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A new species of *Heliothrips* (Thysanoptera, Panchaetothripinae), based on morphological and molecular data

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Abstract

Heliothrips longisensibilis **sp. n.** is described from the tropical regions of southern China, Yunnan and Hainan, based on morphology and data from mitochondrial and nuclear genes. However, specimens that are identical in colour and structure are reported from northern Brazil, and this is presumably the area of origin of this new species. The area of origin within South America of the Greenhouse Thrips, *Heliothrips haemorrhoidalis*, is discussed and remains in doubt. An identification key to the four species of *Heliothrips* is provided.

Key words: *Heliothrips longisensibilis*, new species, mitochondrial genes (*COI*), nuclear genes (ITS2+28S+*EF-1α*)

Introduction

The greenhouse thrips, *Heliothrips haemorrhoidalis*, is one of the most well-known species of Thysanoptera and is recorded widely around the world. Despite this, all three species currently included in the genus *Heliothrips* are presumed to be native to South America (ThripsWiki 2019). The discovery of a new species in this genus in southern China (Yunnan and Hainan) was thus a considerable surprise. Detailed structural comparisons are given here to distinguish this new species from *haemorrhoidalis*, and in view of the many similarities between the two species comparisons were also made using both mitochondrial and nuclear genes. The original manuscript was sent to our colleague, Dr Elison Lima at Piauí in northeastern Brazil, for comment and review. He replied that he had collected specimens of both sexes in several States of central-western and northeastern Brazil (Piauí, Goiás, Maranhão and Mato Grosso), and that these specimens matched the colour and structural description in the draft manuscript. We are happy to accept his identification, and we conclude that the population from southern China has been introduced from Brazil.

One of the most obvious character states of the new species is the uniformly brown to dark brown colour of abdominal segments VIII–X in females. This contrasts with the condition in the common pest species, *haemorrhoidalis*, in which segments VIII–IX are yellowish-brown, and segment X is bicoloured with a dark ring at the apex. In this connection, the image of a female *haemorrhoidalis* given by Hoddle *et al*. (2012) is of a specimen from Tambopata, Peru. Re-examination of this female indicates that it shares many character states with the new species, including colour and the form of antennal segments IV and V, but has polygonal reticulation on the mesoscutum. The population recorded from Peru (Mound & Marullo 1996; Mound & Monteiro 1998) is therefore misidentified as *haemorrhoidalis*, and probably represents either the species described below or a further undescribed species. The pattern of *Heliothrips* radiation in Brazil is worth further study, incorporating more extensive molecular data. The new species described below is from northern Brazil, whereas *H. zucchi* is known from the south east (Mound & Monteiro 1998). In contrast, *H. similis* has been recorded in eastern Brazil between Bahia and Rio de Janeiro (Nakahara *et al.* 2015), and has also been seen from near Porto Alegre (Adriano Cavalleri pers. comm. 2019). This raises an important question about the area of origin of the pest species *haemorrhoidalis*,

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Elison Lima has pointed out to us that trade out of Brazil in the early 19th century would have been primarily from Rio de Janeiro. Presumably, *haemorrhoidalis* was therefore sent to Europe in shipments from that port. However, *similis*, *zucchi* and *haemorrhoidalis* have all been reported from around Rio, and so the area of origin of the Greenhouse Thrips remains conjectural. Trade from northern Brazil is more recent, and the populations described below from southern China may result from a relatively recent introduction. Prior to the report below only a single *Heliothrips* male had been reported from outside of the Americas, and this was from Thailand (Mound 1976). It remains possible that this male was misidentified, and the same may be true for some of the records of *haemorrhoidalis* by Wilson (1975), also the record from Colombia (Mound 1976). A further possible source of error that needed to be checked was the long-accepted synonymy of *ceylonicus* with *haemorrhoidalis*. However, the original description of *ceylonicus* refers to antennal segment II being yellowish-brown and paler at the apex, in contrast to the dark brown antennal segment II of the new species described here. It should be noted that teneral females of *haemorrhoidalis* are commonly encountered, and these have the abdomen pale, almost golden. In contrast, teneral females of the new species are uniformly light brown (Fig. 2). The objectives of the study presented here were to describe this new species of *Heliothrips*, to distinguish it from other members of the genus using morphological and molecular data, and to discuss its probable area of origin.

Material and methods

DNA extraction, amplification, and sequencing. An abdominal intersegmental membrane of each thrips was pierced with a fine micro-needle before genomic DNA was extracted, using the TIANamp Genomic DNA kit (TIANGEN BIOTECH CO., LTD, Beijing, China), following manufacture protocols. The carcasses were retrieved and stored in 75% ethanol at -20°C until slide mounted in Canada balsam for morphological work. Six individuals of *H. haemorrhoidalis*, each from six different provinces in southern China, and ten individuals of the new species were used for DNA extraction and subsequent sequencing. One individual of *Retithrips javanicus* from Java was used as outgroup, since *Retithrips* is one of the most closely related genera to *Heliothrips* (Mound *et al.* 2001). Approximately 3μL of the extraction DNA was used to amplify *COI*, ITS2, 28S rDNA and *EF-1α* sequences. The primers used for amplifying above sequences are given in Table 1. All loci were amplified by polymerase chain reactions (PCRs), which were run on Eppendorf Mastercycler Thermal Cyclers under the following profile: an initial denaturation at 95°C for 5 min, 35 cycles at 95 °C for 10 sec, 47 °C for 30 sec, 72 °C for 1 min, and a final extension step at 72 °C for 10 min. PCR products were then sequenced in both forward and reverse directions using an ABI 3730xl DNA Analyzer (Applied Biosystems) at Sangon Biotech Co., Ltd. (Shanghai, China). Sequences were checked, aligned, and trimmed with CHROMAS 2.31 (Technelysium, Helensvale, Australia), SeqMan package (DNASTAR Lasergene software), and MEGA 7.0 (Kumar *et al.* 2016). The three nuclear genes ITS2, 28S and *EF-1α* were concatenated by SequenceMatrix -Windows-1.7.8 (Vaidya *et al.* 2011).

TABLE 1. Primers used to amplify *COI*, ITS2, 28S rDNA and *EF-1α.*

Gene	Name	Sequence (5'-3')	Source
COI	LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer *et al*. 1994
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer *et al.* 1994
ITS2	P1	ATCACTCGGCTCGTGGATCG	Moritz *et al.* 2002
	52R	GTTAGTTTCTTTTCCTCCCCT	Moritz *et al.* 2002
28S rDNA	28SS	GACCCGTCTTGAAMCAMGGA	Chen *et al.* 2003
	28SA	TCGGARGGAACCAGCTACTA	Inoue & Sakurai 2007
EF1-α	EF1	GACAACGTTGGCTTCAACGTGAAGAACG	Palumbi 1996
	EF2	ATGTGAGCAGTGTGGCAATCCAA	Palumbi 1996

All sequences have been deposited in GenBank under accession numbers: *COI* (MK484658–MK484674); ITS2 (MK484624–MK484640); 28S (MK484641–MK484657); *EF-1α* (MK484675–MK484691). Some available mtDNA *COI* sequences of *H. haemorrhoidalis* from GenBank acquired by Buckman *et al*. (2013), Nguyen *et al*. (2015) and Tyagi *et al*. (2017) were also included in the analysis. Sequence divergences were calculated using the Kimura 2-parameter (K2P) distance model (Kimura 1980). Neighbour-joining trees (Saitou & Nei 1987) of mitochondrial

genes (*COI*) and the concatenated nuclear genes (ITS2+28S+*EF-1α*) were constructed using MEGA 7.0 with K2P distance.

Key to *Heliothrips* species
[based on Nakahara *et al*. 2015]

1. Metascutal triangle with posteromarginal flange absent, or distant from anterior margin of metascutellum; abdominal tergite I with median minute setal pair arising on reticulate area; tergites III–VIII with reticulation in front of antecostal ridge weakly developed or absent . . . . . *similis*
-. Metascutal triangle with posteromarginal flange extending to or over anterior margin of metascutellum (Figs 11, 12); tergite I with median minute setal pair arising anterior to reticulate area (Fig. 7); tergites III–VIII with reticulation in front of antecostal ridge strongly developed (Fig. 5) 2
2. Femora dark brown; antennal segment VI dark brown with base slightly paler . . . . . *zucchi*
-. Femora yellow; antennal segment VI yellow at least in basal half 3
3. Female abdominal tergite X uniformly brown, segments VIII–IX uniformly brown to dark brown not yellowish (Fig. 15); antennal segment II dark brown, base of antennal segments IV and V stout, slender dorsal sense cone on IV extending to middle or apical third of VI (Fig. 13); mesoscutum with reticles anterior to median setae narrowly transverse (Fig. 11) . . . . . *longisensibilis* **sp.n.**
-. Female abdominal tergite X basal half light brown to yellow brown, with darker apical band, segments VIII–IX often yellowish (Fig. 16); antennal segment II yellowish-brown with apex paler; base of antennal segments IV and V narrowed to basal neck (Fig. 14), slender dorsal sense cone on IV rarely extending as far as middle of VI; mesoscutum with reticles anterior to median setae irregularly polygonal (Fig. 12) . . . . . *haemorrhoidalis*

***Heliothrips longisensibilis* sp. n.**
(Figs 1–10, 11, 13, 15)

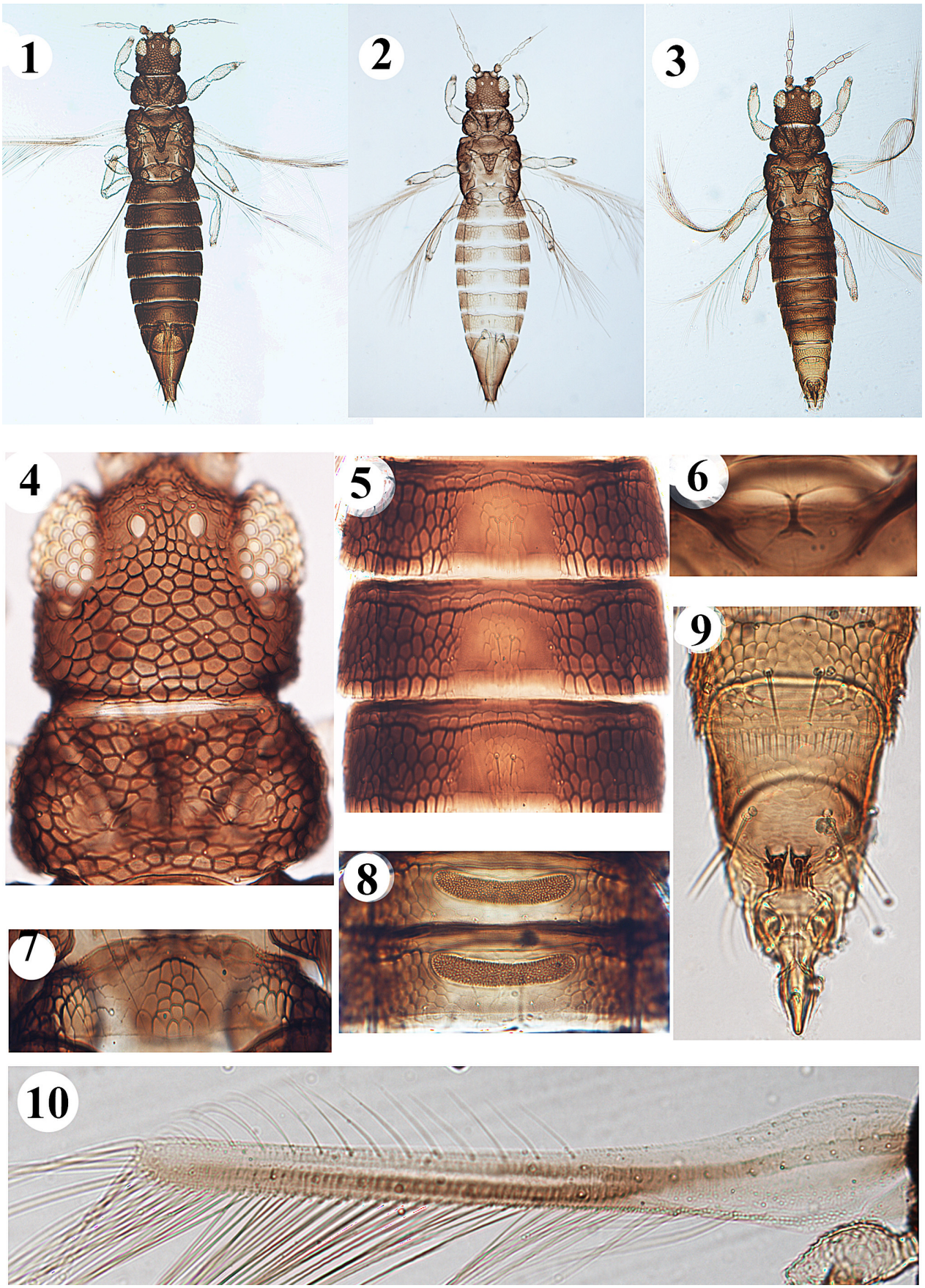
Female macroptera. Colour brown to dark brown (Fig. 1); legs completely pale yellow; antennal segment I brown, II dark brown, III–V and basal two-thirds of VI yellow, apical third of VI brown, VII–VIII light brown (Fig. 13); fore wing pale with extreme base and veins brown, clavus pale with base light brown (Fig. 10).

Head sculptured with polygonal reticulation, slightly wider than long, anterior margin triangular, genae almost parallel but slightly concaved in the middle and constricted at base (Fig. 4). Ocellar region not elevated, fore ocellus depressed in sculpture, setae minute. Antennae 8-segmented, constricted base of III much longer than the base of IV and V; III and IV each bearing simple sense cones, with the slender dorsal sense cone on IV much longer and extending to the middle or even third apical of VI (Fig. 13); segment VIII much longer than VII; microtrichia absent. Mouth cone rounded apically, not extending beyond fore coxae, maxillary palps 2-segmented.

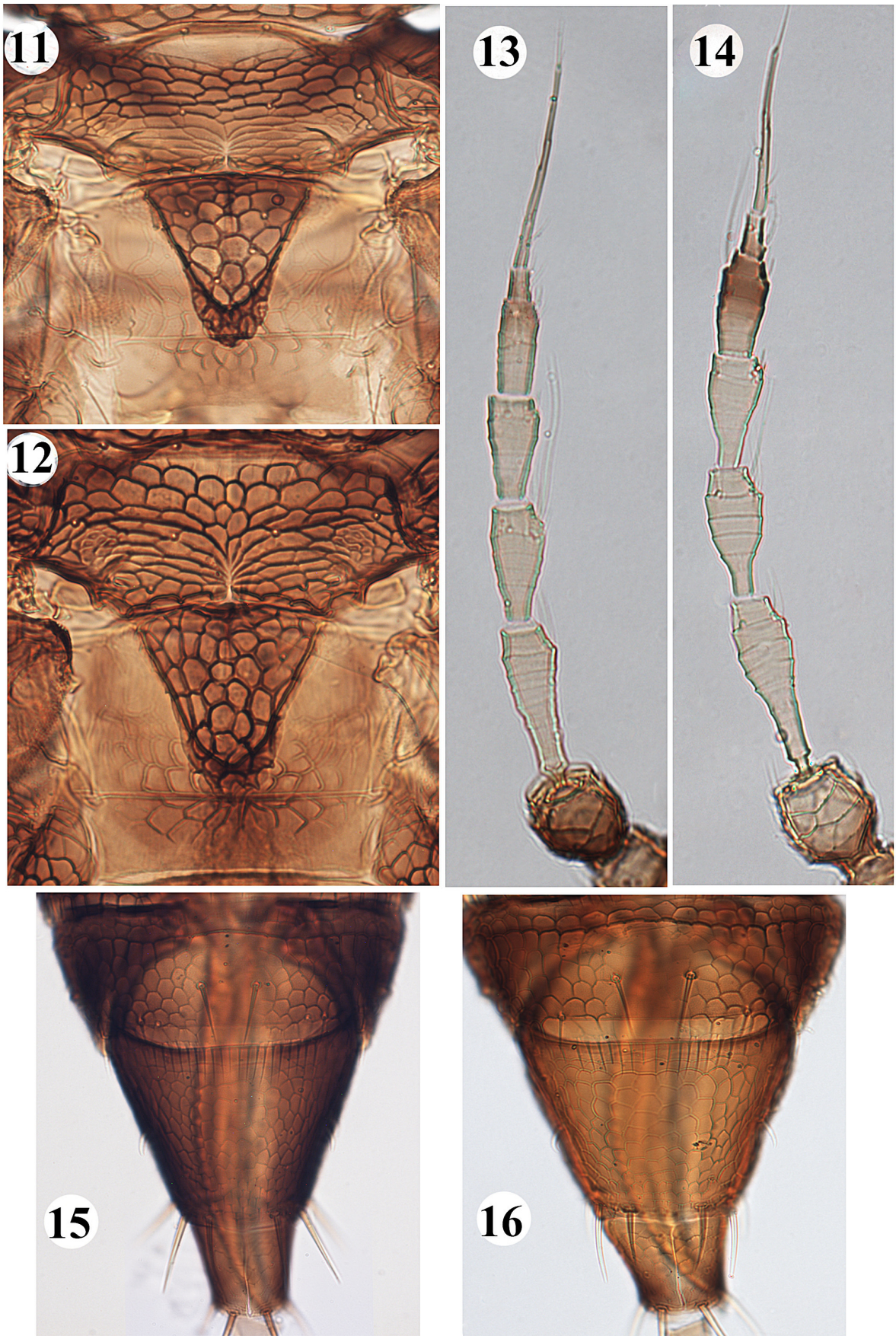
Pronotum transversely rounded, entirely covered with reticulations, setae small, prospinasternum forked apically (Fig. 6). Mesoscutum with 4–5 narrow transverse rows of reticulation before median setae, almost rectangle (Fig. 11), posteromedian short cleft. Metascutum with median sculptured triangle, posterior flange present, reaching anterior margin of metascutellum. Fore wing widened at base and rounded at apex, costal vein fused to first longitudinal vein with costal vein bearing approximately 7 setae, first vein 11–14 setae, second vein sparse 5–7 setae, setae minute; posterior fringe cilia straight except several cilia near cross vein wavy. Tarsi 1-segmented.

Abdominal tergites extensively reticulate, except submedian area of I–VII smooth, III–VIII with reticulations in front of antecostal ridge strongly developed (Fig. 5); tergite I with a pair of median setae arising anterior to reticulate area (Fig. 7), II–VIII median setae long and the base distance between them gradually wider on the posterior tergites, submedian setae close to campaniform sensilla (CPS); posterior margin of VIII with complete comb of long teeth (Fig. 15); IX with 3 pairs of thorn-like setae, equal in size and length to the setae on X, tergites IX and X with microtrichia posteriorly, X with a complete longitudinal split. Sternites completely reticulate and with 3 pairs of small setae situated in front of posterior margin. Ovipositor long and well developed.

Measurements (holotype female in microns): Body length 1466. Head, length 165; width across genae 184. Pronotum, length 114; width 230. Fore wing length 600. Tergite V median setae length 32. Tergite VIII median setae length 45. Tergite IX length 135. Tergite X length 60. Antennal segments I–VIII length 20, 32, 62, 42, 38, 35, 12, 70; III simple sense cone length 25, IV dorsal sense cone length 69, ventral sense cone length 23.



FIGURES 1–10. ***Heliothrips longisensibilis*** **sp. n.** (1) Female; (2) Teneral Female; (3) Male; (4) Head and pronotum; (5) Abdominal tergites IV–VI; (6) Prospinasternum; (7) Abdominal tergite I; (8) Abdominal sternites III–IV of male; (9) Abdominal tergites VIII–X of male; (10) Fore wing.



FIGURES 11–16. *Heliothrips* species from China. Meso– and metanotum, 11–12: (11) *longisensibilis* sp. n.; (12) *haemorrhoidalis*; Antennae, 13–14: (13) *longisensibilis* sp.n.; (14) *haemorrhoidalis*; Abdominal tergites VIII–X of female, 15–16: (15) *longisensibilis* sp. n.; (16) *haemorrhoidalis*.

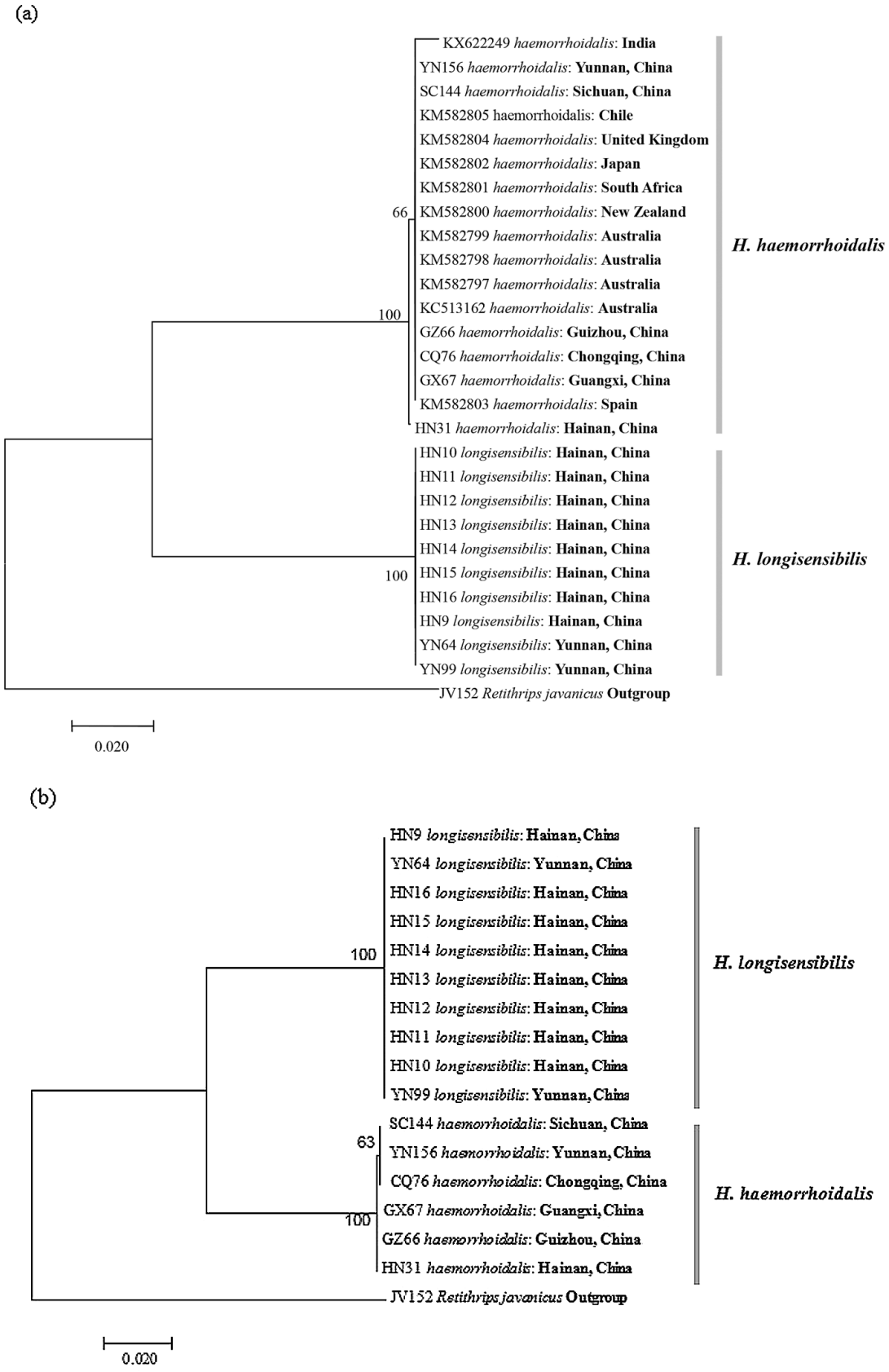


FIGURE 17. Neighbour-joining tree of two species of *Heliothrips* based on mitochondrial *COI* sequences (a) and combined nuclear ITS2+28S+*EF-1α* sequences (b), using Kimura 2-parameter distance. Bootstrap values are calculated in MEGA7.0 with 1000 replicates. Voucher number, species name, origin and GenBank Accession number are shown in the right column.

Male macroptera. Similar to female but smaller (Fig. 3). Body brown but with terminal abdominal segments paler (Fig.3). Abdominal tergite IX with three pairs of stout thorn-like setae and the base of anterior pair nearly contiguous, each posterior pair of setae slightly slender and lateral to preceding pair (Fig. 9). Sternites III–VII each with a transverse oblong pore plate (Fig. 8).

Measurements (paratype male in microns): Body length 1138. Head, length 133; width across genae 165. Pronotum, length 101; width 212. Fore wing, length 600. Antennal segments I–VIII length 19, 30, 58, 37, 31, 29, 11, 62; III simple sense cone length 23, IV dorsal sense cone length 58, ventral sense cone length 20. Anterior pair of thorn-like setae on tergite IX length 19. Pore plate on sternite III length 95; width 14, on sternite VII length 59; width 14.

Material studied. Holotype female, **CHINA**, **Yunnan Province**, Xishuangbanna Tropical Botanical Garden, from leaves of *Tecoma stans*, 24.x.2017 (Xie Yanlan & Liu Hui), in collection of Yunnan Agricultural University, Kunming.

Paratypes: 14 females with same data as holotype; same location, 1 female from *Melastoma candidum*, 11.iii.2017; 1 female from *Fagopyrum dibotrys*, 11.iii.2017; 1 female from *Cerasus glandulosa*, 11.iii.2017; 10 females from *Ixora chiensis,* 24.viii.2018; 1 female from *Albizia julibrissin,* 30.v.2018. **CHINA, Hainan Province**, Jianfengling National Forest Park, 11 females and 2 males from *Lithocarpus corneus* leaves, 27.vi.2018 (with about 10 female specimens preserved in alcohol). [1 female, 1 male deposited in Australian National Insect Collection]

Molecular data. The DNA sequences were approximately 669 bp for *COI*, 620 bp for ITS2, 380 bp for 28S rDNA and 189 bp for *EF-1α*, respectively, no pseudogene sequences were amplified. The neighbour-joining tree based on mitochondrial and concatenated nuclear datasets revealed that *H. longisensibilis* is well separated from *H. haemorrhoidalis* (Fig. 17). Ten individuals of *H. longisensibilis* clustered into one branch and all *H. haemorrhoidalis* from different populations formed another clade. The genetic divergences between *H. longisensibilis* and *H. haemorrhoidalis* were 12.7–13.6% for mitochondrial *COI* sequences, 19.1–19.6% for ITS2 sequences, 3.8–4.4% for *EF-1α* sequences, and 1.9% for the highly conserved 28S D3 domain sequences, respectively. After concatenating the three nuclear genes (ITS2+28S+*EF-1α*), genetic divergences between *H. longisensibilis* and *H. haemorrhoidalis* were 10.3–10.4%, supporting the hypothesis that these are distinct species.

Conclusions

The molecular data support the morphological data presented in the key above in distinguishing this new species from the widespread pest species, *H. haemorrhoidalis*. Concerning the probable origin of this new species, Dr Elison Lima has pointed out that there is variation among specimens available to him from different localities in northern Brazil, particularly in the form of the metascutum. A more extensive study in Brazil of molecular and morphological variation within and among *Heliothrips* populations would be of considerable interest.

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