Three new genera, two new species and one new combination of family Hystrignathidae (Nematoda: Thelastomatoidea) from Ceracupes fronticornis (Westwood) (Insecta: Passalidae) in China

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Abstract Two new genera and two new species of family Hystrignathidae were collected from Ceracupes fronticornis (Westwood) from the Yunnan Province, China. Pseudoxyo yunnanensis gen. et sp. nov. differs from the related genera by having the cervical region armed with alternating rows of spines, with 22 spines in the first row, and by lacking the first cephalic annule. Sinospinata chitwoodi gen. et sp. nov. can be easily distinguished from the related genera by having the cervical region of females armed with irregularly arranged spines, two or three spines clustered together in their roots in some spines. Meanwhile, Huntinema gen. nov. was proposed to replace Huntia Zhang, Yin, Carreno & Zhang, 2021 because the name of this genus was preoccupied by Huntia Gray & Thompson, 2001 for placement of two new species of spiders. In addition, the 18S and 28S rDNA partial sequences of two new species were obtained.

Introduction

The members of Hystrignathidae Travassos, 1920 (Nematoda: Thelastomatoidea) are only parasitic in passalid beetles. To date, 36 genera have been described with more than 100 species (Zhang et al. 2021, 2022). The species are mainly distributed in North and South America, Africa, and Australia (Adamson & Van Waerebeke 1992; Morffe & García 2011, 2013a, b; Garduño-Montes de Oca & Oceguera-Figueroa 2020). However, recent studies on thelastomatoid nematodes from two Chinese passalid beetles have been found 4 new genera and 5 new species of hystrignathid nematodes (Zhang et al. 2021, 2022), which indicated it is highly probable that there is a great richness of nematodes to be discovered in passalid beetles in China. In the present study, nematode specimens were collected from Ceracupes fronticornis (Westwood) from the Yunnan Province, China. Two new genera and two new species of Hystrignathidae were confirmed and described here. In addition, Huntinema was proposed to replace Huntia Zhang, Yin, Carreno & Zhang, 2021 because the name of Huntia was preoccupied. Moreover, the 18S and 28S rDNA partial sequences for the new species were also generated.
Materials and methods

**Light and scanning electron microscopy**

The passalid beetle, *Ceracupes fronticornis* (Westwood), were collected from the Yunnan Province, China. They were dissected and examined for nematodes. The collected nematode specimens were killed with hot water (60–70 °C) and then fixed in 80% ethanol. For light microscopical examination, preserved nematodes were placed in a 5% solution of glycerin in 95% ethanol. These were left uncovered for 48 h to allow the ethanol to evaporate, thereby leaving the specimens in 100% glycerin. This was done to limit any damage to the worms caused by rapid transfer to pure glycerin. Measurements were taken with the aid of a calibrated eyepiece micrometer. De Man’s ratios a, b, c, V%, and V’% were calculated. All measurements are given in micrometers as range values followed by mean values in parentheses. Drawings were made with the aid of a Nikon microscope drawing attachment. For scanning electron microscopy studies, specimens were fixed in 7.5% glutaraldehyde, post-fixed in 1% OsO4, dehydrated through ethanol and acetone, and then subjected to critical point drying. The specimens were coated with gold and examined with a S-4800 Field Emission scanning electron microscope at an accelerating voltage of 15 kV. The specimens have been deposited in the College of Life Sciences, Hebei Normal University (HBNU), Hebei Province, China.

**Molecular Procedures**

Randomly selected samples were used for further molecular analysis. Genomic DNA from each sample was extracted using a Column Genomic DNA Isolation Kit (Shanghai Sangon, China) according to the manufacturer’s instructions. The 18S sequence was amplified by PCR using the forward primer 18SF (5’-CCCGATTGATTCTGTCGGC-3’) and the reverse primer 18SR (5’-TGATCCTTCTGCAGGTTCACC-TAC-3’) (Floyd et al. 2005). The 28S sequence was amplified by PCR using the forward primer D2A (5’-ACAAGTACCGTGAGGG AAAGTTG-3’) and the reverse primer D3B (5’-TCGAAGGAACCAGC-TACTA-3’) (Morffe et al. 2019). The PCR reactions for both 18S rDNA and 28S rDNA were performed in a total volume of 25 μL, containing 2 μL of template, 0.5 μL each of forward and reverse primers, and 12.5 μL of 2×Taq MasterMix (Beijing Bio-Lab, China). The 28S rDNA PCR cycling parameters were as follows: an initial enaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 56 °C for 30 s, and 2 °C for 70 s, followed by a final extension step at 72 °C for 7 min. The 18S rDNA PCR cycling parameters were as follows: an initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 70 s, followed by a final extension step at 72 °C for 7 min. PCR products were checked on GoldView-stained 1% agarose gels. Samples were sent to Shanghai Sangon, China for sequencing. Sequencing for each sample was carried out for both strands. Sequences were aligned using ClustalX and adjusted manually. The 18S rDNA and 28S rDNA sequences determined were compared (using the algorithm BLASTn) with those available in the National Center for Biotechnology Information (NCBI) database.

**Systematics**

**Family Hystrignathidae Travassos, 1920**

*Huntingema* gen. nov.

*Syn. Huntia* Zhang, Yin, Carreno & Zhang, 2021

**Diagnosis**: Female body robust. Cervical cuticle unarmed, without annulation. Body cuticle with distinct transverse striations and longitudinal striations. Lateral alae present. Head well developed, continuous with body but without transverse and longitudinal striations. Head bearing eight rounded papillae arranged in 4 pairs. First cephalic annule absent. Oesophagus consisting of a muscular, subcylindrical procorpus, short isthmus, and spherical basal bulb with valve plate well developed. Vulva located near mid-body. Didelphic-amphidelphic. Eggs ovoid, ornamented with rough longitudinal ridges on shell. Tail conical, subulate, ending in a sharp tip. Male unknown.

**Type species**: *Huntia morffei* Zhang, Yin, Carreno & Zhang, 2021; *Huntingema morffei* (Zhang, Yin, Carreno & Zhang, 2021) comb. nov.

**Distribution**: China.

*Huntingema morffei* (Zhang, Yin, Carreno & Zhang, 2021) comb. nov.
Syn. Huntia Zhang, Yin, Carreno & Zhang, 2021.

Type-host: Ceracupes fronticornis (Westwood)

Type-locality: Bubang Village, Sipsongpanna, Yunnan Province, China (21°35’53”N, 101°34’54”E).

Type-material: Holotype female (HBNU-I-2021013); paratypes: 9 females (HBNU-I-2021014–2021022).

Zhang et al. (2021) described a new genus and species, Huntia morffei Zhang, Yin, Carreno & Zhang, 2021, based on the specimens collected from Ceracupes fronticornis (Westwood). The new genus differs from the related genera by having the head continuous with the body, by the absence of first cephalic annule, and by having the cervical region unarmed, without annulation (Zhang et al. 2021). However, the name of this genus has been preoccupied by Huntia Gray & Thompson, 2001 for placement of two new species of spiders (Gray & Thompson 2001). Therefore, Huntia Zhang, Yin, Carreno & Zhang, 2021 is an invalid genus. We set up a new genus, Huntinema gen. nov., to replace Huntia Zhang, Yin, Carreno & Zhang, 2021. The type and only species of Huntia was also transferred to the new genus, forming a new combination, Huntinema morffei (Zhang, Yin, Carreno & Zhang, 2021) comb. nov.

Pseudoxyo gen. nov.

Diagnosis: Body relatively stout. Cervical cuticle bearing alternate rows of spines. Spines originating a short distance from head. Spines of first row small with 22 elements. Second row of spines longer than the first one. Lateral alae absent. Oral opening nearly circular, surrounded by a cuticular ring. Head bearing 8 papillae and a pair of amphids. Oesophagus consisting of a muscular, clavate corpus, short isthmus and basal bulb. Bulb rounded, valve-plate well-developed. Nerve ring encircling corpus in middle of its length (Fig. 1A). Excretory pore located posterior to basal bulb (Fig. 1D). Intestine simple, its anterior region slightly dilated. Reproductive system amphidelphic. Vulva located near mid-body. Vagina slightly extending anteriorly, connecting with two opposite uteri. Anterior ovary reflexed at excretory pore; posterior ovary reflexed at about two times of body width before anus (Fig. 1C, D). Eggs ovoid, smooth-shelled (Fig. 1E). Tail conical, attenuated, sharply pointed. Male not observed.

Type species: Pseudoxyo yunnanensis gen. et sp. nov.

Distribution: China.

Etymology: The genus name refers to the close resemblance between this genus and Xyo Cobb, 1898.

Pseudoxyo yunnanensis gen. et sp. nov.

Type-host: Ceracupes fronticornis (Westwood)

Type-locality: Tongbiguan Nature Reserve, Yunnan Province, China. (97°39’49”N, 24°36’55”E).

Type-material: Holotype female (HBNU-I-2021023); paratypes: 9 females (HBNU-I-2021024–2021032).

Prevalence: 6.1% (7 infected out of 115 examined).

Intensity: 2–10 (mean 5) specimens.

Site in host: Hindgut.

Representative DNA sequences: One partial 28S and one partial 18S rDNA sequence of the new species are deposited in the GenBank database under the accession numbers ON751930 and ON751935, respectively.

Etymology: The new species is named for its occurrence in Yunnan Province, China.

Description. Female: Body relatively stout. Cervical cuticle bearing alternating rows of spines. Spines originating 20 μm behind head, ending at level of anus (Fig. 2A, E). First row with 22 spines, about 8 μm long; second row of spines longer than the first, about 13 μm long. Size of spines gradually smaller after nerve ring (Fig. 1A). Oral opening rounded, surrounded by a cuticular ring (Fig. 2C). Head bearing eight papillae arranged in 4 pairs, a pair of amphids (Fig. 2C). Length of stoma about 5 times of head (Fig. 1A). Oesophagus consisting of a muscular, clavate corpus, short isthmus and basal bulb. Bulb rounded, valve-plate well-developed. Nerve ring encircling corpus in middle of its length (Fig. 1A). Excretory pore located posterior to basal bulb (Fig. 1D). Intestine simple, its anterior region slightly dilated. Reproductive system amphidelphic. Vulva located near mid-body (Fig. 1D). Vagina slightly extending anteriorly, connecting with two opposite uteri. Anterior ovary reflexed at excretory pore; posterior ovary reflexed at about two times of body width before anus (Fig. 1C, D). Eggs ovoid, smooth-shelled (Fig. 1E). Tail conical, attenuated, sharply pointed. Male not observed.

Measurements. Female (n = 10): a = 9.8–15.6 (12.8); b = 4.8–7.0 (5.9); c = 6.2–8.0 (7.5); V = 46–57 (51); V’ = 54–66 (59). Total body length 2280–3420 (2864); maximum body width 200–240 (224). Stoma 48–60 (54); head 8–13 (11) long.35–38 (36) wide. Total oesophagus length 454–512 (483);
procorpus 328–396 (365) long; isthmus 30–48 (41) long; basal bulb 63–87 (76) long, 72–92 (82) wide. Nerve ring 208–246 (230) from anterior end; excretory pore 560–830 (704) from anterior end. Vulva

Fig. 1 *Pseudoxyo yunnanensis* gen. et sp. nov. A: Anterior part of body, lateral view. B: Tail, lateral view. C: Reproductive system. D: Whole body, lateral view. E: Egg. Scale bars: A–E = 100 \( \mu \)m

![Fig. 1](image1)

Fig. 2 *Pseudoxyo yunnanensis* gen. et sp. nov. Female. SEM images. A: Anterior part of body, sub-lateral view. B: Enlarged anterior part of spines. C: Cephalic end, subapical view. D: Vulva. E: Posterior end of body, ventral view. F: Enlarged part of posterior end, showing the terminal part of spines. G: Enlarged middle part of body, showing the middle part of spines. H: Enlarged middle part of spines

![Fig. 2](image2)
Discussion: The new genus is similar to the following genera of hystrignathid nematodes by having a spiny cervical cuticle and didelphic reproductive system: Batwanema Morffe & García, 2013; Carlosia Travassos & Kloss, 1957; Chokwenema Morffe & García, 2013; Hystrignathus Leidy, 1850; Lepidonema Cobb, 1898; Parahystrignathus Zhang, Yin, Carreno & Zhang, 2021; Paralepidonema Zhang, Yin & Zhang, 2022; Salesia Travassos & Kloss, 1958; Soaresnema Travassos & Kloss, 1958; Urbanonema Travassos & Kloss, 1958 and Xyo Travassos & Kloss, 1958.

Pseudoxyo gen. nov. can be easily distinguished from Batwanema, Chokwenema, Lepidonema, Paralepidonema and Salesia by having females with the cervical region armed with pointed spines instead of having scale-like projections. Carlosia differs from the new genus in the cervical region by having only two longitudinal rows of spines. Pseudoxyo gen. nov. is different from Soaresnema by having females with a clavate vs. sub-cylindrical procorpus, and by having the first row with 22 spines instead of 16 spines. Pseudoxyo gen. nov. differs from Hystrignathus by having the cervical cuticle bearing alternating vs. opposite rows of spines, by having the first row with 22 spines instead of 16 spines, and by lacking the first cephalic annule. Pseudoxyo gen. nov. is different from Urbanonema and Xyo by having the first row with 22 spines instead of 32 spines, and by lacking the first cephalic annule. In addition, Urbanonema differs from Pseudoxyo gen. nov. by having the stoma with a dilated anterior end.

The new genus is very similar to Parahystrignathus by having females with the cervical region armed with alternating rows of pointed spines, clavate procorpus, and similar cephalic structure, however, it can be distinguished from the latter by having the first row with 22 spines instead of 16 spines.

Sinospinata gen. nov.
Diagnosis: Female body relatively stout. Cervical cuticle bearing irregularly arranged spines, spines originating just behind first cephalic annule, extending to level of excretory pore. Two or three spines clustered together in their roots in some spines. First row of spines with 44 elements. Lateral alae absent. Oral opening rounded, surrounded by a cuticular ring. Head bearing 8 papillae, and a pair of amphids. First cephalic annule larger than head. Oesophagus consisting of a muscular, clavate corpus, short isthmus and basal bulb. Reproductive system amphidelphic. Vulva located near mid-body. Eggs ovoid, smooth-shelled. Tail conical, attenuated, sharply pointed. Male unknown.

Type species: Sinospinata chitwoodi gen. et sp. nov.
Distribution: China.

Etymology: The genus name is from the Latin words “sinae” meaning Chinese and “spin” meaning spicule.

Sinospinata chitwoodi gen. et sp. nov.
Type-host: Ceracupes fronticornis (Westwood)
Type-locality: Bubang Village, Sipsongpanna, Yuan-nan Province, China (21°35′33″N, 101°34′54″E).
Type-material: Holotype female (HBNU-I-2021001); paratypes (HBNU-I-2021002–2021012).
Prevalence: 4.3% (5 infected out of 115 examined).
Intensity: 3–20 (mean 9) specimens.
Site in host: Hindgut.

Representative DNA sequences: One partial 28S and one partial 18S rDNA sequence of the new species are deposited in the GenBank database under the accession numbers ON751940 and ON751934, respectively.

Etymology: The new species is named in honor of Professor B. G. Chitwood, Zoological Division, Bureau of Animal Industry, United States Department of Agriculture, for his contributions to the helostomatid nematodes.

Description. Female body relatively stout. Cervical cuticle bearing irregularly arranged spines, spines originating just behind first cephalic annule, extending to level of excretory pore (Fig. 3A). Two or three spines clustered together in their roots in some spines (Figs. 3C, F; 4D, E). First row of spines with 44 elements. There are a few of small spines sparsely distributed before first row (Figs. 3C, 4A). After fifth row, number of spines gradually decreased. Lateral alae absent. Oral opening rounded, surrounded by a cuticular ring. Head bearing 8 papillae, and a pair of amphids (Fig. 4B, C). First cephalic annule cone-like, truncated, larger than head. Length of stoma is about twice of the first cephalic annule. Anterior end of stoma dilated and spherical (Fig. 3G). Oesophagus consisting of a muscular, clavate procorpus, short
Fig. 3 *Sinospinata chitwoodi* gen. et sp. nov. Female. A: Whole body, lateral view. B: Anterior part of female, lateral view. C: Anterior end of body, lateral view (reconstructed from SEM images); D: Posterior end of body, lateral view. E: Cephalic end, *en face* view; F: spines. Scale bars: A, D = 100 μm; B = 50 μm; C = 40 μm; E = 10 μm; F = 5 μm.
Isthmus and basal bulb (Fig. 3D). Bulb rounded, valve-plate well-developed. Nerve ring encircling corpus at 40% of its length. Excretory pore located just posterior to basal bulb. Intestine simple, its anterior region slightly dilated. Reproductive system amphidelphic.

Vulva located near mid-body. Vagina slightly extending anteriorly, connecting with two opposite uteri. Anterior ovary reflexed at excretory pore, posterior ovary reflexes forward at the mid-region between vulva and anus. Eggs ovoid, smooth-shelled. Tail

Fig. 4 Sinospinata chitwoodi gen. et sp. nov. Female. SEM images. A: Anterior end of body. B: Cephalic end, sub-apical view. C: Cephalic end, en face view, showing the cephalic papillae (cp) and amphids (am). D: Anterior part of female, showing the spines. E: Spines. F: Enlarged part of body near the excretory pore, showing the terminal part of spines. G: Anus.
conical, attenuated, sharply pointed. Male not observed.

**Measurements.** Female (n = 12):  a = 10.2–17.5 (13.3); b = 4.4–6.4 (5.6); c = 5.1–7.7 (6.3); V = 48–58 (52); V’ = 58–67 (62); Total body length 1950–2620 (2257); maximum body width 140–200 (172); Stoma 29–54 (46); head 5–14 (11) long, 29–39 (36) wide; first cephalic annule 18–25 (22) long, 45–53 (50) wide; Total oesophagus length 355–457 (404); procorpus 261–343 (302) long; isthmus 14–34 (29) long; basal bulb 60–80 (73) long; 60–90 (77) wide; Nerve ring 174–213 (194) from anterior end; excretory pore 510–620 (551) from anterior end; Vulva 860–1510 (1078) from anterior end.; Eggs 101–126 (118) long; 43–48 (45) wide; Tail 320–390 (358) long.

**Discussion:** *Sinospinata gen. nov.* is similar to *Carlosia, Hystrignathus, Parahystrignathus, Pseudoxyo gen. nov.*, *Urbanonema* and *Xyo* by having females with the cervical region armed with pointed spines, by having clavate procorpus and didelphic reproductive system. However, it can be easily distinguished from above related genera by having the cervical region of females armed with irregularly arranged spines, two or three spines clustered together in their roots in some spines.

In addition, *Carlosia* differs from the new genus in the cervical region in having only two longitudinal rows of spines. *Sinospinata gen. nov.* differs from *Hystrignathus, Parahystrignathus, Pseud oxyo gen. nov.* and *Xyo* by having the stoma with a dilated vs. a narrow anterior end.

*Sinospinata gen. nov.* resembles *Urbanonema* because both genera share the presence of a dilated anterior end of stoma. However, the new genus can be separated from *Urbanonema* by having the cuticular armed with irregular vs. alternating rows of spines, and by the first row with 44 spines instead of 32 spines.

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**Author contributions** The first author finished the identification of the new species, and prepared all figures. The second author wrote the main manuscript text and finished the revision of all text.

**Declarations**

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All applicable institutional, national and international guidelines for the care and use of animals were followed.

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