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Metacyclops woni n. sp., a New Cyclopoid Species (Copepoda: Cyclopoida: Cyclopidae) from Cambodia

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ABSTRACT

A new cyclopoid species belonging to the genus *Metacyclops* Kiefer, 1927 is described, based upon the specimens from a temporary pool in a forest of Mt. Phnom Domnak Dambouk, situated in the southwestern part of Cambodia. *Metacyclops woni* sp. nov. is characterized by its quite short caudal rami, about 2.5 times longer than wide, with a spinule row on anterior quarter of lateral margin. This new species belongs to the species group with the spine formula 3,4,4,3 of swimming legs 1–4. Among the members of the species group with the combination of characters of 11-segmented antennule and a single apical spine on the second endopodal segment of leg 4, it most resembles *M. deserticus* Mercado-Salas and Suárez-Morales, 2013 from Mexico in carrying an outermost caudal seta slightly longer than the innermost caudal seta and an apical spine on the second endopodal segment of leg 4 slightly shorter than the segment. However, it differs clearly from the present new species by much longer caudal rami with lateral caudal seta at nearly halfway of lateral margin of the ramus and smooth posterior margin of intercoxal sclerites of legs 1–4. This is the first record of the genus *Metacyclops* from Cambodia and the fourth one from Southeast Asia.

Keywords: Asia, description, morphology, taxonomy, temporary pool

INTRODUCTION

Since the year 2009, the National Institute of Biological Resources of Korea (NIBR) has been conducting an international cooperative project of "Korean-Cambodian Joint Field Survey and Biodiversity Inventory Project" with the Forestry Administration of Cambodia. Professor Hyosig Won of Daegu University, Korea has engaged in the project, and he thankfully made a zooplankton collection for us in June, 2010. One of the sampling bottles he gave us included enough specimens for confirmation of a new species of *Metacyclops*.

The genus *Metacyclops* Kiefer, 1927 is widespread in tropical and temperate regions, being most prolific in European, African and South American regions; at present the genus comprises 62 nominal species and subspecies, most of them recorded in different groundwater habitats, such as wells, caves, anchialine habitats (Pesce, 2015). However, in Southeast Asia, despite its potentially rich fauna, only three species have been reported yet: *M. malayicus* Kiefer, 1930 from Sumatra (Kiefer, 1930), *M. pectinatus* Shen and Tai, 1964 from Kwangtung, China (Shen and Tai, 1964) and Malaysia (Lim and Fernando, 1985), and *M. ryukyuensis* Ishida, 1995 from Ryukyu Islands, Japan (Ishida, 1995).

The cyclopoid copepod fauna of Cambodia is very poor, "even when compared to its neighboring countries", and this country remains as "one of the least explored area in Southeast Asia"; the information on the taxonomic studies of cyclopoid copepods is rather scarce and fragmentary in Cambodia (for details of history of cyclopoid taxonomy in Cambodia, see Chaicharoen et al., 2011). Recently, two reliable studies on the cyclopoid taxonomy have been accomplished in Cambodia: Ishida and Tomikawa (2007) reported a single species, *Mesocyclops thermocyclopoides* Harada, 1931 from Lake Tonlé-Sap; Chaicharoen et al. (2011) made a very comprehensive study and reviewed eight species of the genus *Thermocyclops*.

In this study, we describe a new cyclopoid species of the genus *Metacyclops* obtained from Professor Won's Cambodian expedition, following the junior author's latest studies on the stygobitic copepods from limestone caves in Vietnam,

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that is, two new harpacticoid species of *Microarthridion thanhi* and *Nitokra vietnamensis* (Tran and Chang, 2012), and a new *Graeteriella* cyclopoid species (Tran and Chang, 2013). This is the first record of the genus *Metacyclops* from Cambodia and the fourth one in Southeast Asia.

MATERIALS AND METHODS

Samples were taken from a temporary pool (11°13'18.1"N, 103°50'30.6"E; 197 m in altitude) at a secondary forest along the trail to the newly built hydrodam situated in Mt. Phnum Domnak Dambouk, Koh Kong Province, Cambodia.

Copepods were collected with a hand net of 64 μ m mesh aperture, and immediately fixed in the field by adding a few drops of saturated formalin. In the laboratory, the specimens were isolated from the samples under a zoom-stereomicroscope (Olympus SZX 12, Tokyo, Japan) at high magnifications (32–90 ×) with a micropipette, and transferred to about 80% ethanol for long-term preservation.

Methods for dissection, double-coverglass preparation using H-S slides (see Shirayama et al., 1993), drawings and measurements followed those in the latest paper for cyclopoid copepods of the junior author (Chang, 2012).

Type specimens are deposited in the National Institute of Biological Resources (NIBR), Incheon, Korea, and the specimen room of the Department of Biological Science, Daegu University (DB), Korea.

General terminology for the morphological description of the new species follows Huys and Boxshall (1991). Abbreviations are used in the text and figure legends, following those in Tran and Chang (2013): enp-1 to enp-2 or exp-1 to exp-2, the first to second endopodal or exopodal segments of each leg; P1–P6, first to sixth legs.

SYSTEMATIC ACCOUNTS

Order Cyclopoida Burmeister, 1835 Family Cyclopidae Rafinesque, 1815 Subfamily Cyclopinae Rafinesque, 1815 Genus *Metacyclops* Kiefer, 1927

Metacyclops woni sp. nov. (Figs. 1-4)

Type locality. A small temporary pool $(11^{\circ}13'18.1''N, 103^{\circ}50'30.6''E; 197 m in altitude; about <math>3 m \times 2 m$ wide, not more than 30 cm in depth) at a secondary forest near a hydrodam in Mt. Phnum Domnak Dambouk, Koh Kong Province, southwestern Cambodia.

Material examined. Holotype ♀ (NIBRIV0000324248), al-

lotype σ^{3} (NIBRIV0000324249), both undissected, ethanolpreserved; from the type locality, 24 Jun 2010 (*leg*. H. Won). Paratypes: $2 \Leftrightarrow \Diamond$ (DB20053, 20054), σ^{3} (DB20055), dissected and mounted on slides in glycerol; from the type locality. **Description. Female:** Body (Fig. 1A) dark gray in ethanol; habitus of holotype 693 µm long (ranging 684–693 µm, mean 687 µm, n=3), length/width ratio 2.62. Prosome-urosome boundary distinctly defined between fourth and fifth pedigerous somites; prosome/urosome length ratio about 1.9; cephalothorax 2.34 times as wide as genital double-somite. Dorsal surfaces of prosomites and urosomites ornamented with innumerable pits and minute hairs.

Prosome comprising cephalothorax incorporating first pedigerous somite and 3 free pedigerous somites; elongate, oval, slightly protruding anteriorly; greatest width at posterior end of cephalothorax. Cephalothorax nearly as long as wide, or slightly longer than wide (length/width ratio 1.05), representing about 40.5% of total body length; posterior lateral angle not strongly produced; 2 pairs of sensilla present dorsally on posterior margin. Nauplius eye not discernible. Pedigerous somites 2–4 with narrow and elongate posterior lateral angle. Posterior margins of prosomites nearly smooth, except for second pedigerous somite with minute undulating fringe; paired minute sensilla situated middorsally on posterior margin of fourth pedigerous somite.

Urosomites ornamented with irregularly crenate hyaline fringe along posterior margin both dorsally and ventrally, except for nearly smooth one of fifth pedigerous somite and anal somite with spinule row. On fifth pedigerous somite, lateral basal seta of P5 issuing subdorsally; paired sensilla present middorsally. Genital double-somite slightly wider than long (about 1.1 times as wide as long); anterior third swollen laterally, with dorsolateral cuticular wrinkles bearing one seta and one small cuticular projection, representing P6; posterior margin with serrated hyaline fringe both dorsally and ventrally. Seminal receptacle showing typical shape of Metacyclops, with narrow anterior part, posterior part much larger than anterior part and expanding along the somite. Third and fourth urosomites much narrower than genital double-somite; with posterior margin heavily serrated. Anal somite (Fig. 1B) much wider than long, length/width ratio 0.42; ornamented with 2 sensilla in middle of dorsal surface and transverse row of 9-12 minute spinules along posterior margin. Anal operculum convex with round and smooth posterior margin; slightly extending beyond posterior margin of anal somite in lateral view; anal operculum 0.53 times as wide as total anal somite.

Caudal rami (Fig. 1B) cylindrical, nearly parallel, stout, about 2.5 times as long as wide (ranging 2.45-2.53, n=3); greatest width at base of caudal ramus, slightly narrowing posteriorly. Coarse pits scattered on dorsal surface; 4 trans-



Fig. 1. *Metacyclops woni*, female (holotype): A, Habitus, dorsal; B, Anal somite and caudal rami, dorsal; C, P5. Scale bars=100 µm (A), 50 µm (B, C).

verse rows of pits on ventral surface. Inner margins smooth; outer margin interrupted by 3 spinule groups, comprising 3 spinules on anterior third of lateral margin, 2–3 spinules at base of lateral caudal seta and 7–8 spinules at base of outer caudal seta. Lateral seta II short, plumose, inserted slightly dorsally near anterior two-thirds (65.5%) of caudal ramus. Outer seta III homogeneously plumose (not spiniform nor pinnate). Terminal setae IV and V well developed, plumose, with breaking plane; outer terminal seta IV about 2/3 times as long as inner terminal seta V, about 6 times as long as caudal ramus. Inner seta VI plumose, 0.84 times as long as caudal rami, about 0.83 times as long as outer seta III. Dorsal seta VII short, plumose distally, about 0.6 times as long as caudal ramus, issuing from inner distal corner of caudal ramus.

Antennule (Figs. 1A, 2A) short, not reaching to halfway of cephalothorax, 11-segmented; numerous pits scattered on surface. Segments 1, 3, 7 and 8 stout and long; segments 2, 4, 5 and 9 relatively short; relative length ratio of antennular segments 11.7: 2.7: 4.7: 2.3: 1.0: 3.3: 6.2: 5.7: 3.3: 4.0: 5.0. Segments 1, 3, 7 and segments 9–11 bearing both plumose and naked setae. Segment 1 armed with 1 oblique spinule row anteroproximally. Segment 5 with short, robust, spiniform seta anterodistally. Segment 8 with 1 long, slender aesthetasc, slightly extending over middle of next segment. Segment 11 with 1 long aesthetasc apically. Setal formula: 1-[8], 2-[4], 3-[6], 4-[2], 5-[1+1 spine], 6-[2], 7-[3], 8-[2+1 aesthetasc].

Antenna (Fig. 2B) 4-segmented, comprising coxobasis and 3-segmented endopod. Coxobasis about twice as long as wide, about 15–16 spinules longitudinally along lateral margin; with 1 long pinnate seta outer distally, representing exopod. Enp-1 about 1.4 times as long as wide; with 1 inner naked seta; outer margin swollen, with 1 spinule row; enp-2 about 1.4 times as long as wide, with spinule row outer distally, armed with 6 naked setae along inner margin, and 1 stout geniculate and 1 naked setae distally; enp-3 about 2.4 times as long as wide, bearing 7 geniculate setae apically, armed with spinule row along outer margin.

Mandible (Fig. 2C) with well developed coxal gnathobase; cutting edge armed with 10–11 strong teeth along distal margin, including 2 bidentate teeth, flanking 1 row of 5 sharp spinules and 1 outer subapical pinnate seta. Mandibular palp reduced to small protuberance, bearing 2 long plumose and 1 small naked setae distally or subdistally.

Maxillule (Fig. 2D) comprising well developed praecoxa and 2-segmented palp. Praecoxa bearing 7 elements along inner face, consisting of 3 small and 1 long naked setae, 1 strong pinnate seta, 1 spine and 1 posteriormost small spinous projection. Praecoxal arthrite armed with 4 strong dentate spines inner distally, consisting of 3 dentate spines basally fused together and 1 separated posteriormost spinous spine; 7 elements along inner margin, proximal pinnate seta longest, others naked. Palp consisting of coxobasis and endopod; coxobasis armed with 3 setae distally on basal endite (2 slender pinnate setae and 1 strong spinous seta); endopod 1-segmented with 2 apical and 1 inner setae; exopod represented by 1 long pinnate seta on outer margin of coxobasis.

Maxilla (Fig. 2E) 5-segmented (praecoxa and coxa partly fused in frontal view). Praecoxa with small proximal endite bearing 1 pinnate and 1 plumose setae apically; distal endite reduced to rounded protuberance, unarmed. Coxa with 2 coxal endites; proximal endite represented by 1 naked seta; distal endite highly mobile, armed with 1 apical spinous element with 3–4 secondary setules along its posterior margin and 1 shorter, unipinnate seta. Basis forming a claw with 5 strong spinules on posterior margin, flanking 1 slender, naked seta basally; 1 curved, pectinate, spinous element inserted at base of distal claw, slightly shorter than claw. Enp-1 with 2 strong, spinous seta on inner margin. Enp-2 small, narrowing distally, with 1 unipectinate apical and 2 naked subapical setae.

Maxilliped (Fig. 2F) 4-segmented, composed of syncoxa, basis and 2-segmented endopod. Syncoxal endite with 2 pinnate and 1 naked setae inner distally; frontal face ornamented with 5–6 oblique rows of minute spinules. Basis bearing 2 pinnate setae inner distally, with curved array of spinules near inner proximal margin and 1 group of sharp spinules at outer distal corner. Enp-1 armed with 1 long and stout seta, pinnate proximally and unipectinate distally. Enp-2 small, with 3 setae (1 lateral and 2 apical).

P1-P4 (Fig. 3A-D), both rami 2-segmented, with pits scattered irregularly on surfaces of enp-2 and exp-2. Setal formula (setal arrangement of exp-2 of P1-P4) 5,5,5,5. Spine formula (arrangement of spines on exp-2 of P1-P4) 3,4,4,3. Seta/spine armature of P1-P4 as follows:

Coxa	Basis	Exopod	Endopod
0-1	1-1	I-1; III,1,4	0-1; 1,I,4
0-1	1-0	I-1; III,I,5	0-1; 1,I,5
0-1	1-0	I-1; III,I,5	0-1; 1,I,5
0-1	1-0	I-0; III,1,4	0-1; 1,I,3
	Coxa 0-1 0-1 0-1 0-1	Coxa Basis 0-1 1-1 0-1 1-0 0-1 1-0 0-1 1-0	Coxa Basis Exopod 0-1 1-1 I-1; III,1,4 0-1 1-0 I-1; III,1,5 0-1 1-0 I-1; III,1,5 0-1 1-0 I-1; III,1,4

P1 (Fig. 3A), intercoxal sclerite with paired lateral lobes produced posteriorly, with 5–6 spinules on inner distal margin; both frontal and caudal surfaces smooth without transverse spinule or setule row. Coxa with transverse row of 7–8 spinules on outer posterior margin; 1 plumose seta present inner distally. Basis armed with 1 pinnate seta distomedially, its tip nearly reaching to middle of enp-2; lateral seta well developed, plumose. Inner seta present on both enp-1 and exp-1; enp-2 bearing 4 plumose setae along inner margin, not modified to pinnate or spinous form.



Fig. 2. *Metacyclops woni*, female (paratype, DB20053): A, Antennule; B, Antenna; C, Mandible; D, Maxillule; E, Maxilla; F, maxilliped. Scale bars = 50 µm (A-F).



Fig. 3. Metacyclops woni, female (paratype, DB20053): A–D, P1–P4. Scale bar=50 μ m (A–D).

P2–P3 (Fig. 3B, C), paired lateral lobes of intercoxal sclerite with 5–6 spinules along posterior margin; lateral seta on basis very short, plumose distally; inner seta present on both enp-1 and exp-1; enp-2 bearing 5 plumose setae along inner margin, not modified to pinnate or spinous form.

P4 (Fig. 3D), intercoxal sclerite with paired lateral lobes weakly produced posteriorly, with 5–6 spinules on inner distal margin; caudal surface with undulating row of 18–20 spinules along posterior margin. Coxa with transverse row of 14–16 sharp spinules along posterior margin; 1 inner distal seta well developed, plumose. Basis, lateral seta very short, plumose. Enp-1 with 1 inner distal seta; exp-1 lacking inner seta. P4 enp-2 elongate, 1.62 times as long as wide; armed with single spine apically, slightly shorter than enp-2 (about 0.8 times as long as enp-2); outer distal seta far exceeding beyond tip of apical spine.

P5 (Fig. 1C), protopodal segment completely incorporated into fifth pedigerous somite, with outer basal seta, situated rather dorsolaterally and flanking 6–7 spinules at its base; free exopodal segment very small, subquadrate, about 1.3 times as long as wide, bearing 1 spine inner subapically and 1 outer apical plumose seta; spine slightly shorter than exopod; outer apical seta about 5.6 times longer than inner spine. **Male:** Body (Fig. 4A) 641 µm in length (ranging 632–641 µm, n=2), shorter and slenderer than females (length/width ratio 3.28), greatest width at posterior end of cephalothorax; length ratio of prosome to urosome 1.65. Prosome elongateoval. Cephalothorax slightly longer than wide, 0.3 times as long as body.

Genital somite (Fig. 4D) slightly swollen laterally, 1.42 times wider than long; genital pore located midventrally, large, transversely elongate. Paired genital opercula well developed, ornamented with numerous pits; distolateral corner produced to small protuberance, armed with 1 stout inner spine and 1 short plumose seta, representing P6.

Caudal rami (Fig. 4B) nearly parallel, slightly shorter than those of female, about 2.2 times as long as wide. Coarse pits scattered on dorsal surface; 4 transverse rows of pits on ventral surface. Longitudinal cuticular ridge running along lateral margin from just inside lateral seta II to basis of outer seta III. Inner margins smooth; 1 transverse spinule row situated at anterior quarter of lateral margin. Lateral seta II plumose, inserted slightly dorsally near anterior two-thirds (63%) of caudal ramus. Outer seta III plumose, about 1.2 times as long as ramus. Terminal setae IV and V well developed, plumose, with breaking plane. Inner seta VI plumose, slightly longer than ramus. Dorsal seta VII short, about 0.87 times as long as ramus.

Antennule (Fig. 4C) 16-segmented, relatively longer than in female; dorsal surface ornamented with numerous pits; geniculate between segments 8–10, and between segments 14 and 15; segments 4, 5, 6 and 9 relatively short; segments 14, 15 and 16 elongate; segment 11 large and swollen; segment 1 bearing 3 long aesthetascs, segments 4, 9, 13 and 16 each bearing 1 slender aesthetasc; segment 9 armed with strong cuspid-like spine anterodistally; segments 11 and 12 each with 1 pinnate seta anteriorly. Setal formula: 1-[8+3 aesthetascs], 2-[4], 3-[2], 4-[2+1 aesthetasc], 5-[1], 6-[2], 7-[2], 8-[2], 9-[1+1 aesthetasc], 10-[2], 11-[2], 12-[2], 13-[1+1 aesthetasc], 14-[0], 15-[1], 16-[12+1 aesthetasc].

Other characters of mouthparts and P1-P5 nearly same as those of female.

Etymology. The specific name *woni* is dedicated to the collector, Professor Hyosig Won (Daegu University, Korea).

Remarks. This new species is manifestly assigned as a member of the genus *Metacyclops* Kiefer, 1927 based on the combination of characters: 2-segmented exopod of P1–P4 with 1,II (or I+1, or I), 3 armature on P4 enp-2, and free segment of P5 exopod armed with 1 short spine and 1 long seta implanted close together, which diverges from its related genera *Apocyclops* Lindberg, 1942, *Muscocyclops* Kiefer, 1937, *Speocyclops* Kiefer, 1937, *Cochlacocyclops* Kiefer, 1955, *Goniocyclops* Kiefer, 1955, and *Hesperocyclops* Herbst, 1984 (Dussart and Defaye, 1995; Boxshall and Halsey, 2004; Karanovic et al., 2011; Pesce, 2015).

Members of the genus are classified into four groups, based on the spine formula, that is, the spine armature on the terminal exopodal segments of P1-P4 (see Herbst, 1988; Reid, 1991; Karanovic, 2004). Among the groups, the absolute majority of members (54 of 62 species recognized in this genus) belong to the group with 3,4,4,3 formula, as well as the new species does.

The new species is distinguishable from the most species of the species group with spine formula 3,4,4,3 by the character combination of 11-segmented antennule and a single apical spine on P4 enp-2, sharing the characters with 12 species of the group: *M. communis* Lindberg, 1938, *M. curtispinosus* Dussart, 1984, *M. denticulatus* Dussart and Frutos, 1986, *M. deserticus* Mercado-Salas and Suárez-Morales, 2013, *M. grandispinifer* Lindberg, 1940, *M. hannensis* Defaye, 1992, *M. lusitanus* Lindberg, 1961, *M. malayicus*, Kiefer, 1930, *M. minutus* (Claus, 1863), *M. prolatus* Kiefer, 1935, *M. pectiniatus* Shen and Tai, 1964, and *M. subdolus* (Kiefer, 1938).

Considering the *Metacyclops* species generally have relatively elongate caudal rami (ranging from about 3 to 5 times as long as wide), with few exceptions such as in *M. malayicus* (about 2 times) and *M. ryukyuensis* Ishida, 1995 (2.3 times), this new species is characterized by its quite short caudal rami, about 2.5 times longer than wide. Moreover, this new species is very characteristic in bearing a spinule row on the proximal quarter of lateral margin of caudal rami, Jimin Lee, Cheon Young Chang



Fig. 4. *Metacyclops woni*, male (paratype, DB20055): A, Habitus, dorsal; B, Anal somite and caudal rami, dorsal; C, Antennule; D, P5, P6 and genital somite, ventral. Scale bars = $100 \,\mu m$ (A), $50 \,\mu m$ (B-D).

which brings to mind the empty position of caudal seta I. As far as we are aware, this structure has not been known yet in this genus.

Among the members of the species group with the combination of characters of 11-segmented antennule and a single apical spine on P4 enp-2, M. woni sp. nov. most resembles M. deserticus from a desert in northern Mexico in carrying a caudal seta III slightly longer than seta VI and an apical spine on P4 enp-2 slightly shorter than the segment (see Mercado-Salas et al., 2013). However, M. deserticus differs clearly from the new species, beside its elongate caudal rami (3.5-3.8 times as long as wide), by the shape of caudal rami (caudal seta II issuing from nearly halfway the lateral margin of ramus, against from posterior third of lateral margin in the new species) and smooth posterior margin of intercoxal sclerites of P1-P4. Metacyclops woni sp. nov. is also similar to M. denticulatus from Argentine in sharing cirrated posterior margins of urosomites and caudal seta III longer than seta VI (see Dussart and Frutos, 1986). However, it is distinguished from the new species by the apical spine on P4 enp-2 slightly longer than enp-2 (against slightly shorter than enp-2 in M. woni sp. nov.) and the dorsal caudal seta VII much longer than seta VI (against much shorter in M. woni sp. nov.).

In Asia, eight species of Metacyclops are currently recorded: M. malayicus from Sumatra, M. margaretae Lindberg, 1938 and M. communis from India (Lindberg, 1938), M. amoenus (Mann, 1940) and M. grandispinifer from Turkey (Mann, 1940; Lindberg, 1940), M. pectiniatus from Kwangtung, China (Shen and Tai, 1964) and from Malaysia (Fernando and Ponyi, 1981, as M. minutus; Lim and Fernando, 1985), M. minutus from Inner Mongolia, Pakistan and Syria (Tai and Chen, 1979), and M. ryukyuensis from Ryukyu Islands, Japan (Ishida, 1995). Among them, only M. margaretae displays the spine formula 3,3,3,3, and others do 3,4,4,3. The present new species is similar to *M. pectiniatus* in having the not-elongate caudal rami (less than 3 times as long as wide) with long caudal seta III (longer than setae VI and VII), however, M. pectiniatus is discernible from the new species by transverse rows of spinules on ventral surface of genital double somite, smooth posterior margins of urosomites and an elongate apical spine on P4 enp-2 (longer than enp-2, against slightly shorter in M. woni sp. nov.). Metacyclops minutus, which is distributed over wide area in Eurasia and Africa, evidently differs from the new species by elongate caudal rami (about 4 times), remarkably long apical spine on P4 enp-2 (more than 1.5 times as long as enp-2), lack of medial basipodal seta of P1 and smooth posterior margins of urosomites (Tai and Chen, 1979). Metacyclops malayicus is easily distinguished from the new species by much shorter caudal rami (about 2 times) armed with seta VI longer than seta III. *Metacyclops ryukyuensis*, the only Japanese species, has very short caudal rami (2.3 times) similar to those of the new species, but differs from it by 12-segmented antennules and the caudal seta VI much longer than seta III (against vice versa in *M. woni* sp. nov.).

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