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First report of *Tuberaphis xinglongensis* (Zhang, 1982) from India on *Areca catechu* Linnaeus with morphological re-description and molecular characterization

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ABSTRACT: *Tuberaphis xinglongensis* (Zhang and Zhong) is recorded for the first time from India on *Areca catechu* Linnaeus. The aphid is re-described, illustrated and measured. The identity of species is confirmed with help of molecular characterization. A key to the species under this genus from India is provided. Arecanut is new host plant record for this aphid.

Keywords: Aphid, new distribution, arecanut, *Tuberaphis*, India

INTRODUCTION

In India, Karnataka stands first among areca growing states in the country. Insect pests are a major constraint in areca cultivation. Among different sucking insect pests of arecanut, scale insects, *viz*., *Aspidiotus destructor* Signoret and *Ischnaspis longirostris* (Signoret) and mealybug, *viz*., *Palmicultor palmarum* (Ehrhorn) and *Dysmicoccus finitimus* Williams are of common occurrence in India. Two species of *Cerataphis* Lichtenstein, *viz*., *Cerataphis brasiliensis* (Hempel) and *Cerataphis lataniae* (Boisduval) have been recorded as species of regular occurrence in arecanut growing areas of the world (Blackman and Eastop, 2006). Only *C. lataniae* has been recorded from Karnataka infesting arecanut (Joshi, 2008). In the present paper, we report, for the first time, occurrence of *Tuberaphis xinglongensis* (Zhang) from India on *A. catechu*, which also is new host record for this species.

Smith *et al.* (2007) have described sternorrhynchan as the most successful groups of invasive insects that are commonly transported in the plant trade. During last few years, India has experienced accidental introduction of two species of aphids, *viz*., *Wahlgreniella nervata* (Gillette) on *Rosa* spp. (Joshi *et al*., 2014) and *Liosomaphis ornata* Miyazaki on *Berberis lyceum* Royle (Nadda and Joshi, 2015). In addition, there had been a serious event of rapid spread of *Ceratovacuna lanigera* Zehntner within the country (Joshi and Viraktamath, 2004).

Subsequent to its record on unidentified plant belonging to Arecaceae in China and description as new species, *T. xinglongensis* has been recorded on *Cocos nucifera* Linnaeus also (Chen *et al*., 2014). The original description of this species by Zhang and Zhong (1982) as *Astegopteryx xinglongensis* Zhang is very brief and later the species was transferred to the genus *Tuberaphis* by Jiang *et al*. (2012) without adding much to the description but by providing few microphotographs. Qiao (2018) redescribed the same species in Fauna Sinica Insecta series, however it is in Chinese language and hence difficult to understand for Indians and researchers from other countries, and hence we are re-describing the species along with key for species of *Tuberaphis* occurring in India.

MATERIALS AND METHODS

The pest was first time recorded during a survey in the month of April 2017, from Hullehal village (14° 18'N; and 75° 54'E; 731 MSL) of Chitradurga district (Karnataka state). The arecanut variety was 'Maidan Local' and the age of the garden was five years. Observations on pest intensity were recorded in selected four spots. In each spot, five spindle portions were selected from five plants and the observations on number of aphids were made at fifteen days intervals. The number of aphids in 2.5 x 2.5 sq cm areas at 3 equidistant spots on each spindle were counted and recorded. The aphid sample for this study was collected in 2017 on *Areca catechu* L. Karnataka, India. It was preserved in 70% ethanol and specimens were mounted in Canada balsam following the methods described by Blackman and Eastop (2000). Illustrations of slide-mounted specimens were taken by digital camera (Nikon Digital Sight DSVI -1) attached

Table 1. Population dynamics of *Tuberaphis xinglongensis* on arecanut during 2017 at Hullehal village of Chitradurga district

Month	Fortnight	No. of aphids/2.5 sq. cm (on spindle)
April	I	27.46
	II	30.18
May	I	33.78
	II	27.46
June	I	33.10
	II	36.00
July	I	29.30
	II	27.46
August	I	30.03
	II	33.25
September	I	18.53

to a Nikon Eclipse 80 I microscope. Measurements for each specimen were taken from the digital image using the M205 A Leica Application Suite. DNA extraction, DNA barcode region amplification and sequencing was done as per the methods and materials enlisted by Kinyanjui *et al.* (2016). DNA sequence was submitted to BankIt and accession number was generated through GenBank. Further DNA sequence was submitted to BOLDSYSTEMS to generate valid BOLD-ID and DNA barcode for *T. xinglongensis*.

RESULTS AND DISCUSSION

Damage and incidence of aphid

Both adults and nymphs were found sucking the sap from the spindle portion but less from leaf portion. The more infestation was noticed on spindle region than on leaf and the population was more during summer months. The pest intensity ranged from 18.53 to 36.00 aphids/2.5 sq. cm. From fifth week of May onwards, a gradual increase in the build-up of aphid population was observed with a peak activity during second fortnight of June with a population of 36.00 aphids/2.5 sq. Thereafter, the population started gradually declining with less activity during first fortnight of September (Table 1). It has been reported from East and South–east Asia and Africa (China, Japan, India, Indonesia, Korea, Somalia and Vietnam) (Blackman and Eastop, 2006). The life cycles of *T. xinglongensis* is not known but has been recorded from an unidentified species of palm, and then from *Cocos nucifera* Linnaeus. (Blackman and Eastop, 2006). This is the first report of *T. xinglongensis* on *A. catechu*.

Morphological description

Species *Tuberaphis xinglongensis* (Zhang, 1982)

Astegopteryx xinglongensis Zhang in Zhang and Zhong (1982: 19); Zhang and Zhong (1983:104); Remaudiére and Remaudiére (1997:180); Tao (1999: 18); Jiang *et al*. (2012:217).

Specimens examined: Five apterous viviparous females and three alate viviparous females.

Live aphid description: The aphid makes crowded colonies on spindle region (Fig. 1A). Nymphs yellowish green in colour, pear shaped. Alates with light brownish yellow body. Wings held horizontal above abdomen. (Fig. 1B). Adult female brownish but gives greyish white appearance due to wax powder deposits on dorsum (Fig. 1C). Weak fringe of white wax surrounding abdomen, which breaks as the aphid walks in the colony. Appendages light brown. Ethanol preserved female looks reddish brown with light yellow colour patches around siphuncular pores. These patches have dark brown borders which almost cover the abdominal area except spinal area which is reddish brown. Appendages light brown; cephalic tubercles can be clearly seen (Fig. 1D).

Morphometrics of apterous female and alate females are given in Table 1 and detailed morphological characters of mounted female are illustrated in Fig. 2.

Apterous viviparous female

Body light brown to brown (Fig. 2; 1), 1.44-1.46 mm long, 1.02-1.026 mm as maximum width. Head fused with prothorax (Fig. 2; 3), median suture on head not prominant, head bearing three lateral frontal tubercles

Table 2. Morphometrics of *Tuberaphis xinglongensis* (in mm)

Body part	Apterous female		Alate female	
	Range	Mean	Range	Mean
Body length	1.440-1.460	1.450	0.192-0.192	0.1924
Body width	1.020-1.026	1.024	0.087-0.090	0.089
Ant. I	0.040-0.060	0.050	0.045	0.045
Ant. II	0.040-0.060	0.050	0.045-0.050	0.046
Ant. III	0.100-0.130	0.114	0.320-0.330	0.323
Ant. IV	0.050-0.070	0.060	0.120-0.130	0.123
Ant. V base	0.060-0.080	0.070	0.080	0.080
PT	0.014-0.016	0.015	0.020-0.040	0.030
Whole antenna	0.335-0.376	0.359	0.640-0.665	0.648
URS base	0.050-0.070	0.056	0.057-0.060	0.059
URS length	0.050-0.060	0.056	0.057-0.060	0.059
Hind tibia	0.028-0.029	0.288	0.410-0.420	0.416
Hind femure	0.250-0.270	0.264	0.330-0.340	0.330
2HT	0.080-0.090	0.088	0.085-0.087	0.085
Siphunculi height	0.024-0.026	0.025	0.020	0.020
Siphunculi opening diameter	0.04-00.050	0.044	0.050-0.056	0.054
Cauda	0.040-0.005	0.042	0.025-0.026	0.025
BW cauda	0.100	0.100	0.100	0.100
Ant. III WD	0.022	0.022	0.035	0.035
MW Hind tibia	0.027	0.027	0.035	0.035
Setae on antenna 3	0.024-0.027	0.025	0.023-0.027	0.025
Setae on tergum I	0.004-0.005	0.004	0.010-0.030	0.020
Setae on tergum VIII	0.050-0.070	0.058	0.065-0.067	0.066
Setae on hind tibia	0.022	0.022	0.030-0.040	0.033
Setae on Ant. I	0.010-0.020	0.012	0.022	0.022
Setae on Ant. III	0.020-0.023	0.021	0.025-0.030	0.027
Setae on Ant. IV	0.017-0.020	0.019	0.022	0.022
Setae on Ant. V base	0.017	0.017	0.012	0.012
Setae on PT	0.012	0.012	0.015	0.015
Setae on URS	0.025-0.027	0.025	0.025	0.025
Setae on tergum VIII	0.017- 0.020	0.019	0.037	0.037
Setae on cauda	0.042-0.044	0.042	0.045-0.050	0.046
Setae on anal plate	0.062-0.063	0.062	0.037-0.040	0.038
Setae on genital plate	0.037	0.037	0.037-0.037	0.037
Cephalic tubercle I	0.010-0.020	0.012	0.005	0.005
Cephalic tubercle II	0.012	0.012	0.005	0.005
Cephalic tubercle III	0.014-0.016	0.015	0.005	0.005
Seta on tubercle I	0.020-0.030	0.022	0.012	0.012
Seta on tubercle II	0.012-0.012	0.012	0.012	0.012
Seta on tubercle III	0.012	0.012	0.012	0.012
Longest hair on cauda	0.075-0.076	0.075	0.065-0.070	0.066
Tarsal segment I	0.027-0.037	0.030	0.0375-0.038	0.037
Rhinaria on segment III	-	-	25-27	26.660
Rhinaria on segment IV	-	-	11-12	11.660
Rhinaria on segment V	-	-	7-8	7.500
Hair on siphunculi	0.017-0.020	0.015	0.017-0.030	0.025
Tarsal digitule	0.027-0.030	0.032	0.030-0.037	0.035
	Ratios			
PT/Base Ant. V	0.200-0.233	0.214	0.250-0.500	0.375
PT/Ant III	0.107-0.150	0.131	0.062-0.121	0.092
URS/2HT	0.555-0.777	0.636	0.660-0.705	0.690
URS/Ant	0.133-0.208	0.156	0.089-0.093	0.091
Hind femure/Ant III	1.923-2.700	2.135	1.000-1.062	1.030
Hind tibia/Body length	0.191-0.201	0.198	2.133-2.180	2.164
URS/BW URS	1.000-1.200	1.076	0.958-1.200	1.109
Cauda length/BW Cauda	0.400-0.500	0.420	0.250-0.260	0.253
Diameter of siphunculi/Antenna IIIWD	1.777-2.222	1.956	1.428-1.600	1.542

Fig 1. A. *Tuberaphis xinglongensis* (Zhang and Zhong) colony on areca nut spindle; B. colony of *T. xinglongensis* showing nymphs, adults and alates; C. Close up view of grown up females; D. Ethanol preserved adult female

(Fig. 2), each lateral tubercle bears a single spine, height of the tubercles range from 0.01 to 0.02 mm and height of the spine on these tubercles ranges from 0.01 to 0.03 mm; dorsal cephalic hairs with acute apices, 0.04-0.06 mm long. Antennae 5-segmented (Fig. 2; 5), dark brown with 5th segment darkest, 0.23-0.25× as long as the body; segment I with 1 or 2 hairs 0.01 – 0.02 mm long and II with 2 hairs 0.02 – 0.024 mm long, segment III without hairs but with spinulose imbrication on terminal portion; segment IV with 2 hairs measuring 0.0175 – 0.02 mm; segment V with 1 hair on base (0.017 mm long) and 3 hairs on processus terminalis (0.012 mm long); processus terminalis at most 0.2 to 0.23 × as long as base of last antennal segment (Fig. 2; 6). Eyes three faceted (Fig. 2; 7). Rostrum reaches midcoxae, ultimate rostral segment 0.6 -0.7× as long as second segment of hind tarsus and bears a pair of accessory hairs (0.025 – 0.027 mm long) (Fig. 2; 8), abdominal segments 1st to 6th laterally separated by intersegmental pale areas but fused together and lateral pale area extends to spino-pleural region; 7th and 8th tergite distinctly separated from anterior tergites; spiracles with roughly oval aperature with elongate lateral sclerite; tergum brown, dermal pattern formed with clear and dark areas, hairs on the dorsum of abdomen with fine apices; each of 1st-3rd abdominal tergites with 2 marginal. 1 submarginal, 2 pleural and 2 spinal hairs arranged in a transverse row on each side, 4th and 5th tergite with 6th with 4 hairs and 7th and 8th tergite with 2 hairs, these being 0.004-0.02 mm long. Roughly oval wax plates with numerous small darker cells present from cephalothorax to 8th abdominal tergite. Legs dark, short; trochanters difficult to separate from femora and perhaps fused with femora; femora and tibiae with short and long hairs (Fig. 2; 9), longest one on hind tibiae 0.022-0.023 mm long; first tarsal segments with 4, 2, 2 hairs (Fig. 2 ; 10, 11, 12); dorso-apical hairs on second tarsal segment expanded at apices. Siphunculi in the form of broad pore, placed on shallow cones (Fig. 2; 13), rim of the pore protruding outward in side view (Fig. 2; 14), bearing 6-7 hairs. Body margin bearing a row of marginal wax gland cells from cephalothorax to 8th abdominal tergite, these wax glad cells with 2 or 3 rows smaller darker cells in them (Fig. 2 ;15, 16). Cauda broadly rounded with 8 hairs (Fig. 2; 17). Subanal plate bilobed bearing 14-16 long and stout hairs (Fig. 2; 18). Genital plate oval shape bearing 14 hairs (Fig. 2; 19).

Alate viviparous female

Body elongate oval, light brown; head, thorax, legs and antenna dark brown (Fig. 2; 2). Body length 0.192 mm and body width 0.089 mm. Head bearing two lateral tubercles with single spine and one spine without a tubercle directly placed on the derm. Height of the tubercles 0.005 mm and spine on them 0.013 mm. The third spine 0.018 mm in length (Fig. 2; 20). Eyes multifaceted (Fig. 2; 21). Antennae 5-segmented 0.930 mm long; segment III longest with over 25-28

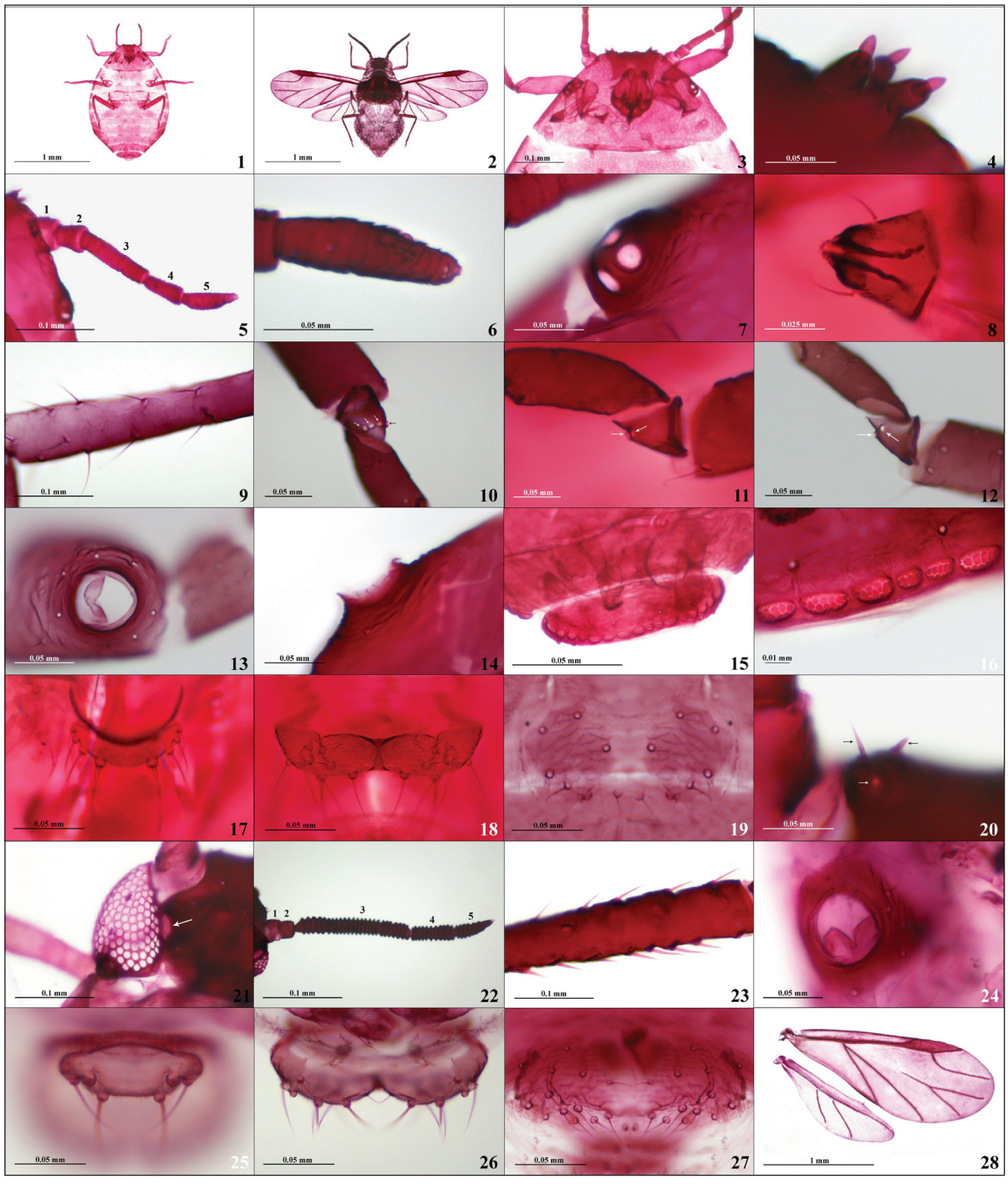

Fig. 2. Taxonomic character of *Tuberaphis xinglongensis* (Zhang): 1. Apterous viviparous female; 2. Alate female; Characters of apterous female 3. Head and prothorax fused; 4. Three tubercles bearing setae on head; 5. Five segmented antenna; 6. Last segment of antenna showing processus terminalis smaller than base; 7. Three faceted eye; 8. Ultimate rostral segment with a pair of accessory hair; 9 Hair on hind leg; 10. Four hairs on the first tarsal segment of fore leg; 11. Two hairs on the first tarsal segment of middle leg; 12. Two hairs on the first tarsal segment of hind leg; 13. Siphunculus surrounded by six or seven hairs; 14. Siphunculus side view; 15. Marginal wax gland around body margin; 16. Close up view of marginal wax glands; 17. Cauda; 18; Anal plate; 19. Genital plate; Characters of alate female 20. Three tubercles bearing setae; 21. Multi-faceted eye; 22. Five segmented antenna with rhinaria; 23. Hair on hind leg, 24. Siphunculus; 25. Cauda; 26. Anal plate; 27. Genital palte; 28. Wings

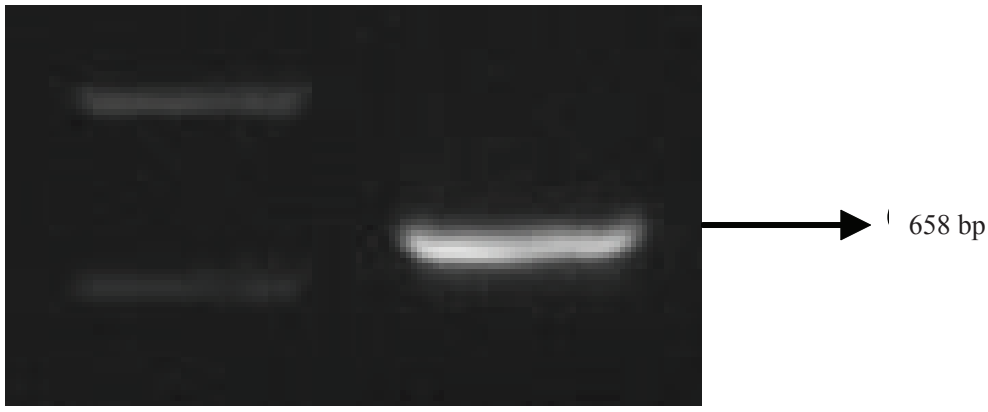

Fig. 3 PCR amplification of *T. xinglongensis* at 700 bp compared to a Low Range DNA ruler (M)

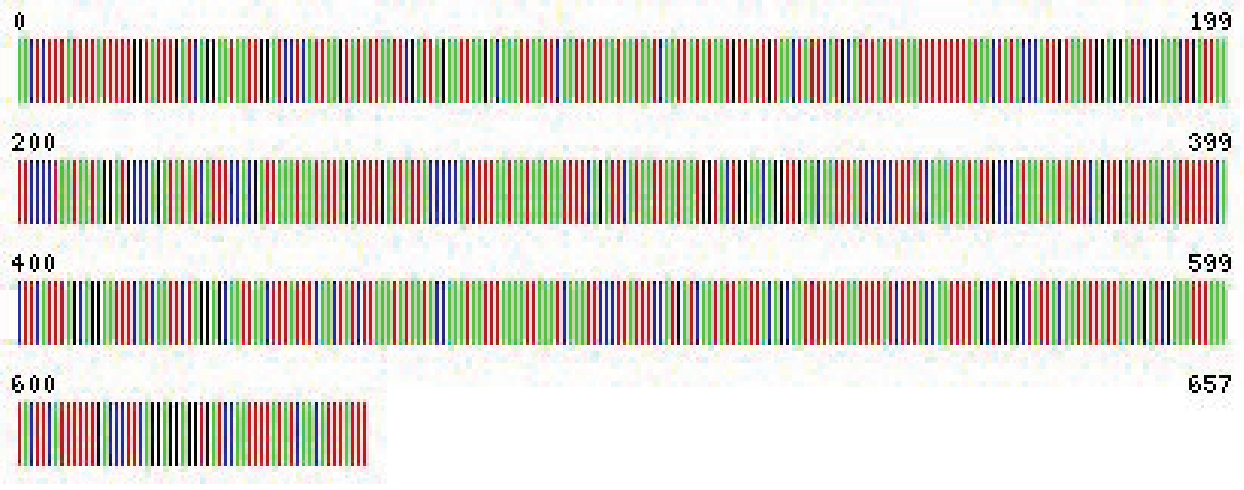

Fig. 4. DNA Barcode of *T. xinglongensis* with ID AGIRI322-17

annular secondary rhinaria; segments IV and V subequal with 11-12 rhinaria on segment IV and 7 rhinaria on segment V (Fig. 2; 22). Dorsum without wax pores on abdomen. Dorsal hairs fine; lateral abdominal hairs up to 0.065-0.067 mm long. Legs slender, much longer than in apterae; hairs on femora and tibiae fine (Fig. 2; 23), longest one on hind tibiae 0.033 mm long; dorso-apical hairs flattened at apices. First tarsal chaetotaxy 4, 3, 2 hairs. Siphunculi similar to apterous female but with 5 hairs (Fig. 2; 24). Cauda semicircular bearing six hairs (Fig. 2; 25). Subanal plate bilobed with 10 - 11 hairs (Fig. 2; 26). Genital plate with 26-28 hairs (Fig. 2; 27). Fore wing showing costa, subcostal, pterostigma, radial sector, media branched once, Cu1a and Cu1b (anal) emerging from same point and a claval fold; hind wing with hamuli, media and cubitus vein (Fig. 2; 28).

Genomic DNA extracted and confirmed on 0.8% agarose gel from *T. xinglongensis* was subjected to mtCO1 PCR and 658bp of mitochondrial DNA product was obtained (Fig. 3). Nucleotide sequence of *T. xinglongensis* checked for homology by using BLAST tool of NCBI. As no insertions, deletions or stop codons were observed in the 2nd frame of DNA sequence was chosen from ORF finder for submission to GenBank. GenBank Accession number "MG197811" was obtained and the DNA sequence was submitted to BOLDSYSTEMS, DNA barcode with BOLD-ID "AGIRI322-17" was successfully generated by providing both specimen and sequencing details (Fig. 4).

Key to adult females of the Indian species of *Tuberaphis* Takahashi

1. Body 2.6 – 2.7 mm long. Marginal hairs on abdomen 0.076 mm long. 8th tergite with fine hairs measuring 0.066 – 0.070 mm long. Antennal segment III 0.26 – 0.28 mm long. Ultimate rostral segment 0.13 – 0.14 mm long……..*T. indica* Ghosh, Ghosh and Raychaudhuri

– Body small 1.3 – 1.46 mm long. Marginal hair variable. Body pale or dark…….....2

2. Marginal hairs on the abdomen very small measuring up to 0.013 – 0.016 mm.

Abdominal dorsum pale with indistinct wax glands. Hairs on legs 0.006 – 0.015 mm. Ultimate rostral segment without any accessory hairs……
…….....*T. breviseta* Ghosh

– Marginal hairs on the abdomen variable. Abdominal dorsum dark with distinct wax glands. Hairs on legs variable. Ultimate rostral segment with accessory hairs……3

3. Marginal hairs on the abdomen up to 0.100 mm long. Hairs on 8th abdominal tergite up to 0.117 mm long. Hairs on legs up to 0.033 – 0.040 mm long. Ultimate rostral segment with 2 accessory hairs. Length of ultimate rostral segment up to 0.11 mm. Head bearing a median tubercle and two lateral frontal tubercles on each side. Siphunculi surrounded by 10–25 hairs …..……...*T. loranthi* (van der Goot)

– Marginal hairs on the abdomen up to 0.02 mm long. Hairs of 8th tergite 0.05 – 0.07 mm long. Hairs on legs up to 0.0075–0.025 mm long. Ultimate rostral segment with 2 accessory hairs. Length of ultimate rostral segment 0.05 mm. Head bearing three lateral frontal tubercles on each side, median frontal tubercles absent. Siphunculi surrounded by 6 – 7 hairs……..*T. xinglongensis* (Zhang)

Note: Morphological characteristics used for diagnosing *T. indica* and *T. breviseta* were taken from Ghosh (1988) and for *T. loranthi* from Jiang *et al*. 2012.

As the pest was recorded for the first time in India on arecanut, no chemical options were tried for direct control of this pest. No natural enemies were encountered during surveys conducted in Karnataka, India. There are no records of any natural enemies of this species from any part of the world. Absence of aphidocolous ants attending this species makes it more suitable for biological control.

These types of introductions are generally associated with movement of plant material. The species is known to occur only in China and it is not known how the species entered in India, as India generally exports arecanut to China and imports it from countries like Sri Lanka, Bangladesh, Nepal and Indonesia (Vinayak, 2016). It can be speculated that the species already exists in these countries without having been recorded there and has crossed the ports unnoticed. Standard phytosanitary procedures should be implemented to reduce risk of spread of this species within the country and neighboring countries growing arecanut. Existing quarantine regulations that regulate movement of plant material between countries should be reviewed.

More surveys need to be conducted to record natural enemies of this pest and to study potential of major natural enemies. The results of this program could be the basis of the development of management strategies of this species in India, if and when it arrives here again in future.

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