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DESCRIPTION OF A NEW SPECIES OF THE GENUS *Hyperaxis* Germminger & Harold, 1874 (Coleoptera: Chrysomelidae: Eumolpinae) FROM VIETNAM WITH AN UPDATE KEY TO SPECIES

Dinh Thi Nguyen[1,2,*]

[1]Institute of Ecology and Biological Resources, VAST, Vietnam
[2]Graduate University of Science and Technology, VAST, Vietnam

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ABSTRACT

A new species *Hyperaxis phuquocnia* sp. nov. is described and illustrated from Phu Quoc National Park, Kien Giang province, southern Vietnam based on female specimens. Color photos of habitats and genitalia, and DNA Barcoding data of the new species are presented. An updated key to all species of the genus *Hyperaxis* in Vietnam is provided.

Keywords: Asia, biodiversity, Barcoding, islands, taxonomy.

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**Corresponding author email*: ntdinh.iebr@gmail.com

INTRODUCTION

The genus *Hyperaxis* Germminger & Harold, 1874 (*Metaxis* Baly, 1863, nec Chaudoir, 1850) belongs to the subfamily Eumolpinae, erected to accommodate the type species *Hyperaxis sellata* (Baly, 1863) described in Borneo (Germminger & Harold, 1874). *Hyperaxis* distributes in the Indo-Malayan region and is identified by the following characteristics: the anterior margin of pro-episterna is straight or concave; claws are bifid; the head has no sulcus above the eye; the dorsum is covered with scales and intermixed on the elytra with short rigid erect setae; the prothorax is much broader than long and lateral sides are not marginate and serrate; anterior and posterior femora are thickened, the middle femur is slender, each femur has a large tooth (Baly, 1863; Jacoby, 1908; Kimoto & Gressitt, 1982). Subsequently, Moseyko & Medvedev (2017) found that the coloration and pattern of scaly vestiture and waxy blooms are too variable to be used for species identification, while the characters most important for identification including the shape of the posterior femur, aedeagus, spermatheca, pygidium and the 5^{th} ventrite in both sexes.

The genus *Hyperaxis* is closely related to two genera *Hemiplatys* Baly, 1863 and *Demotina* Baly, 1863 (Baly, 1863; Jacoby, 1908; Kimoto & Gressitt, 1982) but *Hyperaxis* can be distinguished from these genera by the following characteristics: *Hyperaxis* has an oblong body, subcylindrical, clothed above with regular scales, intermixed on the elytra with short rigid erect setae, whereas *Hemiplatys* and *Demotina* do not have short rigid erect hair on the elytra. Kimoto & Gressitt (1982) distinguished them by the size of the femora: in the genus *Hyperaxis*, a thickened anterior and posterior femur, intermediate femur slenderer; posterior femur, in some cases the anterior femur, each with a large tooth while in the genera *Hemilatys* and *Demotina*, anterior and posterior femora not thicker than the intermediate femur and posterior femora with or without minute teeth.

Currently, 30 species of the genus *Hyperaxis* have been recorded from the Indo-Malayan region (Baly, 1863; Medvedev, 2001; Moseyko & Medvedev, 2017; Jacoby, 1904, 1908; Chujo, 1956) with 11 among (one of them with two subspecies) have been recorded in mainland Vietnam (Kimoto & Gressitt, 1982; Moseyko & Medvedev, 2017).

In the present study, a new species *Hyperaxis phuquocnia* **sp. nov**. from Phu Quoc National Park, Kien Giang province, southern Vietnam is described, and update a key to all species of the genus *Hyperaxis* in Vietnam.

MATERIALS AND METHODS

Collecting samples: The specimens were collected by the beating method in a tree canopy in the tropical forest in Phu Quoc National Park, Kien Giang province, southern Vietnam, and transferred immediately to vials containing 96% ethanol.

Photographs of the new species were prepared using a Nikon SMZ800N stereomicroscope and a Nikon Ds - Fi3 camera and processed with NIS - Element imaging software. Images of the same objects in different focal planes were combined using the Helicon Focus 7 software.

DNA was extracted from the whole three identified specimens using the QIAamp DNA Investigator (QIAGEN) kit according to the manufacturer's protocol. Primers LepF1 (forward direction) (5'-ATTCAACCAAT CATAAAGATATTGG-3') and LepR1 (Reverse direction) (5'-TAAACTTCTGGAT GTCCAAAAAATCA-3') (Hebert et al. 2004) were used to amplify 658 bp of the *COI* fragment. Each PCR reaction mixture contained 2.5 µL of 10x reaction buffer (Evrogen, Russia), 0.5 µL of 10 mM dNTPs, 0.5 µL of 10 µM forward primer, 0.5 µL of 10 µM reverse primer, 1 µL of 25 mM Mg^{2+}, 2 µL of template DNA, 0.2 µl of thermostable Taq DNA polymerase (Evrogen, Russia), and 17.8 µL deionized water. The PCR protocol is as follows: initial denaturation at 94 ^{o}C for 3 min; 35 cycles of denaturation at 94 ^{o}C for

30 s, annealing at 42 ^{o}C for 40 s, elongation at 72 ^{o}C for 60 s; and final elongation at 72 ^{o}C for 5 min. The amplicons were checked by 1.5% agarose gel electrophoresis and were sequenced in both directions using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City CA, USA) with the same PCR primers. Specimens after DNA extraction were mounted dry and labelled with a voucher number for future reference at the Institute of Ecology and Biological Resources (IEBR).

Forward and reverse Sanger sequences were assembled in a consensus sequence (Geneious Prime 2019.0.4) and then uploaded to GenBank and BOLD for use in future studies.

RESULTS

Hyperaxis phuquocnica sp. nov. (Figs. 1–4)

Material examined. Holotype ♀ (IEBR), "VIETNAM, KIEN GIANG/Phu Quoc National Park, 10.36534 N 103.99071E/33 m, 27.XI.2019, Leg. N.T.Dinh and T.D.Cuong". Paratypes: 1 ♀ (IEBR) "VIETNAM, KIEN GIANG/Phu Quoc National Park, 10.36534 N 103.99071E/33 m, 7.VII.2019, Leg. N.T.Dinh and T.D.Cuong". 1 ♀ (IEBR) "VIETNAM, KIEN GIANG/Phu Quoc National Park, 10.33572 N 104.0445E/45 m, 30.XI.2019, leg. N.T.Dinh". Each type specimen was provided with one additional printed label: "HOLOTYPE (or PARATYPE)/*Hyperaxis/phuquocnica*/Nguyen [red label]".

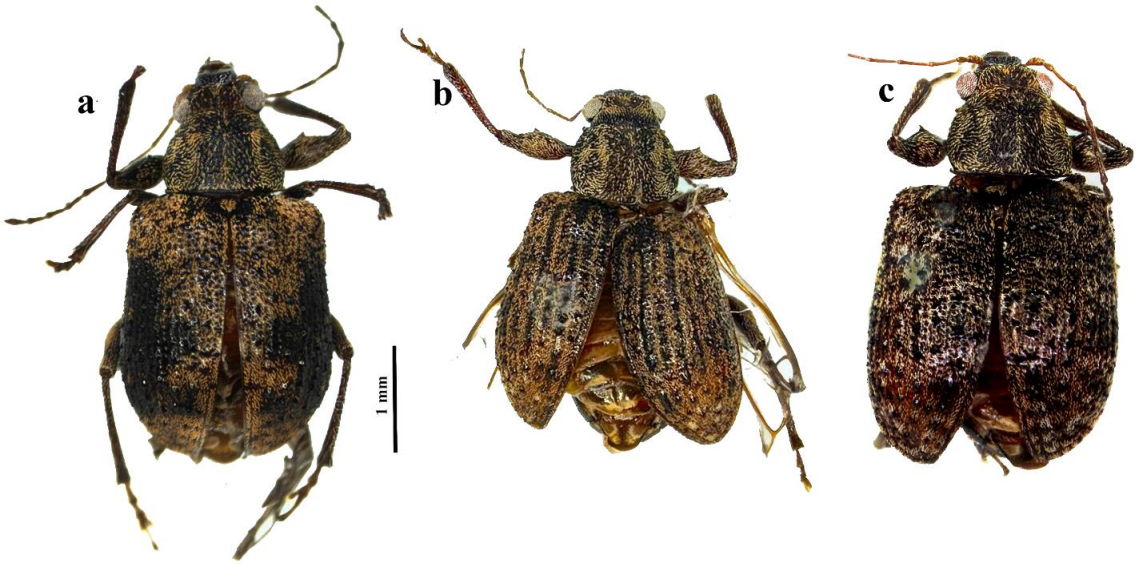

Figure 1. Habitus of *Hyperaxis phuquocnica* **sp. nov**.: (a, b) paratype; (c) holotype

Diagnosis: Coloration. Body reddish-brown; head, pronotum covered with alternate orange and dark scales; elytra with the base of the elytral hair emerging clearly as a spine, disc of elytra covered with bicolored densely punctate and covered with orange, white and dark scales. Scutellum sub-pentagonal narrowed posteriorly, round at the apex, and covered with an orange scale (Figs. 1a–1c). Legs robust, red-brown with a large tooth. Ventral reddish-brown, the pygidium without longitudinal rib, 5th segment densely covered with a long lanugo, lateral margins with excavations of hind angles in the near posterior margin, posterior margin with small denticles and long lanugo.

Description. Measurements. Females: 3.45–3.66 mm.

Head (Fig. 2a) densely punctate and covered with the orange scale on the vertex and dark scale near the anterior base of the

pronotum. Fronto-clypeus trapeziform, clearly punctate, transverse, separate from frons. The inter-antenna area is concave triangle and covered with orange scales; after, this area to the vertex is an upside-down heart shape not covered with scales. Eyes medium-sized, not emarginated, prominent. Antennae dark brown with first and second antennomeres brown, nearly 3/4 as long as the body. The first segment was robust, somewhat club-shape, the second segment short, from segments 3 to 11 slender, the ratio of the antennomeres lengths 100: 60: 140: 150: 150: 150: 150: 120: 120: 120: 120 (Fig. 2b).

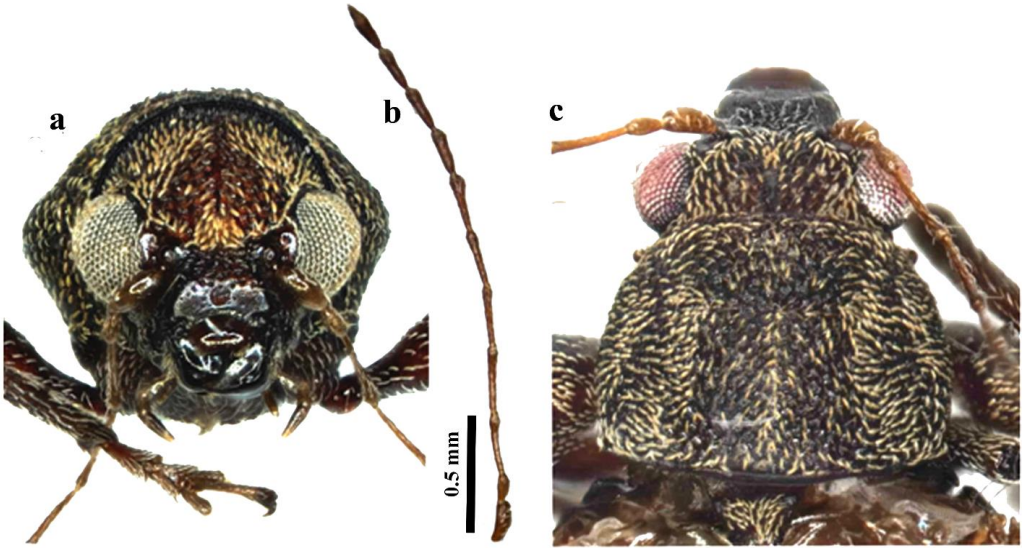

Figure 2. Details of *Hyperaxis phuquocnica* **sp. nov.**: (a) head; (b) antenna; (c) pronotum

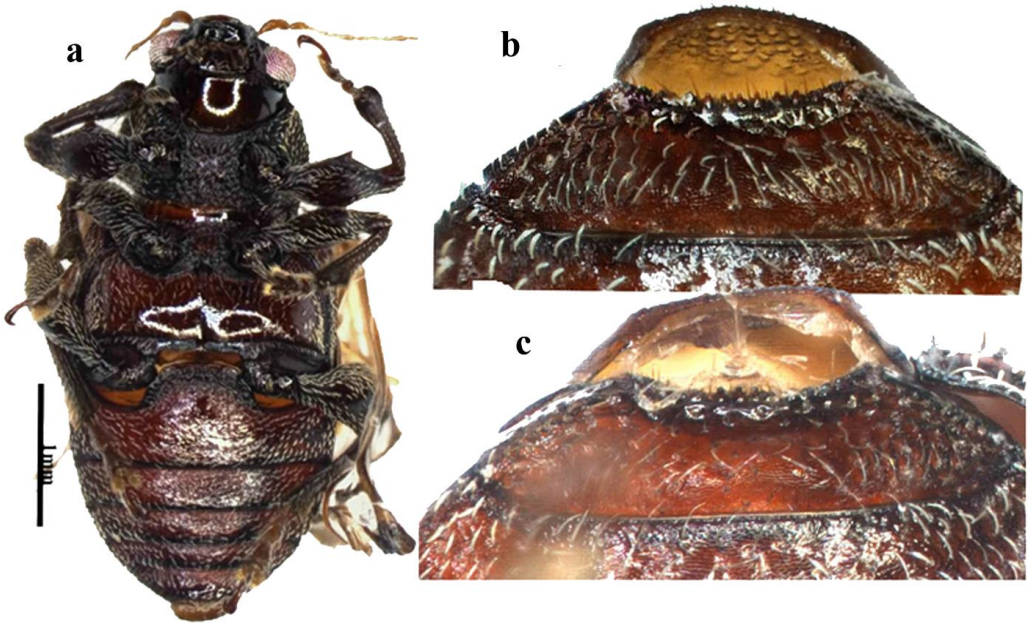

Figure 3. Details of *Hyperaxis phuquocnica* **sp. nov**.: (a) ventral view; (b) 5th ventrite of paratype; (c) 5th ventrite of holotype

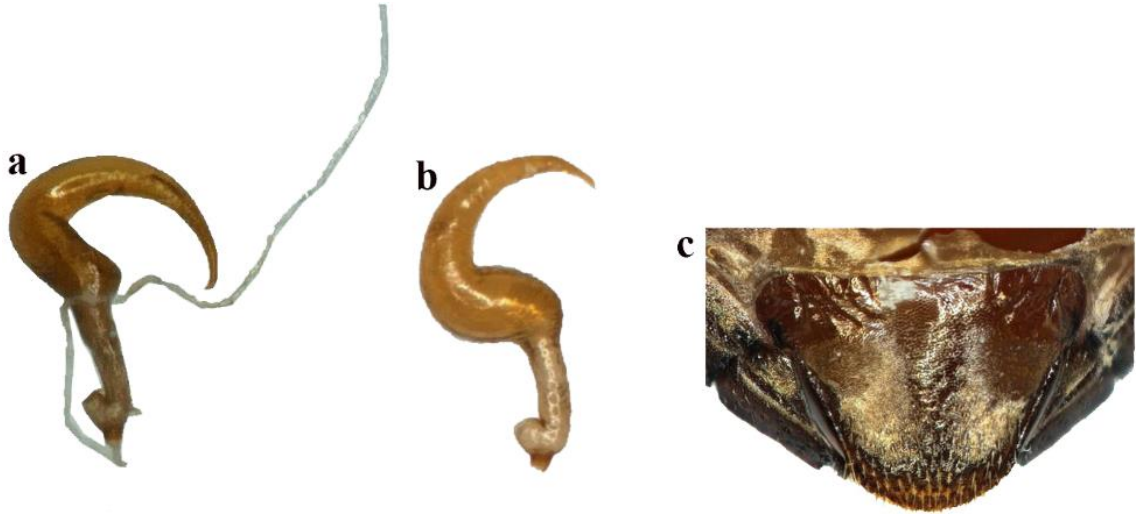

Figure 4. Details of *Hyperaxis phuquocnica* **sp. nov**.: (a) spermatheca of holotype; (b) spermatheca of paratype; (c) pygidium

Pronotum (Fig. 2c) 1.34–1.43 times as wide as long, with sides slightly curved almost subparallel, widest in the middle. Surface densely punctate and densely covered with alternate orange and dark scales. Prosternum between coxae longer than wide, the surface of pro-mesosternum covered with dense lanugo; metasternum covered with sparse lanugo in the middle, two sides covered with alternate orange and white scales. Mesepisternum, mesepimeron, metepisternum and lateral part of metathorax densly covered with alternate white and orange scalesScutellum: subpentagonal, narrowed posteriorly, round at the apex, and covered with an orange scale.

Elytra (Figs. 1a–1c) 1.63–1.68 times as wide as pronotum, slightly narrowed behind humeri and rounded at the apex. The dorsum is strongly convex, with humeral callus, well-developed, basal prominence, and basal impression. Elytra is covered by erect hair with the base of the setae emerging clearly as the spines. Elytra is densely punctate; punctuation is arranged in 11 rows, slightly confused in the center of elytra. Interstices between rows covered by scales and pattern of scales varied from (1) Disc of elytra with the base of the hair emerging clearly as spines but not arranged in lines, the elytra covered with dark and orange scales, partly arranged in stripes, the orange poculiform strip, and alternate white scales are scattered (2) the base of the hair emerging clearly as the spine and arranged in 8 rows from the suture and humeral callus covered with dark scale; interstices between rows covered with orange scale; apex covered scattered white scale accepting around the scutellum covered dark scale.

Legs (Fig. 3a) robust, red-brown with a large tooth, the middle leg shorter and smaller than the anterior and posterior legs. The surface of the abdomen of the femora was covered with white scale, the surface of the dorsal femora covered with orange-white; tibias covered with alternate orange and white scales.

Abdomen (Figs. 3a–3c). 1^{st}–4^{th} abdominal segments are covered with scales smaller than those on the dorsum, denser on the two sides, and thin in the middle. The fifth segment is densely covered with a long lanugo, with two excavations of hind angles on the outer side of the posterior margin, a posterior margin with three or more three small denticles, and long lanugo between the excavations.

Female genitalia: Spermatheca falciform with the proximal part of the spermathecal capsule is vertical and as long as the distal part of the spermathecal capsule (Figs. 4a–4b). The pygidium without longitudinal rib (Fig. 4c).

Etymology

Phuquocnica, a noun in the apposition case, is derived from the type locality of the new species, Phu Quoc National Park.

Remarks

Hyperaxis phuquocnica **sp. nov.** can be separated from other *Hyperaxis* species by the following characters: the basal part of elytral erect setae of the new species is hardened like spine, which is not found in other *Hyperaxis* species. The fifth abdominal segment and pygidium are also key characters for distinguish this new species from its congeners (Figs. 1, 3, 4).

The fifth segment of *Hyperaxis phuquocnica* **sp. nov.** has two excavations on hind angles on the outer side of the posterior margin, which has at least three small denticles (Figs. 3a–b). *Hyperaxis feae*, *Hyperaxis pallidipes*, *Hyperaxis buonloica buonloica*, and *Hyperaxis buonloica darlaki* also have two excavations of hind angles on the outer side of the posterior margin of the fifth segment, but they are different from *Hyperaxis phuquocnica* **sp. nov.** in the absence of small denticles on the posterior margin of the fifth segment. The presence of small denticles on the posterior margin of the fifth segment of *Hyperaxis phuquocnica* **sp. nov.** is similar to that of *Hyperaxis dentifemur*, but the posterior margin of the fifth segment of the latter has no excavation.

Regarding the pygidium, *Hyperaxis phuquocnica* **sp. nov**. has no longitudinal rib on it (Fig. 4c), while the species mentioned above have a longitudinal rib. The longitudinal rib is also absent in several the species, such as *Hyperaxis nigrita*, *Hyperaxis harmandi*, *Hyperaxis longipilosa*, *Hyperaxis sonlangi*, *Hyperaxis phanrangi*, and *Hyperaxis yaosanica*, but unlike *Hyperaxis phuquocnica* **sp. nov**., these species have neither excavation nor small denticles on the posterior margin of the fifth segment.

Distribution

Vietnam (Phu Quoc National Park, Kien Giang province, southern Vietnam).

DNA Barcoding data

Hyperaxis phuquocnica **sp. nov**. is a polymorphic species and only female specimens have been obtained, so this DNA Barcoding data is complete for the description of this species. Three DNA sequences of the 658 bp fragment of the COI gene of *Hyperaxis phuquocnica* **sp. nov.** obtained were uploaded in BOLD and GENBANK; three accession numbers in Genbank: MW429279–MW429281; the sequences were assigned in BOLD with the BIN ID: BOLD: AEH1987 with average distance is 0.43% and the maximum distance is 0.65% (calculate in BOLD).

Key to species of the genus *Hyperaxis* of Vietnam
(The key based on Moseyko & Medvedev, 2017)

1. 5th ventrite with small denticles ……...……...……...…….2

- 5th ventrite without small denticle ...…….4

2. 5th ventrite with excavations of hind angles, pygidium without longitudinal rib spermatheca as Figs. 4a–4b. Body length: females 3.45–3.66 mm…*H. phuquocnica* **sp. nov**.

- 5th ventrite without excavations of hind angles…..…….……..….3

3. Pygidium with longitudinal rib, male 4.1 mm.……...…… ……..*H. dentifemur* Moseyko & Medvedev, 2017

- Pygidium without longitudinal rib, spermatheca as Fig. 5a. Body length: males 2.9–3.1 mm, females 3.9–4.2 mm …….……. *H. nigrita* (Eroshkina, 1992)

4. 5th ventrite with excavations of hind angles, pygidium with thin longitudinal or some females without thin longitudinal rib…….……..……..…….5

- 5th ventrite without excavation of hind angles, pygidium without longitudinal rib…...….8

5. Pygidium always with thin longitudinal rib.…….…..…….……6

- Pygidium without thin longitudinal ribs in some females. Coloration contrasting, body blackish with white or yellowish and blackish scales, forming a pattern; legs reddish with dark apices of tibiae; spermatheca as in Fig. 5l. Body length: males 2.8–3.2 mm, females 3.6–4.2 mm ……. *H. feae* Jacoby, 1904

6. Excavation of the 5th ventrite small. Scales on elytra long, about 3.5 times as long as wide. Body length: males 3.2–3.4 mm, females 3.8–4.2 mm...……. *H. pallidipes* (Pic, 1929)

- Excavation of 5th ventrite larger. Elytra often with a brownish pattern and whitish wax powder …….……....7

7. Color reddish, spermatheca as Fig. 5m. Body length: males 2.7–3.2 mm, females 3.3–4.0 mm ……...……..…. *H. buonloica buonloica* Moseyko & Medvedev, 2017

- Color dark grayish, elytra with a weak brownish tint. Body length: males 2.5–2.8 mm, females 3.0–3.2 mm……..*H. buonloica darlaki* Moseyko & Medvedev, 2017

8. Scales on elytra short, about twice as long as wide. Spermatheca as in Fig. 5b. Body length: females 4.3 mm ...…….……..….. *H. harmandi* Lefèvre, 1893

- Scales on elytra longer, at least 2.5 times as long as wide……..…….…….……9

9. Elytra with a very long setae 0.3–0.4 mm in length. Body fulvous to reddish-brown, with darker pattern...…….……..……10

- Elytra with moderately long setae 0.1–0.2 mm in length…..……..…….....11

10. Setae on the elytra are very long and up to 0.4 mm. Spermatheca as in Fig. 5c. Body length: males 3.4–4.0 mm, females 4.0–4.4 mm....*H. longipilosa* Moseyko & Medvedev, 2017

- Setae on elytra shorter, about 0.3 mm long. Spermatheca as in Fig. 5d. Body length: males 3.8–4.1 mm, females 4.2–4.8 mm …..…….…….. *H. sonlanga* Moseyko & Medvedev, 2017

11. Body less than 4 mm…….…….……..13

- Body large, males 4.1–4.7 mm, females 4.8–6.3 mm. Scales on the pronotum arranged in 3 poorly delimited longitudinal tripes, elytra of male with scales mostly arranged in a few spots, one near the middle, 2 behind middle and 1 at the apex; female with a chessboard pattern on the elytra or with the same as in males, spermatheca as Fig. 5e …….. *H. maculata* Kimoto & Gressitt, 1982

13. Hind femurs were widened with large tooth, spermatheca as in Fig. 5f. Body length: males 3.3 mm, females 3 mm .....……...…… *H. phanrangi* Moseyko & Medvedev, 2017

- Hind femur narrow, with small tooth. Body completely fulvous, or two colored, pronotum brown with two longitudinal fulvous stripes, or fulvous with dark median and lateral stripes; elytra with contrasting pattern, apices of femora dark. Antennae comparatively short about 2/3 body length. Spermatheca as Figs. 5g–k. Body length: males 2.6–3.1 mm, females 3.1–4.1 mm …...……. *H. yaosamica* Chen, 1940

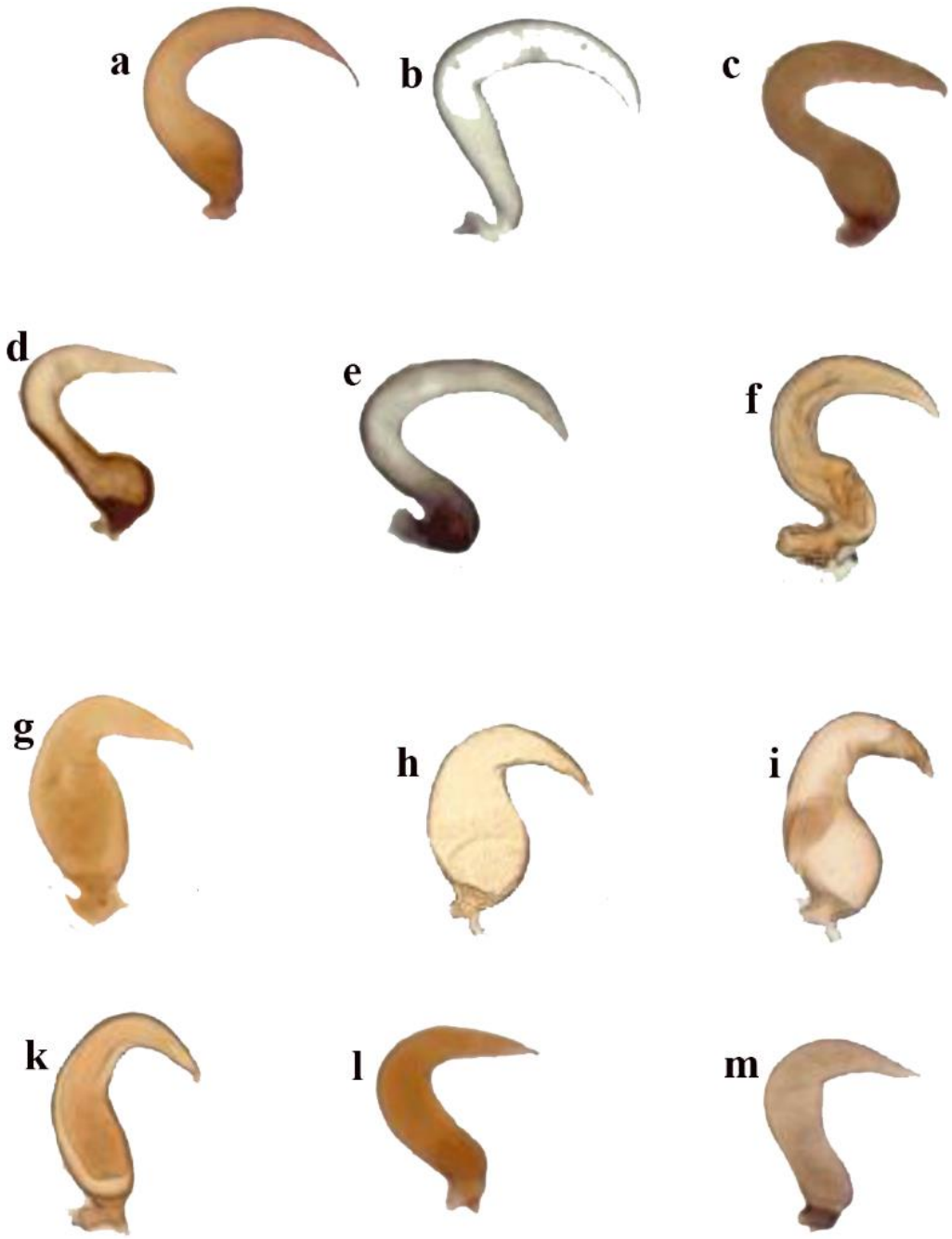

Figure 5. Hyperaxis spp., spermatheca of females: (a) *Hyperaxis nigrita*; (b) *Hyperaxis harmandi*; (c) *Hyperaxis longipilosa*; (d) *Hyperaxis sonlanga*; (e) *Hyperaxis maculata*; (f) *Hyperaxis phanrangi*; (g-k) *Hyperaxis yaosanica*; (l) *Hyperaxis feae*; (m) *Hyperaxis buonloica buonloica* (Moseyko & Medvedev, 2017)

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