








Discovery of *Aleuroclava canangae* (Corbett) (Hemiptera: Aleyrodidae) on *Coleus* in India and the establishment and review of the *Aleuroclava canangae* species group

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ABSTRACT

Aleuroclava canangae (Corbett, 1935) was discovered on an ornamental *Coleus* species in Kerala, India, and represents the first occurrence of this species in India and on this plant host. The species is redescribed and illustrations of the puparium and adult males and females are provided along with morphometric data. A molecular analysis of the species using the *COI* gene was conducted to characterise it, determine its genetic lineage and compare it to seven other species of *Aleuroclava* Singh, 1931 and those of other whitefly genera. Results of the DNA analysis indicate that *A. canangae* forms a clade with *Aleuroclava indica* (Singh, 1932) which also has two pairs of very elongate, tuberculate setae which appear two-jointed because they have a wider basal part separated from a long slender apical part by a fine suture. The *Aleuroclava canangae* species group is established and an illustrated key is provided for the 12 species in the group, all of which were once placed in the genus *Martiniella* Jesudasan and David, 1990, now a synonym of *Aleuroclava*. The molecular analysis indicates that *A. psidii* (Singh, 1931), *A. guyavae* (Takahashi, 1932), *A. montanus* (Takahashi, 1939) and *A. gordoniae* (Takahashi, 1932) form a clade within the genus *Aleuroclava* and share similar morphological characters. Based on the results of the molecular and morphological analyses, *Aleurothrixus trachoides* (Back, 1912) is transferred back to *Aleurotrachelus trachoides* (Back, 1912) based on its morphology and the results of the DNA analysis.

<http://www.zoobank.org/urn:lsid:zoobank.org:pub:634C8003-1755-4CB1-B351-00F83D1E5BF9>

ARTICLE HISTORY


Received 20 April 2023
Accepted 17 April 2024

KEYWORDS

Whitefly; illustrated key to species; new host; new distribution; fauna; adult whiteflies; molecular; nucleotide analysis; *COI* gene

Introduction

Whiteflies (Hemiptera: Aleyrodidae) comprise several of the major plant pests worldwide due to their direct feeding on plants, indirectly by secreting honey dew on which sooty mould deposits are formed that impair photosynthesis and, most importantly, as vectors of plant diseases (Gilbertson *et al.* 2015). There are 1702 whitefly species belonging to 197

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genera in the family Aleyrodidae known globally (Evans *et al.* in press). David *et al.* (2021) reported 476 species of whiteflies under 73 genera in India.

During a search of exotic whiteflies associated with coconuts in India, an ornamental *Coleus* species growing within the grove that was infested by *Aleuroclava canangae* (Corbett, 1935) and associated with sparse sooty mould was discovered. This represents the first record of the distribution of this species in India (Kerala), and to our knowledge is also the first record of a whitefly found on a *Coleus* species. Notes on the morphological features of the puparium and adult of *Aleuroclava canangae* are provided along with data from a molecular analysis of the mitochondrial cytochrome c oxidase subunit 1 (*COI*) gene.

Detailed studies of the morphological characteristics of the puparia and adults of a whitefly species may provide supporting evidence to their identity and relationships with other taxa. In addition, nucleotide (DNA) analyses have been used to gain deeper insights into the identity of the species, which may be difficult to discern based on the morphology of the puparium due to the presence of cryptic species and a lack of understanding of the degree of inter- and intraspecific variation occurring in the species.

Aleuroclava Singh, 1931

Singh (1931) erected the genus *Aleuroclava* with *Aleuroclava complex* Singh 1931 as the type species. It is by far the largest genus of whiteflies, with 148 nominal species, followed by *Dialeurodes* with 121 species (Evans 2007, personal communication). The Indomalayan fauna of *Aleuroclava* is the most rich and diverse with 132 species, of which 66 species were described from India, followed by 15 species from the Eastern Palearctic region, primarily from China and Japan. Three species, *A. aucubae* (Kuwana, 1911), *A. jasmini* (Takahashi, 1932) and *A. similis* (Takahashi, 1938) have been introduced in the Nearctic and the Western Palearctic regions; *A. jasmini* (Takahashi) and *A. similis* (Takahashi) were introduced in the Neotropical region; and *A. jasmini* (Takahashi) is the only species recorded from the Pacific Islands (Table 1). The Western Palearctic, Afrotropical, Malagesian and Australian regions have two, two, two and one species described, respectively.

Characteristics of the genus *Aleuroclava*

Currently, the genus *Aleuroclava* contains 148 species and is very diverse in structure and in the shape of the body and setae. Dubey and Sundararaj (2005) provided a review of the genus *Aleuroclava* in India. Pushpa and Sundararaj (2010, p. 96) characterised the puparia of *Aleuroclava* species as being small; elliptic or pyriform or subelliptic; pale yellowish to white in colour; lateral margin faintly crenulate with little wax; submarginal area not separated from dorsal disc; dorsum generally granulated and with tubercles; submargin with papilla-like markings in some species; transverse moulting suture not reaching

Table 1. Distribution of *Aleuroclava* species.

| NA | NT | WP | AF | MA | EP | IM | AU | PI | WW |
|----|----|----|----|----|----|-----|----|----|-----|
| 3* | 2* | 5 | 2 | 4 | 15 | 132 | 1 | 1* | 148 |

NA: Nearctic, NT: Neotropical, WP: Western Palearctic, AF: Afrotropical, MA: Malagesian, EP: Eastern Palearctic, IM: Indomalayan, AU: Australian, PI: Pacific Islands, WW: worldwide. * all species in the NA, NT and PI regions were introduced.

margin; thoracic tracheal pores generally smooth shallow emarginations; eighth abdominal segment generally longer than seventh; caudal furrow distinct ending in a cleft; vasiform orifice cordate, subcordate or subcircular and generally notched posteriorly; lingula obscured by operculum filling the orifice; and, generally, with a minute seta on submargin cephalolaterad of caudal setae.

Characteristics that appear to be constant or nearly constant in this genus include: (1) prothoracic setae (Ts1), mesothoracic setae (Ts2) and metathoracic setae (Ts3) and submarginal setae absent, except in *Aleuroclava siamensis* (Takahashi, 1942) which has thoracic setae and a row of submarginal setae, the latter also being present in *Aleuroclava hindustanicus* Meganathan and David (1994); (2) cephalic setae (Cs1) and first abdominal segment setae (As1) present in 136 and 141 species, respectively; (3) tracheal opening in the shape of a cleft (shaped like a shallow bowl with a wide base) with smooth inner margins, not pore-like, with or without teeth such as in *Dialeurodes* (in 112 species); or undifferentiated (in 36 species); (4) submarginal ridge absent; most species with the dorsum entire; however many species have the submargin separated from the rest of the dorsum by a thin, concentric suture just inside the lateral margin that is interrupted at the caudal furrow. This differs from the submarginal ridge that separates the submargin from the rest of the dorsum in a relatively wide submarginal band as in *Aleurolobus*, *Aleurothrixus*, *Tetraeurodes* and similar genera; (5) colour: most species (121) are pale, 21 species are black and six species are bicour (usually pale with a dark streak down the centre); (6) submarginal papillae present or absent; (7) cephalothoracic tubercles present or absent; (8) vasiform orifice cordate, subcordate or subcircular with posterior margin closed, and is notched medially in most species; caudal furrow present, narrow, parallel sided, relatively short (1–2.5× the length of the vasiform orifice) and terminating in a cleft.

Similar genera

Manzari and Quicke (2006), in their cladistical analysis of over 430 species of whiteflies belonging to 124 different genera, found that *Aleuroclava* is most similar to *Minutaleyrodes* Jesudasan and David, 1990. The latter genus differs by having the transverse moulting suture which bends sharply cephalad on subdorsum, reaching the lateral margin; dorsum tassellated; submargin without papillaelike structures; pupal case broadest at the basal part of abdomen, which is somewhat shorter than the cephalothorax; vasiform orifice rounded and subcordate; cephalic setae (Cs1) present and relatively short, and the first abdominal pair of setae (As1) absent (except in *Minutaleyrodes kolliensis* which has As1 and lacks the submarginal suture; vs *Aleuroclava* species which have the transverse moulting suture without sharp anterior bending on subdorsum, not reaching margin; dorsum without tassellation; submargin generally with papillae; abdomen longer than or equal to cephalothorax; pupal case broadest at the metathoracic region; vasiform orifice cordate, subcordate or subcircular. *Aleuroclava* is also similar to *Dialeurodes*, *Massileurodes* and *Singhiella* but differs by lacking submarginal setae and the structure of the tracheal opening which is pore-like, with or without fleshy ‘teeth’ along its margin.

The *Aleuroclava canangae* group has long tuberculate setae on the cephalus (Cs1) and first abdominal segment (As1) which are characterised by having a wider elevated base arising from the dorsum that is separated by a fine suture from the slender filamentous apical part, thus appearing to be two-jointed. These are similar to, but less numerous than, the dorsal setae found on elongate digitiform tubercles in *Tuberaleyrodes* Takahashi, 1932

which includes *T. glutae* (Corbett), formerly *Dialeurodes glutae*, which also has the tuberculate setae with a wider basal part and a long slender apical part (Dubey and Martin, 2018). These are also similar to those in *Acanthaleyrodes* Takahashi, 1931. Dubey *et al.* (2014) observed that the development of tuberculate setae on the cephalus and the eighth abdominal segment of *Acanthaleyrodes callicarpae* Takahashi begin in the second instar with a slight elevation of dorsal cuticle. During the same stage, the sockets are formed in which the dorsal setae are fixed, and they continue to develop in the third instar. In the fourth instar (puparium), the cuticular elevation extends dorsally to produce the tuberculate setae. However, the formation of tuberculate-like setae is not restricted to these genera and has been shown to be induced by environmental conditions. Neil and Bentz (1999) showed that individuals of *Bemisia tabaci* (Gennadius) found on smooth-leaved hosts had short dorsal setae, whereas those found on hairy leaves had long, dorsal setae set on raised tubercles.

Generic synonyms of *Aleuroclava*

Five genera have been synonymised with *Aleuroclava*; of these, *Taiwanaleyrodes* Takahashi, 1932, *Aleurotuberculatus*, 1932 and *Martiniella* Jesudasan and David, 1990 have included species with two pairs of very elongate tuberculate setae having a wider basal part and a long slender apical part, of which one pair is on the cephalus (Cs1) and the other is on the first abdominal segment (As1).

Taiwanaleyrodes Takahashi, 1932

Takahashi (1932) erected the genus *Taiwanaleyrodes* with *Taiwanaleyrodes meliosmae* Takahashi, 1932, a species with non-tuberculate setae, which appears one-jointed and with lateral margin undifferentiated, as the type species. Corbett (1935) described *Aleurotuberculatus canangae* and *Aleurotuberculatus macarangae*, two species with tuberculate setae and tracheal clefts, and *Taiwanaleyrodes canangae*, *Taiwanaleyrodes baccaureae* and *Taiwanaleyrodes fici*, three species with tuberculate setae and the lateral margin undifferentiated at the opening of the tracheal furrow. He compared the latter species to *Taiwanaleyrodes indica* (Singh, 1931) which also has tuberculate setae and lacks a tracheal cleft. Subsequently, other species, some with non-tuberculate setae and others with tuberculate setae, and some species with and others without tracheal clefts, were placed in the genus; however, all species of *Aleuroclava* that have tuberculate setae were placed in *Martiniella* in the past; Manzari and Quicke (2006) synonymised *Taiwanaleyrodes* with *Aleuroclava*. The new synonymy created a homonym of *Aleurotuberculatus macarangae* Corbett, 1935 described on pages 828–829, with *Taiwanaleyrodes macarangae* Corbett, 1935 described on pages 840–841 of the same publication; Martin and Mound (2007) renamed the latter *Martiniella sepangensis* based on page priority.

Aleurotuberculatus Takahashi, 1932

Takahashi (1932) erected the genus *Aleurotuberculatus* Takahashi, 1932 with *Aleurotuberculatus gordoniae* Takahashi, 1932 as the type species. He stated that it is 'closely allied to *Aleuroclava* Singh, and may be its synonym, but differs from Singh's

description and figures in possessing thoracic tracheal clefts, as well as, in the submarginal area more or less separated from the dorsal disk'; Martin (1999, p. 31) opined that the 'submarginal/subdorsal definition is so variable that this character should not be used for the separation of these genera', and synonymised the genus with *Aleuroclava*.

Japaneyrodes Zahradnik, 1962

Zahradnik (1962) erected the genus *Japaneyrodes* Zahradnik, 1962 with *Aleurotuberculatus trachelospermi* Takahashi, 1938 as the type species, distinguishing it from *Aleurotuberculatus* by the absence of a submarginal suture and of cephalic tubercles. Mound and Halsey (1978, p. 340) synonymised *Japaneyrodes* with *Aleurotuberculatus*, stating that 'in the genus *Aleurotuberculatus*, both these characters are variable. It is possible that *Aleuroclava* is the oldest available name for this group but the type-species of that genus is known only from the inadequate original description'.

Hindaleyrodes Meganathan and David, 1994

Meganathan and David (1994) erected the genus *Hindaleyrodes* Meganathan and David, 1994 with *Hindaleyrodes hindustanicus* as the type species, distinguishing it from *Aleuroclava* by its 12 pairs of submarginal setae, the dorsum with minute pores and porettes and abdominal segment VII shorter than VIII. Martin and Mound (2007) synonymised the genus with *Aleuroclava* but did not comment on their reasoning. The type species is unusual in that it has 12 pairs of submarginal setae which are lacking in almost all of the other *Aleuroclava* species.

Martiniella Jesudasan and David, 1990

Jesudasan and David (1990) erected the genus *Martiniella*, designating *Aleurotuberculatus canangae* Corbett, 1935 as the type species of the genus whilst also placing *Aleurotuberculatus macarangae* Corbett, 1935 in the genus. They characterised the genus as being

closely allied to *Aleuroclava* Singh in possessing dorsal tubercles, submarginal papillae and other characters but differs from it by having distinct tuberculate setae on cephalic and first abdominal regions. It is closely related to *Taiwanaleyrodes* Takahashi, 1932 but differs from it in having dorsal tubercles, submarginal papillae and thoracic fold and cleft.

Sundararaj and David (1993) described *Martiniella ayyari* and *Martiniella lefroyi* from India, which both have a tracheal cleft. Martin (1999, p. 31) synonymised *Martiniella* with *Aleuroclava* Singh, 1931, stating that 'although unusual, setae of this type are sometimes present in species of *Taiwanaleyrodes* and *Dialeurodes*, and this character has also been seen to vary within samples (personal observations)'. Manzari and Quicke (2006) upheld the synonymy; however the synonymy was not accepted by authors Sundararaj and Dubey (2004) who upheld *Martiniella* as a valid genus and provided a diagnosis of the genus and a key to three species known from India, and described *Martiniella papillata* infesting *Xeromphis spinosa* from Goa, India which also has a pair of tracheal clefts. Sundararaj and Pushpa (2011) transferred to *Martiniella* two species that were

previously placed in *Taiwanaleyrodes* which have tuberculate setae with a wider basal part and a long slender apical part, *T. fletcheri* Sundararaj and David, 1992 and *T. tripori* Dubey and Sundararaj, 2006; and Vimala and Sundararaj (2015) reinstated *Martiniella* as a valid genus based on the constant presence of the tuberculate setae with a wider basal part and a long slender apical part as well as the thoracic cleft in all specimens they had observed since 1976. They provided a description of the genus and a key to species, and assigned five species then belonging to *Aleuroclava* to the genus: *Martiniella baccaureae* (Corbett, 1935), *Martiniella fici* (Corbett, 1935), *Martiniella macarangae* (Corbett, 1935), *Martiniella sepangensis* (Martin and Mound, 2007) and *Martiniella srilankaensis* (David, 1993).

Whether the species of *Aleuroclava* that have tuberculate Cs1 and As1 setae represent a valid genus (as some authors have treated them), a subgenus of *Aleuroclava*, or a species group is still debatable, primarily due to varying opinions regarding the importance assigned to this character and its variability. Herein, we propose the *Aleuroclava canangae* species group to accommodate these species. We consider them to belong to *Aleuroclava* because other than their tuberculate Cs1 and As1 setae with a wider basal part and a long slender apical part, they are very similar to the many other species in the genus, and because the length and shape of the dorsal setae has been shown to vary among individuals of the same species. For example, some individuals of *Aleuroclava jasmini* (Takahashi, 1932) have long Cs1 and As1 setae, whereas those setae are short in other individuals. *Aleuroclava* is the largest genus of whiteflies, and other species groups within the genus might be distinguished; however, further studies on the morphology of puparia and adults as well as molecular characterisation of other *Aleuroclava* species are needed to better understand the relationship among species of the genus.

The present study analyses and characterises the DNA and morphology of the puparia and adult stages of *Aleuroclava canangae* and compares them to those of other *Aleuroclava* and non-congeneric species; the *Aleuroclava canangae* species group is proposed for *Aleuroclava* species having long, tuberculate Cs1 and As1 setae with a wider basal part and a long slender apical part.

Methodology

Puparia and adult whiteflies were collected from an infested ornamental *Coleus* species and observed under a LEICA EZ4W stereomicroscope, and images were taken. Permanent slide mounts of selected specimens were prepared as per Martin *et al.* (2000) and relevant images were taken of the puparium, male genitalia, cement gland, antennal features, ommatidium and leg features using Nikon Eclipse Ni trinocular research microscope. The different taxonomic features were studied in detail. Based on the puparium features, the whitefly species was identified as *Aleuroclava canangae* by Dr Gregory A. Evans, United States Department of Agriculture/Animal and Plant Health Inspection Service, USA, and the identification was confirmed by Dr R. Sundararaj, Institute of Wood Science and Technology, Bengaluru, India. Voucher specimens of puparia and the adult male genitalia on permanent slides are deposited with Indian Council of Agriculture Research-National Bureau of Agricultural Insect Resources, Bengaluru, India repository as NIM/NBAIR/HEM-1/ 17721 and NIM/NBAIR/HEM-2/ 17721, respectively.

Molecular characterisation

Genomic DNA was isolated from adult whiteflies that emerged from puparia identified morphologically as *Aleuroclava canangae* collected from ornamental *Coleus* sp. found in a coconut system. The primers LCO1490 5'-GGTCAACAAATCATAAAGATATTGG-3' and HC02198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer *et al.* 1994) (Eurofins Genomics India, Bangalore) in salt-free status were used for the amplification of the mitochondrial *COI* gene, and a polymerase chain reaction (PCR) was performed following the procedure detailed by Sambrook and Russell (2001). All the PCR amplifications were carried out in a thermal cycler (Techne Flexigene) as follows: initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min 30s, and final extension at 72°C for 10 min for end filling (Josephraj Kumar *et al.* 2020). The reaction products were analysed in 1.0% agarose gel, and the gel was documented using a molecular imaging system (Molecular Imager® Gel Doc XR+, Bio-Rad, Hercules, California, USA). The amplicons obtained were purified from each reaction mixture using the PCR purification kit from Qiagen. The purified products were sequenced at AgriGenomeLabs Pvt. Ltd., Kochi, Kerala, and compared with the nucleotide sequences of previously identified whiteflies for validation. The *COI* nucleotide sequences of different whitefly species used for comparison were retrieved from GenBank (Benson *et al.* 1999) including nucleotide sequences deposited from our laboratory as well. BLAST (Altschul *et al.* 1990) was used to identify related sequences available in the GenBank database. Multiple sequence alignments were made using ClustalW. A phylogenetic tree was constructed with *COI* gene sequences using Mega XI software by the neighbour-joining method, with 1000 replications for bootstrap analysis and aphids and psyllids as an outgroup.

Results and discussion

Life stages

All life stages of *A. canangae* were found only on the underside of the leaves of ornamental *Coleus* plants during March–May of 2021 in Kayamkulam, Kerala, India. The life stages of the pest are mostly prevalent on the older leaves, covering the entire leaf surface (Figure 1B). Eggs are brown and laid on stalks along the leaf veins in rows (Figure 1B). Upon eclosion, the mobile crawlers (first-instar nymphs) move around the leaf surface and upon finding a suitable feeding site, shed their long legs and long antennae as they develop into second-instar nymphs, become sedentary and remain in the same site through the third and fourth nymphal stages. The mature nymph (puparium, fourth-instar nymph) possesses two reddish spots on the anterior region (Figure 1B (inset)) which we believe are the eyes of the adult developing inside the puparium (none of the species in the *A. canangae* group have eye spots in the nymphal stage) and has a pair of very elongate, conspicuous tuberculate setae on the cephalus and another pair on the first abdominal segment, which are also observed on the remains of the exuviae of the puparium after adult emergence (Figure 1C). Due to continuous feeding, honey dew excreted gets deposited on the upper leaf surface which leads to the development of sooty mould, interfering with photosynthesis and weakening the plant. Damage by this

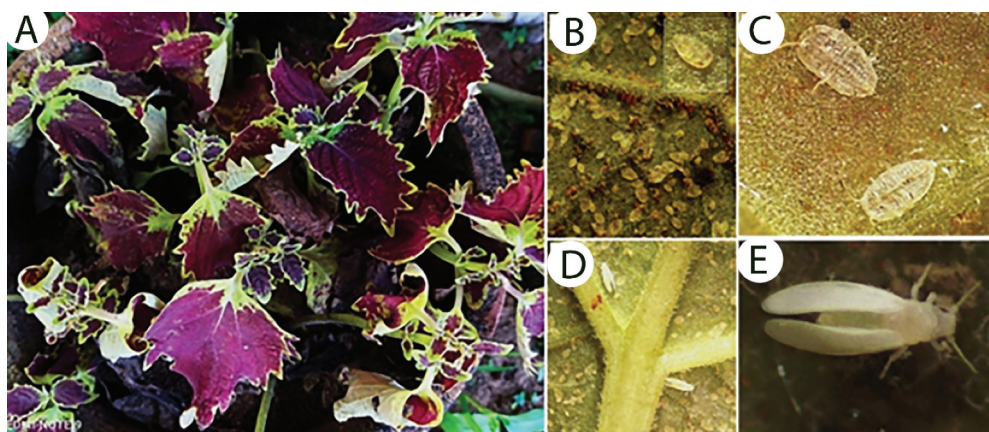


Figure 1. Feeding symptoms and life stages of *Aleuroclava canangae*. (A) damaged *Coleus* sp. plant; (B) eggs and immature stages on undersurface of leaf, with detail inset of a puparium showing its reddish spots; (C) exuviae with remnants of the thoracic and abdominal setae; (D) adults; (E) closer view of adult.

pest to *Coleus* plants is not very severe, causing neither death of the plant nor any visible feeding lesions (Figure 1A). Adult whiteflies are very tiny and have white wings that they hold roof-like over their body when resting (Figure 1D–E).

Molecular identity

To the best of our knowledge, our sequence (MX295258.1) is the first GenBank nucleotide deposit of *A. canangae* which has had its identity confirmed by the unique set of morphological characteristics of its puparium and it is the first description of the adults of the species. The *COI* sequences of *A. canangae* (MX295258.1) showed 83.28% identity with that of *A. indica* isolate YM (JQ340180). A phylogenetic tree was constructed with GenBank nucleotide sequences of select whitefly species belonging to the Aleurodicinae and Aleyrodinae subfamilies; two aphid species, *Acyrtosiphon pisum* Harris, 1776; *Cervaphis quercus* Takahashi, 1918 and the citrus psyllid, *Diaphorina citri* Kuwayama, 1908 were used as the outgroups (Figure 2). Different clades belonging to whitefly genera *Aleurodicus*, *Aleuronudus*, *Paraleyrodes* (Aleurodicinae), *Aleurocanthus*, *Aleuroclava*, *Aleurolobus*, *Aleurothrixus*, *Aleurotrachelus*, *Bemisia*, *Dialeurodes*, *Neomaskiella*, *Trialeurodes*, and *Vasdavidius* (Aleyrodinae) emerged with clear demarcation. At the base of the tree are seven species belonging to three genera of the Aleurodicinae subfamily. *Lecanoideus floccissimus* was described by Martin, Hernández-Suarez and Carnero, 1997 and was transferred to the genus *Aleurodicus* following the synonymisation of the genus with *Aleurodicus* by Martin (2008). The synonymy is supported by the results of the DNA analysis which shows the species between *Aleurodicus rugioperculatus* Martin, 2004, which has six pairs of compound pores on the abdomen; and *Aleurodicus dispersus* Russell, 1965 which, like *Aleurodicus floccissimus*, has four pairs of compound pores on the abdomen. Above these species is *Aleuronudus melzeri* Bondar, 1928, a genus and species

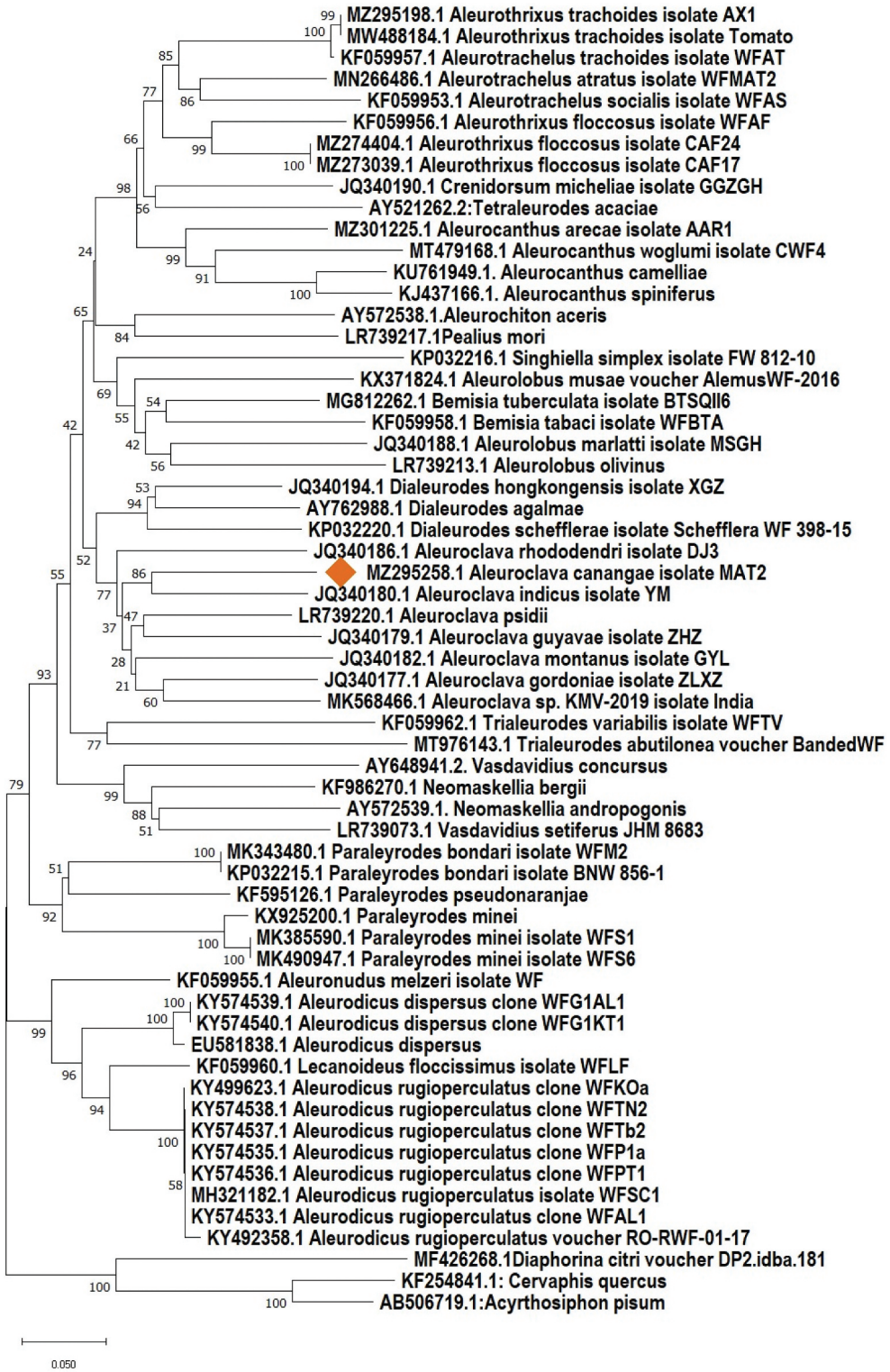


Figure 2. Phylogenetic tree derived from DNA analysis of select whitefly species.

that is the most similar to *Aleurodicus* in this analysis and is also corroborated based on the morphology of the puparia and adults (Evans *et al.* [in press](#)). These are followed by three aleurodicine species of the genus *Paraleyrodes* – *P. minei* Iaccarino 1990, *P. pseudonaranjae* Martin, 2000 and *P. bondari* Peracchi, 1971. Morphologically, *Paraleyrodes* falls between the two subfamilies Aleyrodinae and Aleurodicinae in that the forewing of the adults has 1 central vein (R) that is not forked (similar to almost all species of Aleyrodinae), whereas the R vein is forked in most aleurodicine species. The remainder of the tree comprises aleyrodine species. *Vasdauidius setiferus* (Quaintance and Baker), 1917, *Vasdauidius concursus* (Ko), 1998, *Neomaskellia andropogonis* Corbett, 1926 and *Neomaskellia bergii* (Signoret), 1868 are all Asian species found on grasses (Poaceae). Between these and the *Aleuroclava* species are two species, *Trialeurodes vaporariorum* (Westwood), 1856 and *Trialeurodes variabilis* (Quaintance), 1900. *Trialeurodes* species are similar to *Aleuroclava* species in that many of them have submarginal papillae and tubercles on the dorsum.

The *Aleuroclava* clade falls between species in the genus *Dialeurodes* and *Trialeurodes*. Manzari and Quicke (2006), in their cladistic analysis based on the morphological characteristics of over 430 species of whiteflies which included 14 species of *Aleuroclava* (none in the *A. canangae* species group), found *Aleuroclava* to be most closely related to *Dialeurodes* and *Minutaleyrodes* and distantly related to *Trialeurodes* species.

Next on the phylogenetic tree (Figure 2) are four *Aleuroclava* species – *A. gordoniae* (Takahashi, 1932), *A. guyavae* (Takahashi, 1932), *A. montanus* (Takahashi, 1939) and *A. psidii* (Singh, 1931) – and one unnamed *Aleuroclava* species. Morphologically, the four named *Aleuroclava* species are similar to each other in that they are black in colour, except for *A. psidii* which is variable in colouration, often pale with a dark streak down the centre of the body. Except for *A. montanus* which Takahashi (1939) described in the genus *Taiwanaleyrodes*, the other species were described in the genus *Aleurotuberculatus*, now a synonym of *Aleuroclava*. Takahashi (1932) erected the genus *Aleurotuberculatus* with *A. gordoniae* (Takahashi, 1932) as the type species. He distinguished it from *Aleuroclava* based primarily on the narrow submarginal band that is differentiated from the remainder of the dorsal disk by a suture. In addition, the cephalus of many species in this group has a sculptured arch which forms a T-shape with the longitudinal moulting suture; the cephalic and first abdominal segment setae are short to medium in length. Takahashi (1939) described *Aleuroclava montanus* as a *Taiwanaleyrodes* species but did not illustrate it. Suh (2009) provided photographs of *A. montanus* based on the identification confirmed by Dr Chiun-Cheng Ko, which show a dark species with a submarginal ridge and that appears to have a faint T-shaped figure on the cephalus.

Interestingly, among the *Aleuroclava* species, *Aleuroclava canangae* forms a sub-clade with *Aleuroclava indica* (Singh, 1931), which also has two pairs of very elongate, tuberculate setae. Since *Aleuroclava* is a feminine genus (ending in an 'a') the species epithet should be *indica* to reflect the female gender (Pellizzari and Williams, 2013). Herein, we propose the *Aleuroclava canangae* species group which includes 12 species previously assigned to *Martiniella*. Species in the *Aleuroclava canangae* species group can be characterised as being similar to other *Aleuroclava* species in that most have the lateral margin modified at the tracheal opening as a shallow, smooth and flat tracheal cleft without 'teeth' along its margins, the vasiform orifice cordate and with posterior margin incised medially and are pale in colour as in most species in the genus and differ by having two

pairs of tuberculate setae. It contains 12 species, all described from the Indomalayan region, with two species (*A. ayyari* and *A. lefroyi*) reported to occur also in China (Wang and Du 2015).

Species in the *A. canangae* group form a clade with *A. rhododendri* (Takahashi, 1935) which has the cephalic and first abdominal setae very elongate, albeit non-tuberculate, and appearing one-jointed, and provides evidence that supports the synonymy of *Martiniella* with *Aleuroclava*. However, the length of the dorsal setae has been shown to vary among individuals of the same species. For example, some individuals of *Aleuroclava jasmini* (Takahashi, 1932) have long Cs1 and As1 setae, whereas these setae are short in other individuals. Similarly, the shape and length of the dorsal setae of *Bemisia tabaci* (Gennadius, 1889) has been shown to vary based on the characteristics of the hosts that they are found on and other environmental factors. *Aleuroclava* is the largest genus of whiteflies, and other species groups within the genus might be distinguished; however, further studies on the morphology of puparia and adults as well as molecular characterisation of other *Aleuroclava* species are needed to better understand the relationship among species of the genus.

Directly above the *Dialeurodes* species are two species of *Aleurolobus* – *Aleurolobus olivinus* (Silvestri), 1911 and *Aleurolobus marlatti* (Quaintance), 1903 – which are followed by *Bemisia tabaci* (Gennadius), 1889, *Bemisia tuberculata* Bondar, 1923 and *Aleurolobus musae* Corbett, 1935. Morphologically, we do not see why the latter species is closer in the tree to the *Bemisia* species than the other *Aleurolobus* species.

Singhiella simplex (Singh), 1931 is an Asian species that has become an important pest of *Ficus* in several regions of the world. It is very similar to *Pealius mori* (Takahashi, 1932) and was placed in the genus *Pealius* for many years. These are followed by *Aleurochiton aceris* (Modeer, 1778); a genus is found on *Acer* species in Europe. The remaining species have the lateral margin undifferentiated at the tracheal opening and most of them are dark coloured species with or without a submarginal ridge. The four *Aleurocanthus* species, *Aleurocanthus arecae* David and Manjunatha, 2003, *Aleurocanthus camelliae* Kanmiya and Kasai, 2011, *Aleurocanthus spiniferus* (Quaintance), 1903 and *Aleurocanthus woglumi* Ashby, 1915, cluster together; members of this genus lack the submarginal ridge and have long spine-like dorsal setae. *Crenidorsum micheliae* (Takahashi), 1932 is a pale species that has a pair of submedial sutures on the cephalothorax similar to that of *Aleurotrachelus* species. The three isolates of *Aleurothrixus floccosus* (Maskell), 1895 formed a clade separate from the *Aleurotrachelus* species – *Aleurotrachelus socialis* Bondar, 1923, *Aleurotrachelus atratus* Hempel, 1922 and *Aleurotrachelus trachoides* (Back, 1922). Dubey and Sundararaj (2015) transferred the latter species to the genus *Aleurothrixus*; however, the results of the DNA analysis indicate that it clusters with the other *Aleurotrachelus* species, which is supported by the morphological characters of having the vasiform orifice cordate, the submarginal ridge divided under the vasiform orifice, lack of submarginal setae and presence of a pair of submedial sutures on the cephalothorax, as opposed to *Aleurothrixus* species which have the vasiform orifice transverse (wider than long), the submarginal ridge not divided under the vasiform orifice, submarginal setae present at least on the cephalothorax, and lacking a pair of submedial sutures on the cephalothorax (Arulappan *et al.* 2022).

The following key to puparia of the species in the *Aleuroclava canangae* species group was created based on the original illustrations, species descriptions and keys to species in

this group provided by the respective authors; only actual specimens of *A. canangae* collected in our study were examined in detail. Admittedly, there are shortcomings to this course of action: important details for each species may have been overlooked. However, we decided to take this course of action because the type specimens of almost all of the species were not available to the authors for examination and we considered it important to bring all of this data together in one place so that the species can be compared and contrasted.

Key to puparia of the species in the *Aleuroclava canangae* species group

1. Lateral margin differentiated at thoracic tracheal opening as a pore or cleft 2
- 1b. Lateral margin undifferentiated at thoracic tracheal opening 6
- 2(1) Tracheal opening large, round and drop-shaped; submargin without a row of papillae; dorsum smooth; vasiform orifice (vo) cordate; body 1.3× as long as wide; caudal setae (Cas) 1.3× as long as the vo; China, India on *Litsea* and *Mussaenda* (Figure 3A) *ayyari* Sundararaj and David
- 2b. Tracheal opening cleft shaped; submargin with or without a row of papillae; dorsum and vo variable; body 1.4–1.7× as long as wide; Cas/vo 1.0–6.0× as long as the vo 3
- 3(2b) Submargin without a row of papillae; dorsum punctate; vo subrectangular; Cas 1.0× vo; setae on first abdominal segment (As1) 2.0× radius; China, India on *Elatostema* (Figure 3G). *lefroyi* Sundararaj and David
- 3b. Submargin with a row of papillae; Cas 2× as long as the vo (except 0.5× in *A. fletcheri*); As1 setae less than 2.0× radius 4
- 4(3b) Submargin with punctate sculpture and with about 40 pairs of tall papillae with pointed apices extending to the lateral margin; dorsum of abdomen without rows of round pores; body 1.7× as long as wide; Cs1 1.8× radius; As1 1.5× radius; India on *Xeromphis* (Figure 3I) *papillata* Sundararaj and Dubey
- 4b. Submargin smooth and with about 20 pairs of short papillae with rounded apices not extending to the lateral margin; body 1.4–1.5× as long as wide; Cs1 1.6–1.7× radius 5
- 5(4b) Dorsum pale with a dark central area; Cas 2.4× vo; submedian area of cephalothorax with three pairs of enlarged tubercles; entire dorsum with microtubercles; India, Malaysia on *Cananga*, *Coleus*, *Psidium* (Figure 3C). *canangae* (Corbett)
- 5b. Dorsum entirely pale; Cas 6.0× vo; submedian area of cephalothorax without enlarged tubercles; microtubercles present only on the submarginal or submedial region; dorsum of abdomen with 3 rows of 5–6 evenly spaced round pores; Malaysia on *Macaranga* (Figure 3H) *macarangae* (Corbett)
- 6(1b) Submargin with three pairs of large subcircular lobes; dorsum with reticulate sculpture; lateral margin where thoracic tracheal furrow undifferentiated from lateral margin; submargin without a row of papillae; Cs1 and As1 subequal, each about 1.9× vo; India on *Ficus* (Figure 3L) *tripori* (Dubey and Sundararaj)

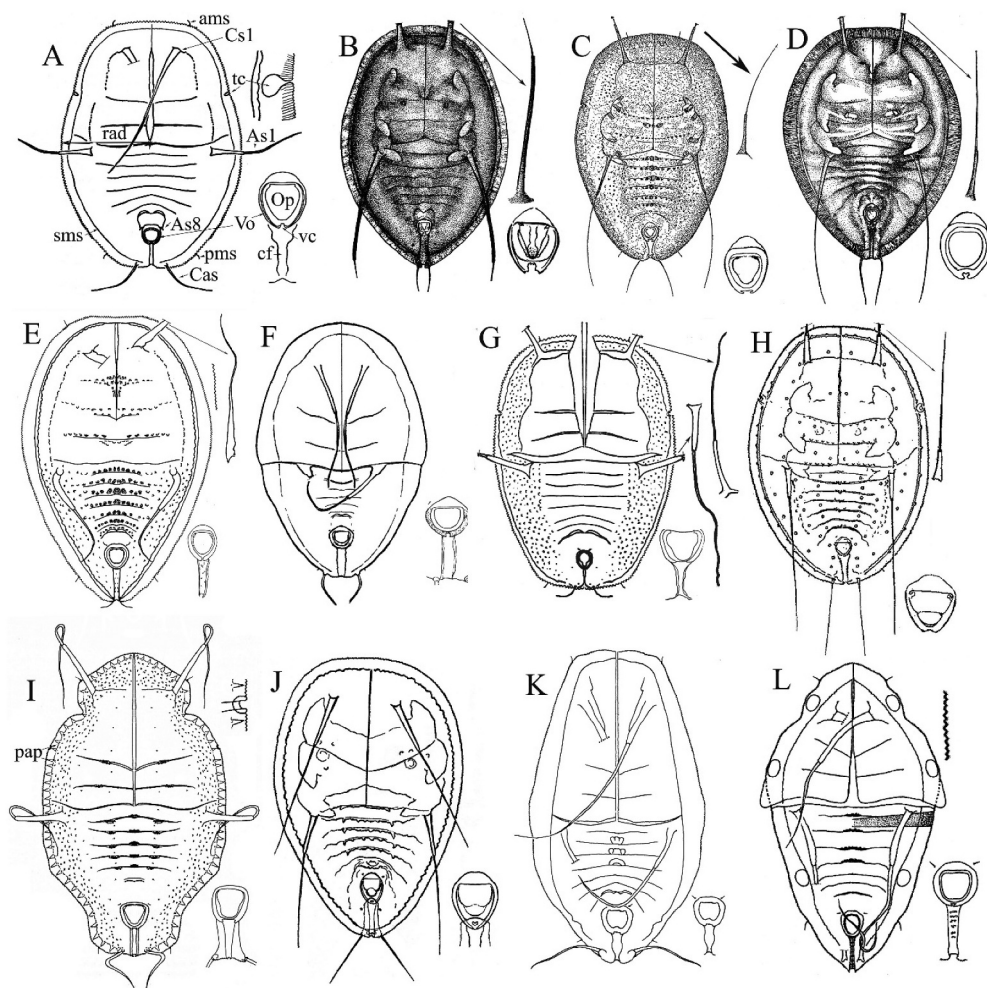


Figure 3. Puparia of the *A. canangae* species group (A) *ayyari*, (B) *baccaureae*, (C) *canangae*, (D) *fici*, (E) *fletcheri*, (F) *indica*, (G) *lefroyi*, (H) *macarangae*, (I) *papillatus*, (J) *sepangensis*, (K) *srilankaensis*, (L) *tripori*. Abbreviations: ams (anterior marginal setae), As1 (first abdominal setae), As8 (eighth abdominal seta), Cas (caudal setae), cf (caudal furrow), Cs1 (cephalic setae), op (operculum), pap (papillae), pms (posterior marginal setae), tc (tracheal cleft), vc (vasiform orifice cleft), vo (vasiform orifice). Sources: A, B, E: Sundararaj and David (1993); C, D, J–L: Corbett (1935); E, F: Sundararaj and Dubey (2004); G: Sundararaj and David (1992); H: David and Subramaniam (1976); I: David (1993).

- 6b. Submargin without large subcircular lobes; dorsum not reticulate; lateral margin variable; submargin with or without a row of papillae; Cs1 and As1 not subequal and each about 1.9× vo 7
- 7(6b) Submargin with striate sculpture; cleft along the posterior margin of vasiform with a medial tooth 8
- 7b. Submargin smooth; cleft along the posterior margin of vasiform without a medial tooth 9

- 8(7) Apical joint of Cs1 setae much longer (about 2×) as long as the basal joint; Cas 5× vo; Cs1 and As1 subequal, each 1.6× radius; Malaysia on *Ficus* and *Euphorbia* (Figure 3D) *fici* (Corbett)
- 8b. Apical joint of Cs1 setae much shorter (about 0.3×) as long as the basal joint; Cas 2.0× vo; Cs1 2.1× and As1 1.6× radius; Malaysia on *Baccaurea* (Figure 3B) *baccaureae* (Corbett)
- 9(7b) Cas short, 0.5–1.3× vo; Cs1 1.5× radius; As1 1.1–1.2 radius 10
- 9b. Cas longer, 3.0× vo; Cs1 1.9–2.0× radius; As1 1.5–1.9 radius 11
- 10(9) Vo wider than long; dorsum smooth; abdominal segments without median tubercles; Hong Kong, India, Malaysia on various hosts (Figure 3F) *indica* (Singh)
- 10b. Vo longer than wide; dorsum smooth with punctate areas, abdominal segments with median tubercles extending along the abdominal sutures; India, various hosts (Figure 3E) *fletcheri* (Sundararaj and David)
- 11(9b) Abdominal segments II to IV with median tubercles; vo quadrate; caudal furrow not closed at its anterior end; As1 1.9× radius; Sri Lanka on *Macaranga* (Figure 3I–K) *srilankaensis* (David)
- 11b. Abdominal segments II–V and VII with chitinised thickened areas and extending into subdorsal area; vo longer than wide; As1 1.5× radius; India, Malaysia on *Macaranga* (Figure 3J) *sepangensis* (Martin and Mound)

Aleuroclava canangae species group

Diagnosis

Oval shaped, 1.3–1.7× as long as wide; cephalic (Cs1) and first abdominal segment setae (As1) extremely long (1.1–2.0× radius) and tuberculate and set on tubercles; vasiform orifice (vo) cordate with a cleft along the posterior margin medially; operculum filling almost the entire orifice except less so in *A. indica*; setae on eighth abdominal segment (As8) short 0.1–0.7× length of the vo; caudal setae (Cas) long in most species 0.5–6.0× the length of the vo and set on tubercles; tracheal furrow terminating in a pore, cleft or undifferentiated from the lateral; and dorsal tubercles and submarginal papillae often present. All of the known species in this group are primarily pale in colour (some species such as *A. canangae* with faint brownish areas medially) and have the dorsum entire (submarginal furrow/fold absent). Species in the *A. canangae* species group are most similar to *Aleuroclava* species that have the Cs1 and As1 setae very long, but are non-tuberculate and not set on tubercles (Table 2). Measurements were taken from illustrations of each species. The radius is measured from the junction of the longitudinal furrow on the cephalothorax and the transverse moulting suture that separates the cephalothorax from the abdomen following a straight line perpendicular to the longitudinal furrow, across half of the body to the lateral margin (Figure 3A). The distribution column shows the country from which the species was described followed by subsequent country records.

Table 2. Characteristics of the *Aleuroclava canangae* species group.

| Species | BL/BW | Cs1/rad | As1/rad | Cas/vol | As8/vol | Tracheal cleft | Distribution |
|----------------------|-------|---------|---------|---------|---------|----------------|----------------------------|
| <i>ayyari</i> | 1.3 | 1.6 | 1.1 | 1.3 | 0.3 | Present | India |
| <i>baccaureae</i> | 1.4 | 2.1 | 1.6 | 2.0 | 0.7 | Absent | Malaysia |
| <i>canangae</i> | 1.5 | 1.6 | 1.8 | 2.4 | 0.2 | Present | Malaysia; India; Indonesia |
| <i>fici</i> | 1.3 | 1.6 | 1.6 | 5.0 | 0.3 | Absent | Malaysia |
| <i>fletcheri</i> | 1.5 | 1.5 | 1.1 | 0.5 | 0.3 | Absent | India |
| <i>indica</i> | 1.7 | 1.5 | 1.2 | 1.3 | 0.2 | Absent | India; Hong Kong; Malaysia |
| <i>lefroyi</i> | 1.5 | 1.8 | 2.0 | 1.0 | 0.4 | Present | India; China |
| <i>macarangae</i> | 1.4 | 1.7 | 1.9 | 6.0 | 0.3 | Present | Malaysia |
| <i>papillata</i> | 1.7 | 1.8 | 1.5 | 2.2 | 0.1 | Present | India |
| <i>sepangensis</i> | 1.5 | 1.9 | 1.5 | 3.0 | 0.2 | Absent | Malaysia; India |
| <i>srilankaensis</i> | 1.6 | 2.0 | 1.9 | 3.0 | 0.2 | Absent | Sri Lanka |
| <i>tripori</i> | 1.8 | 1.9 | 1.9 | 2.5 | 0.2 | Absent | India |

BL/BW = body length/body width; Cs1/rad = length of Cs1 setae/body radius; As1/rad = length of As1 setae/radius; Cas/vol = length of caudal setae (Cas)/length of the vasiform orifice; As8/vol = length of 8th abdominal setae/length of vasiform orifice; cleft: absent, present. Measurements taken from illustrations of each species. The radius is measured from the junction of the longitudinal furrow on the cephalothorax and the transverse moulting suture that separates the cephalothorax from the abdomen following a straight line across half of the body to the lateral margin.

Aleuroclava canangae (Corbett, 1935)

Diagnosis

Aleuroclava canangae can be distinguished from other species in the *canangae* species group by the following combination of characteristics: lateral margin differentiated at the opening of the thoracic tracheal furrow as a cleft; submedian area of the dorsum of the cephalothorax with three pairs of enlarged tubercles; and the entire dorsum with micro-tubercles. It is most similar to *A. macarangae* (Corbett) and *A. papillata* Sundararaj and Dubey, but can be distinguished from those species which lack the three pairs of enlarged tubercles on the submedian area of cephalothorax and have microtubercles on the subdorsum only.

Puparium

Colouration. Colouration is variable, some specimens entirely pale while other specimens pale with a faint dark brown streak down the centre of the body, covering the area above the mouthparts, the medial region of the first to third abdominal segments and the area above the vasiform orifice.

Dorsum. Mean length and width 590.65 and 403.41 μm , respectively; body elliptical with entire dorsum covered with microtubercles and granules (Figure 4A, F); lateral margin crenulated, tracheal furrow terminating in a cup-shaped sunken cleft (Figure 4C, G) along the lateral margin; submarginal region with row of conical papillae around the entire body, except within the caudal furrow, and separated from each other by about the width of their base; submarginal fold wavy with conspicuous stipples and with short setae along the fold. **Cephalothorax:** base of cephalic setae with long, slender U-shaped sclerotisation; submedian area with three pairs of enlarged tubercles; transverse moulting suture extends to submedian region. **Abdomen:** eight-segmented, medial region with transverse rows of rounded microtubercles along the abdominal sutures; caudal furrow smooth, about as long as the vasiform orifice with well-defined sclerotised sides. **Vasiform orifice**



Figure 4. *Aleuroclava canangae* puparium. (A–E) USNM specimen (intercepted on *Psidium guajava* from the Philippines) – (A) habitus, with cephalic setae (Cs1), first abdominal segment setae (As1), caudal setae (Cas); (B) As1 two-jointed seta; (C) tracheal cleft; (D) vasiform orifice (vo) with operculum (op) and eighth abdominal segment setae (As8); (E) caudal furrow with vasiform orifice cleft (vc); (F–J) Indian specimen – (F) habitus; (G) thoracic cleft; (H) crenulate lateral margin with submarginal papillae; (I) vasiform orifice with caudal furrow (cf) and posterior marginal setae (pms); (J) vo showing position of As8 setae.

(vo): orifice subcordate, slightly wider ($41.21\ \mu\text{m}$) than long ($32.53\ \mu\text{m}$) and with a wide notch/cleft located medially along the posterior margin, operculum filling nearly the entire orifice obscuring the lingula (Figure 4D, E, I). *Chaetotaxy*: lateral margin with one pair of very short ($20.33\ \mu\text{m}$) anterior marginal setae (ams) and posterior marginal setae (pms) similar in length (Figure 4H); cephalic pair of setae (Cs1) ($421.41\ \mu\text{m}$) separated from each other by $75.05\ \mu\text{m}$ (Figure 4B); first abdominal segment setae (As1) extremely long ($433.50\ \mu\text{m}$) slightly shorter than the width of the body, tuberculate, set on elevated long tubercles (Figure 4A) and separated from each other by $165.39\ \mu\text{m}$; eighth abdominal setae (As8) very short ($15.56\ \mu\text{m}$) (Figure 4J), rod-like and located just above the lateral margin of each side of the orifice; caudal setae prominent ($100.45\ \mu\text{m}$), about $2\times$ as long as the length of the vasiform orifice ($34.53\ \mu\text{m}$) and set on tubercles (Figure 4I).

Adult female (Figure 5A–C)

Colouration. body pale yellow; forewing and hindwings entirely white. *Antennae* (Figure 5A): seven-segmented consisting of a short basal segment (scape), pedicel, and a long five-segmented flagellum (F3–F7); pedicel about as long as wide; F3 $9.3\times$ as long as wide with two annulated-round, flat sensoria at its distal end, aligned one under the other and a long sensorium arising from the mid position and extending up to the first round sensorium (pores not seen); F4 $2.5\times$ as long as wide and without sensoria; F5 $3.2\times$ as long as wide with a small, annulated-round, flat sensorium; F6 $3.3\times$ as long as wide with a long sensoria; F7 (terminal segment) very long, $6.0\times$ as long as wide, tapering distally and terminating with a seta at the tapering end, with a very long sensorial cone arising at about the basal third of the segment and extending past the round flat sensorium located towards the tip of the segment (Figure 5A). *Eyes*: Upper to the lower eye connected by 3–4

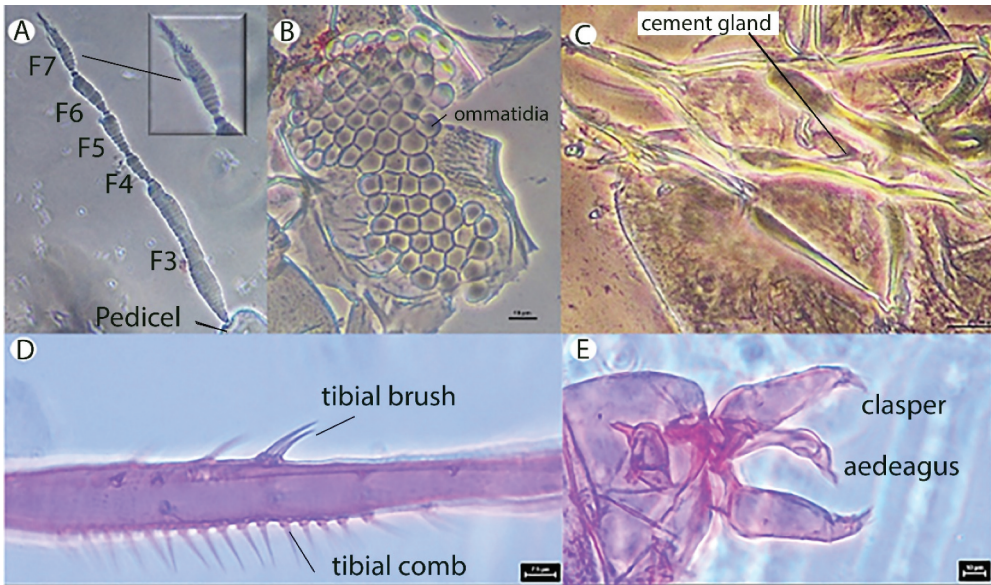


Figure 5. *Aleuroclava canangae* adults. (A) Antenna (female), inset – closer view of terminal antennal segment with long sensorial cone; (B) upper and lower eye (female); (C) female genitalia with cement gland; (D) tibia of leg II with one row of comb and brush with two setae (male); (E) male genitalia.

ommatidia (Figure 5B). *Legs*: Hind tibia with a conspicuous tibial comb consisting of 17 setae in a row, tibial brush with two setae (Figure 5D).

Abdomen. With two pairs of wax plates, *vasiform orifice*: Cordate to triangular in shape, operculum trapezoidal, lingula included, concealed by the operculum; cement gland $63.2 \pm 2.8 \mu\text{m}$ long which has a cup-shaped unipolar head and a long and wavy shaft bulbous medially (Figure 5C).

Adult male (Figure 5D, E)

Similar to female in colouration and shape, but has four pairs of wax plates on the abdomen which is the typical number of wax plates for the males and females in species in the Aleyrodinae subfamily. *Genitalia*: Claspers stout $66.7 \pm 6.4 \mu\text{m}$ long with swollen pads terminating with a terminal spine and one spine on the lateral side. Aedeagus $47.3 \pm 3.5 \mu\text{m}$ long, has a characteristic constriction before bulging at the terminal end with a few prominent horns (Figure 5E).

Distribution

Prior to it being found on the ornamental *Coleus* plant in Kayamkulam, Kerala, India, during 2020–2021, *A. canangae* was known only from Malaysia on the cananga tree, *Cananga odorata*, a fast-growing tree which is native to Southeast Asia, and on guava, *Psidium guajava* (Corbett, 1935). It was also recently reported on salak (*Salacca zalacca*) and *P. guajava* from Bogor, Indonesia (Hidayat *et al.* 2018). The United States Museum of

Natural History (USNM) has specimens of this species intercepted at a US Port of Entry on *Psidium guajava* from the Philippines and Thailand in 1985 and 1981, respectively, and one specimen from Thailand on *Lansium domesticum* intercepted in 1980.

Abbreviations

COI, cytochrome c oxidase subunit 1; DNA, deoxyribonucleic acid; NA, Nearctic; NT, Neotropical; WP, Western Palearctic; AF, Afrotropical; MA, Malagasian; EP, Eastern Palearctic; IM, Indomalayan; AU, Australian; PI, Pacific Islands; WW, worldwide; Ts1, prothoracic setae; Ts2, mesothoracic setae; Ts3, metathoracic setae; Cs1, cephalic setae; As1, first abdominal segment setae; R, central vein on wings; BL, body length; BW, body width; rad, body radius; Cas, caudal setae; vol, length of vasiform orifice; vo, vasiform orifice; op, operculum; vc, vasiform orifice cleft; cf, caudal furrow; ams, anterior marginal setae; pms, posterior marginal setae; F, flagellum; ICAR-CPCRI, Indian Council of Agricultural Research – Central Plantation Crops Research Institute; ICAR-NBAIR, Indian Council of Agricultural Research – National Bureau of Agricultural Insect Resources; USNM, United States Museum of Natural History; USDA/APHIS, United States Department of Agriculture/Animal and Plant Health Inspection Services; IIWST, Indian Institute of Wood Science and Technology.

Conclusion

The identification of whiteflies to the level of species has become increasingly important due to the great increase in the volume and diversity of plant products which are transported throughout the world each day. This has increased the ability of species to inhabit and thrive in their new environment, separated from their natural enemies, and has often led to major crop losses in the areas where they have invaded. Early detection and accurate species identification are essential to thwarting the introduction or mitigating the impact of invasive species by unlocking all that is known regarding the distribution, hosts and natural enemies of the pest species. Species identification is also key to understanding the fauna of the country and the ecological relationships of the species in their environment.

The identification of whiteflies to the level of species has been almost entirely based on the morphological characteristics of the puparium of the species. Very few studies have been conducted on the morphological characteristics of the adults of whitefly species and only a small fraction of the 1702 known species of whiteflies have undergone DNA analyses; studies such as these are essential to gaining a better understanding of the intraspecific variation that occurs in the species and detecting the presence of cryptic species. Detailed morphological studies of the puparia and adults of many more species of whiteflies, coupled with molecular analyses, need to be conducted to gain a better understanding of the species and the role it plays in the environment.

Acknowledgements

The authors profusely thank ICAR, New Delhi, for the grant in-aid through Institute Research Project No. 1000765041; we also thank the Director, ICAR-CPCRI, Kasaragod, Kerala, India, and Head, ICAR-

CPCRI, Regional Station, Kayamkulam, Kerala, India, for extending all support and facilities for the research programme. We express special gratitude to the Association for Advancement of Entomology, Thiruvananthapuram, Kerala, India, for their permission to use the images of puparium published in the journal *Entomon*, and Dr Ian Stocks, who helped with cleaning up and clarifying some of the images used. We are extremely grateful to Ms N. Preetha and Ms K. Ponnu Sugathan for their assistance in processing the samples of the puparia and recording morphometric data. We express sincere gratitude to the two anonymous reviewers for their assistance in critical re-structuring of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The work was primarily carried out by the senior author (Josephraj Kumar) whose work is supported by his institution, ICAR; and the 2nd author (Evans) by his institution USDA.

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