast microscopy. Drawings were made with a camera lucida. Type specimens (slide number: RZ080422-4 and RZ080422-6, respectively) were deposited in the Qingdao Institute of Oceanology, Chinese Academy of Sciences.

Measurements are in μm. Abbreviations are as follows: a, body length/max. body diameter; a.b.d., corresponding body diameter of anus; b, body length/pharynx length; c, body length/tail length; c.b.d., corresponding body diameter; c′, tail length/a.b.d; M. maximum body diameter; Spic, spicule length along arc; V, corresponding body diameter of vulva; V%, position of vulva from anterior end expressed as a percentage of total body length.

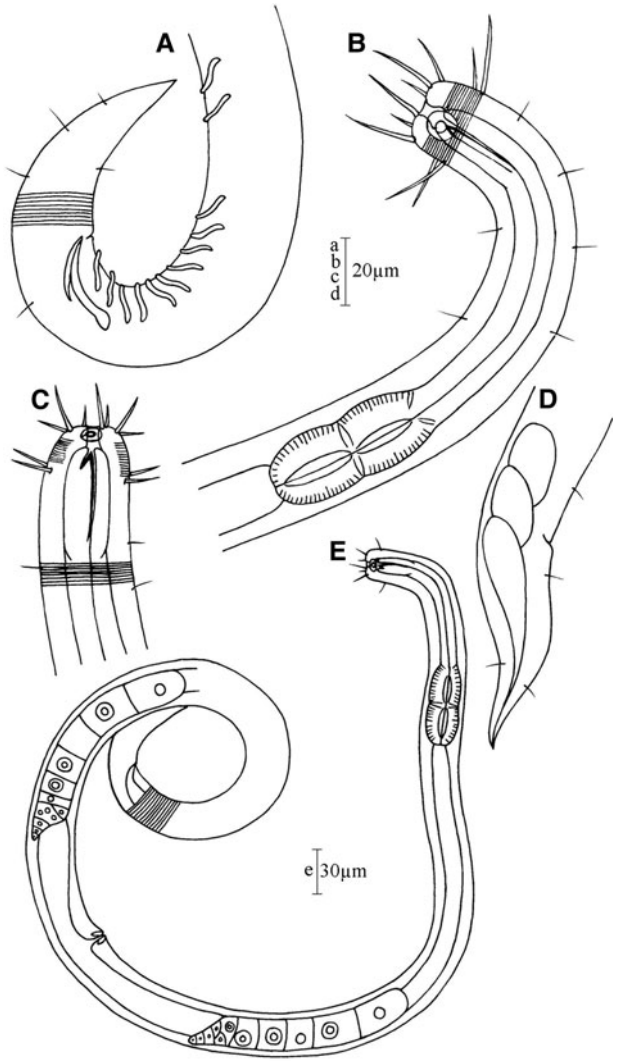

Fig. 1. *Onyx rizhaoensis* sp. nov. (A) Lateral view of holotype posterior end, showing spicule, gubernaculum, precloacal supplememts and part detail of surface body cuticle; (B) lateral view of holotype anterior end, showing setiform anterior sensilla, amphidial fovea, buccal tooth and posterior pharyngeal bulb; (C) lateral view of female head end, showing amphidial fovea and buccal tooth; (D) lateral view of female tail region, showing caudal gland cells; (E) entire view of female body, showing reproductive system.

SPECIES DESCRIPTION

Order CHROMADORIDA Chitwood, 1933
Family DESMODORIDAE Filipjev, 1922
Subfamily SPIRINIINAE Gerlach and Murphy, 1965
Genus *Onyx* Cobb, 1891
Onyx rizhaoensis sp. nov.
(Figures 1 & 2)

TYPE MATERIAL

Two males and four females were collected from the sampling sites in July 2008. Holotype: ♂1 (slide number: RZ080422-4); paratypes: ♂2, ♀1, ♀2 in slide RZ080422-3, RZ080422-1 and RZ080422-4, respectively.

TYPE LOCALITY AND HABITAT

Intertidal sandy sediment at Rizhao coast of the Yellow Sea: 119°34′E 35°26′N.

ETYMOLOGY

This species is named after the sea area Rizhao of the type locality.

MEASUREMENTS (TABLE 1)

Holotype ♂1: $\frac{-\ 185\ M\ 1264}{22\ \ 28\ \ 28\ \ 25}$ 1330 μm; a = 44.3, b = 7.2, c = 20.2, Spic = 30 μm

Paratype ♀1: $\frac{-\ 190\ V\ 1210}{22\ \ 32\ \ 33\ \ 24}$ 1280 μm; a = 38.8, b = 6.7, c = 18.3, V% = 51%

DESCRIPTION

Male. Body cylindrical, tapered in tail to a pointed end. Cuticle with fine transverse striations extending from middle of amphidial fovea to tail tip. Head 22 μm wide, surrounded by one circle of sensilla setae, which consisted of six longer outer labial setae, 20 μm long and four shorter cephalic setae, 10 μm long. Inner labial sensilla not observed. Eight 18 μm subcephalic setae posterior to amphidial fovea edge. Cervicle setae irregularly distributed. Amphidial fovea with a single loop, 10 μm in diameter, located far anteriorly in head region. Buccal cavity cup-shaped with a large spear-shaped dorsal tooth, 20 μm long. Pharynx, anteriorly slightly widened, posteriorly with an elongated double bulb with a constriction in the middle. Cardia not discernible. Secretory-excretory system not observed. Tail short conical with three caudal glands. Two of which extend anteriorly beyond cloacal opening. Caudal setae scattered in tail region, about 15 μm long.

One anterior outstretched testis. Spicules regularly bent, proximal end cephalated and distal end tapered, 30 μm long along arc. Gubernaculum plate-like, 20 μm long in holotype, without apophysis. 12 S-shaped tubular precloacal supplements (11–14 μm) in 10 + 2 arrangement, posterior 10 closely spaced, anterior 2 set apart.

Female. Similar to males in most aspects. Amphidial fovea smaller and more anteriorly, i.e. almost at apical surface of the head. Ovaries paired, opposed, reflexed. Anterior ovary situated on the right side of the intestine, 160 μm long and posterior one 170 μm long. Vulva situated at about midbody.

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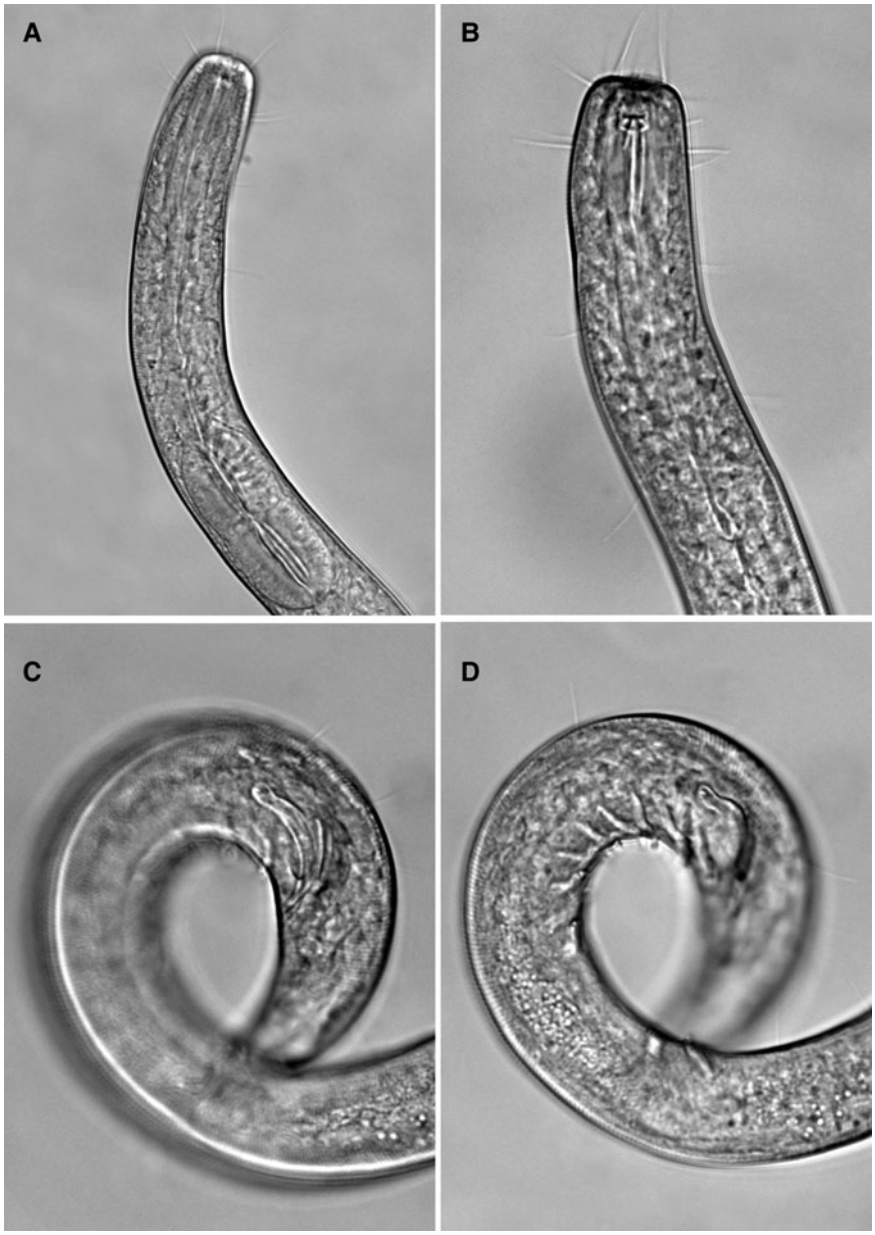

Fig. 2. *Onyx rizhaoensis* sp. nov. (A) Lateral view of female cervical region, showing posterior pharyngeal bulb; (B) lateral view of holotype, showing setae and buccal tooth; (C) lateral view of male tail end, showing spicule and gubernaculum; (D) lateral view of male posterior part, showing precloacal supplememts.

Differential diagnosis

In addition to the genus characters *Onyx rizhaoensis* sp. nov. with relatively long cephalic setae; 12 S-shaped tubular precloacal supplements in 10 + 2 arrangement, posterior 10 closely spaced, anterior 2 set apart; vulva situated at about midbody. The new species most resembles *Onyx perfectus* Cobb, 1891 in most features but differs from it in dorsal buccal tooth length (20 vs 52 μm), spicules length (30 vs 52 μm), the number and arrangement of precloacal supplements (12 in 10 + 2 pattern vs 13 in evenly spaced), relatively posteriorly located vulva (51 vs 47%). In the arrangement of precloacal supplements, the new species is similar to *Onyx potteri* Hourston & Warwick, 2010 but differs from it in the number of precloacal supplements (12 in 10 + 2 pattern vs. 10 in 8 + 2 pattern), amphidial fovea structure (single loop vs2.75 turns), spicules length (30 vs 50 μm) and gubernaculum shape (plate-like vs oval-like).

Onyx minor sp. nov.
(Figures 3 & 4)

TYPE MATERIAL

Three males and four females were collected from the sampling sites in July 2008. Holotype: ♂1 (slide number: RZ080422-6); paratypes: ♂2, ♀1, ♀2 in slide RZ080422-6, RZ080422-7 and RZ080422-8, respectively.

TYPE LOCALITY AND HABITAT

Intertidal sandy sediment at Rizhao coast of the Yellow Sea: 119°34′E 35°26′N.

ETYMOLOGY

Species name refers to small body.

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Table 1. Individual measurements of *Onyx rizhaoensis* sp. nov. (in μm except for ratios).

Characters	Holo-type	Paratypes				
Specimens	♂1	♂2	♀1	♀2	♀3	♀4
Total body length	1330	1213	1280	1290	1200	1320
Maximum body diameter	28	27	33	35	34	32
Head diameter	22	20	22	20	18	18
Length of buccal dorsal tooth	20	22	20	25	24	22
Length of outer labial setae	20	16	14	18	16	16
Pharynx length	185	175	190	180	175	182
Pharynx c.b.d. at base	28	27	32	29	29	30
Length of posterior pharyngeal bulb	55	55	62	54	56	60
Spicule length as arc	30	30	–	–	–	–
Gubernaculum length	20	17	–	–	–	–
Tail length	66	68	70	72	60	70
a.b.d.	25	24	24	22	21	21
c'	2.6	2.8	2.9	3.3	2.9	3.3
Distance of vulva from anterior end	–	–	650	660	635	675
V	–	–	33	35	34	32
V%	–	–	51	51	53	51
a	44.3	44.9	38.8	36.9	35.3	41.3
b	7.2	6.9	6.7	7.2	6.9	7.3
c	20.2	17.8	18.3	17.9	20	18.9

MEASUREMENTS (TABLE 2)

Holotype ♂1: $\frac{-\ 120\ M\ 619}{16\ 19\ 19\ 16}$ 675 μm; a = 35.5, b = 5.6, c = 12.1, Spic = 22 μm

Paratype ♀1: $\frac{-\ 116\ V\ 694}{19\ 25\ 26\ 16}$ 660 μm; a = 25.4, b = 5.7, c = 12.7, V% = 51%

DESCRIPTION

Male. Body cylindrical, tapered in tail to a pointed end. Cuticle with very fine transverse striations. Head 16 μm wide, surrounded by one circle of 10 almost equal sensilla setae, which consisted of 6 outer labial setae and 6 cephalic setae, 7 μm long. Inner labial sensilla not observed. Eight subcephalic setae in one circle, 7 μm long, posterior to amphidial fovea. Cervicle setae irregularly distributed. Amphidial fovea with a single loop, 5 μm in diameter, located anteriorly in head region. Buccal cavity cup-shaped with a large spear-shaped dorsal tooth, 22 μm long. Pharynx, anteriorly slightly widened, posteriorly with an elongated double bulb with a slight constriction in the middle, 40 μm long. Cardia not discernible. Secretory-excretory system not observed. Tail short conical with 3 caudal glands. Two of which extend anteriorly beyond cloacal opening. Several short caudal setae scattered in tail region.

Monorchic. Spicules regularly bent, cephalated proximally and tapered distally, 22 μm long along arc. Gubernaculum slender curving parallel to spicule tip and with a hooked dorsal apophysis. 12 S-shaped tubular precloacal supplements in even distance apart, each one about 10 μm long.

Female. Similar to males in most aspects. Amphidial fovea smaller and slightly more anteriorly on the head. Two opposed and reflexed ovaries. Anterior ovary situated on the right side of the intestine, 96 μm long and posterior ovary 135 μm long. Vulva situated at almost midpoint of the body.

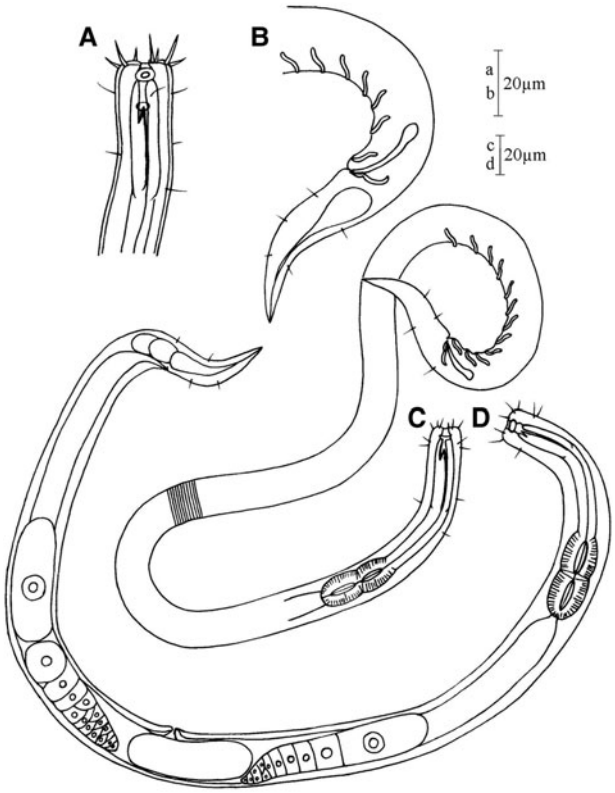

Fig. 3. *Onyx minor* sp. nov. (A) Lateral view of male head end, showing setiform anterior sensilla, amphidial fovea and buccal dorsal tooth; (B) lateral view of male tail end, showing spicule, gubernaculum and precloacal supplements; (C) entire view of male body; (D) entire view of female body, showing reproductive system with eggs.

DIFFERENTIAL DIAGNOSIS

Onyx minor sp. nov. is characterized by a relatively smaller body (600–806 μm long) for the genus, amphidial fovea with a single loop, forward in position, gubernaculum slender parallel to spicule and with a hooked dorsal apophysis, 12 S-shaped tubular precloacal supplements fairly evenly spaced. The new species most resembles *Onyx macramphis* Blome & Riemann, 1994 in the body length (their bodies shorter than 900 μm) but differs from it in amphidial fovea structure (single loop vs spiral with four turns), the number of precloacal supplements (12 vs 14), and anteriorly located vulva (51 vs 55%) in female.

KEY FOR *ONYX* MALES INCLUDING TWO NEW SPECIES

1 Male with atypical sigmoidal precloacal supplements 2
- Male with typical sigmoidal precloacal supplements 3

2 Male with 22 cup-shaped precloacal supplements *O. rugatus* Wieser
- Male with 7 tubular precloacal supplements *O. septempapillatus* Wieser
- Male with 10 conical precloacal supplements *O. dimorphus* Gerlach

3 All supplements in staggered row 4
- All supplements uniform and not in staggered row 6

4 Male with 18 equally sized supplements *O. orientalis* Tu *et al.*

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Fig. 4. *Onyx minor* sp. nov. (A) Lateral view of female head end, showing buccal cavity and dorsal tooth; (B) lateral view of male anterior end, showing setiform anterior sensilla and dorsal tooth; (C) lateral view of male posterior body region showing spicule and gubernaculum; (D) lateral view of male tail region, showing gubernaculum and precloacal supplements.

- Male with different sized supplements 5

5 Male with 18 supplements in 4 different size groups *O. adenophorus* Blome & Riemann
- Male with 15 supplements in 3 different size groups *O. cannoni* Blome & Riemann

6 Amphidial fovea dimorphism in shape, male's elongated-oval, female's spiral with 2 turns *O. cobbi* Tu *et al.*
- Amphidial fovea no dimorphism in shape 7

7 Male amphidial fovea with multispiral 8
- Male amphidial fovea with a single loop 10

8 Male amphidial fovea broader than c.b.d., with 4–5 turns *O. macramphis* Blome & Riemann
- Male amphidial fovea narrower than c.b.d., with 2–3 turns 9

9 Amphidial fovea with 2.5 turns, 8 supplements *O. blomei* Tu *et al.*
- Amphidial fovea with 2.75 turns, 10 supplements *O. potteri* Hourston & Warwick
- Amphidial fovea with 3 turns, 15 supplements *O. paradimorphus* Tu *et al.*

10 Tail short, less than 1.5 a.b.d. *O. mangrovi* Tu *et al.*
- Tail longer than 2 a.b.d. 11

11 Outer labial setae longer than 15 μm (0.5–1 c.b.d.) 12
- Outer labial setae short than 15 μm (0.5 c.b.d.) 14

12 Male with 12 supplements in 10 + 2 arrangement *O. rizhaoensis* sp. nov.
- Male with 13–23 supplements in about evenly spaced 13

13 Presence of two groups of setae anterior to spicules, 15–23 supplements
O. balochiensis Nasira, Rehmat & Shahina
- Absence of groups of setae, 13–17 supplements *O. perfectus* Cobb

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Table 2. Individual measurements of *Onyx minor* sp. nov. (in μm).

Characters	Holo-type	Paratypes					
Specimens	♂1	♂2	♂3	♀1	♀2	♀3	♀4
Total body length	675	806	754	660	660	630	600
Maximum body diameter	19	20	19	26	23	24	29
Head diameter	16	18	15	19	14	18	18
Length of buccal dorsal tooth	22	21	21	22	23	23	23
Length of outer labial setae	7	9	9	7	8	6	9
Pharynx length	120	110	118	116	138	116	125
Pharynx c.b.d. at base	19	20	19	25	22	23	25
Length of posterior pharyngeal bulb	40	33	35	39	46	43	42
Spicule length as arc	22	25	24	–	–	–	–
Gubernaculum length	15	14	13	–	–	–	–
Tail length	56	60	60	52	54	60	56
a.b.d.	16	19	18	16	18	18	17
c’	3.5	3.2	3.3	3.3	3.0	3.3	3.3
Distance of vulva from anterior end	–	–	–	336	340	320	310
V	–	–	–	26	23	24	29
V%	–	–	–	51	52	51	52
a	35.5	40.3	39.7	25.4	28.7	26.3	20.7
b	5.6	7.3	6.4	5.7	4.8	5.4	4.8
c	12.1	13.4	12.6	12.7	12.2	10.5	10.7

14 Male with 12 supplements *Onyx minor* sp. nov.
- Male with 14–16 supplements *O. cangionensis* Tu *et al.*
- Male with more than 22 supplements 15

15 Male with 24 supplements, presence of the cervical field of short setae
O. cephalispiculus Hourston & Warwick
- Male with 22–24 (23) supplements, absence of the cervical field of short setae *O. sagittarius* Gerlach

ACKNOWLEDGEMENTS

The authors would like to thank Ms Guilan Qi for her kind help in samples collection. We are also grateful to Dr Xiuqin Wu for providing the related references. Several anonymous referees provided valuable comments for the paper.

FINANCIAL SUPPORT

This work was supported by National Natural Science Foundation of China (Nos 30770250 and 41176107).

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https://doi.org/10.1017/S0025315414002069 Published online by Cambridge University Press