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A revision of the genus *Orosius* Distant (Hemiptera: Cicadellidae) based on male genitalia and DNA barcoding

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http://zoobank.org/urn:lsid:zoobank.org:pub:6FA164B9-C2D6-4982-B94F-1291EAB15B7E

Abstract A morphological and molecular review of the genus *Orosius* Distant (Deltocephalinae: Opsiini) was undertaken using male genitalia and DNA barcoding. We recognise 14 valid species of the genus, of which two were indeterminate, based on females only and recognised only by DNA barcodes. *Orosius argentatus* (Evans) stat. rev. is removed from synonymy with *Orosius orientalis* (Matsumura) after DNA barcoding demonstrated that the two species are distinct; small but consistent differences in the male genitalia are also found to support the two species hypothesis. The other species, recognised on male genitalia, are confirmed with DNA barcode sequence data. These species are *Orosius albicinctus* Distant, *Orosius lotophagorum* (Kirkaldy), *Orosius canberrensis* (Evans), *Orosius ryukyuensis* (Ishihara) and *Orosius cellulosus* (Lindberg), plus five new species described herein as *Orosius albifrons* Fletcher & Löcker sp. nov. and *Orosius brunneus* Fletcher & Löcker sp. nov. from Barrow Island, Western Australia, *Orosius recurvus* Fletcher & Löcker sp. nov. from New South Wales and *Orosius magareyi* Fletcher & Löcker sp. nov. and *Orosius pallidus* Fletcher & Löcker sp. nov. from Loxton, South Australia. This is the first record of *O. cellulosus* from Australia. *Orosius aegypticus* Ghauri syn. nov. is synonymised with *O. albicinctus*, the synonymy of *Nesaloha cantonis* Oman syn. nov. is transferred to *O. orientalis* from *O. argentatus* and *Orosius argentatus novaebrittaniae* Ghauri syn. nov. is synonymised with *O. orientalis. Orosius minuicus* Dlabola is excluded from the genus. A key for the discrimination and identification of the species based on male genitalia is provided.

Key words Cicadomorpha, integrative taxonomy, leafhopper, Opsiini.

INTRODUCTION

One of the more economically significant leafhopper genera is *Orosius* Distant (Deltocephalinae: Opsiini) which contains at least three species recognised as vectors of serious phytoplasma diseases in the Middle East, North Africa, the Oriental region and Australia. However, there has been confusion with the nomenclature of a number of species within the genus, particularly with the use of the name *Orosius orientalis* (Matsumura, 1914) in Australia (e.g. Trébicki *et al.* 2009), Israel (Klein *et al.* 2001, Weintraub *et al.* 2004), Pakistan (Akhtar *et al.* 2009) and Turkey (Sertkaya *et al.* 2007; Ikten *et al.* 2014).

The most recent revision of the genus was by Ghauri (1966) who recognised seven species, *Orosius albicinctus* Distant, 1918 (India, Middle East, North Africa), *Orosius cellulosus* (Lindberg, 1927) (North Africa), *Orosius lotophagorum* (Kirkaldy, 1907) (Polynesia, Melanesia, Australia), *Orosius argentatus* (Evans, 1938) (Australia, Fiji, Melanesia, Indonesia), *Orosius cantonis* (Oman, 1943) (Canton Island, Eniwetok Atoll), *Orosius canberrensis* (Evans 1938) (Australia) and *Orosius aegypticus* Ghauri 1966 (Egypt). He included description of a new subspecies *novaebritanniae* of *O. argentatus* characterised by darker colouration, and noted that his recognition of *O. aegypticus* as a valid species was tentative because it was based on a single male which may have been deformed. Ghauri (1966) did not treat *O. orientalis* other than to tentatively refer it to *O. cantonis*, based on examination of figures of the species from Taiwan (= Formosa) published by Ishihara (1963).

Linnavuori (1975) synonymised *O. cantonis* with *O. argentatus* and Kwon and Lee (1979) synonymised *O. argentatus* with *O. orientalis* but this latter synonymy was not accepted by Day and Fletcher (1994) for the same reason that

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Ghauri (1966) did not accept the synonymy with *O. cantonis* and that was because the original description was from Japan and no Japanese specimens had been examined. Further complication was added to the nomenclature of the genus by Ishihara (1982) who proposed a synonymy between *O. orientalis* and *O. albicinctus* Distant. As a result, recent publications from the Middle East and central Asia have used *O. orientalis* in preference to *O. albicinctus.*

Examination of specimens identified as *O. orientalis* from Japan and the Middle East has shown that the species known as *O. orientalis* in the Middle East is *O. albicinctus* while the species in Korea treated by Kwon and Lee (1979) as *O. orientalis* and specimens of *O. argentatus* from Australia closely match the male genitalia of *O. orientalis* in Japan. Consequently, recent publications from Australia (Fletcher 2009; Trébicki *et al.* 2009) have used *O. orientalis* in preference to *O. argentatus.*

A number of species which were previously included in *Orosius* have been transferred to other genera. *Orosius maculatus* Pruthi from India was transferred to *Pruthiorosius* Ghauri by Ghauri (1964) and *O. santali* Pruthi, also from India, was transferred to *Acacimenus* Dlabola by Viraktamath (1999). By contrast, *Thamnotettix puellus* Melichar (1911) from Eastern Africa was suggested as possibly being a species of *Orosius* by Ghauri (1966) but none of the type material could be found and the species remains unknown.

Recent surveys of Barrow Island, Western Australia (Majer *et al.* 2008a, 2008b; Gopurenko *et al.* 2013), of grapevines at Loxton, South Australia and of lucerne in New South Wales (Pilkington *et al.* 2004b) have revealed that undescribed species of *Orosius* occur in all three States. This raised the possibility that the Australian fauna of the genus was considerably more diverse than previously suspected.

Arguably, the most significant recent aid to traditional alpha-taxonomy has come from the advent of molecular systematics and DNA barcoding (Hebert *et al.* 2003). DNA barcoding, i.e. sequence analysis of the 5′-region of the mitochondrial cytochrome c oxidase I (COI) gene, is used primarily for faunal species identifications via genetic distance associations. The method also allows independent testing of species hypotheses where there is subtle morphological evidence suggestive of species separations (Bellis *et al.* 2013) and also where morphologically cryptic species cannot be distinguished (Bellis *et al.* 2014). This integrative taxonomic approach allows a rigorous approach to species delimitation by examining for concordance across multiple forms of evidence contained primarily in molecular and morphological data sets (Dayrat 2005; Gopurenko *et al.* 2015).

In an attempt to clarify species identifications among Australian species of *Orosius*, we conducted a reciprocal DNA barcode analysis with morphological examination of specimens from all described taxa except *Orosius argentatus novaebrittaniae* Ghauri, for which fresh material was unobtainable. Specimens were identified to species level using Ghauri's (1966) key and species were examined for evidence of genetic monophyly at DNA barcodes.

MATERIALS AND METHODS

Specimen preparation and morphological analysis

Recently collected specimens of most of the described species of *Orosius* were sourced from North Africa, the Middle East, India, Japan, China, Pakistan and Australia. No recently collected specimens of *O. argentatus novaebrittaniae* Ghauri were available and only morphological examination of the original material was possible. Specimens were catalogued and stored in ASCU (see list of abbreviations below).

The abdomen was removed from adult specimens and digested using a non-destructive (or semi-destructive) technique (Gopurenko *et al.* 2013) which clears the abdomen for subsequent genitalia examination and provides a DNA sample. The abdomens were then washed and transferred to glycerine for examination and, eventually, storage in vials attached to the pin of the specimens from which they had been removed. The male aedeagus was illustrated in lateral and ventral view for all species. In addition to line drawings, photographs of these same views were taken using a Micropublisher 5 RTV digital camera (QImaging) attached to a Leica MZ12.5 dissecting microscope, with montaged images and *habitus* photographs produced using AutoMontage Pro (Synchroscopy P/L) for all species.

Total length measurements (from the anterior tip of the head to the apex of the forewing) are given for each species based on the specimens for which COI sequences were successfully obtained.

Abbreviations of institutions used are: AM, Australian Museum, Sydney, NSW; ASCU, Biosecurity Collections Unit, Orange Agricultural Institute, Orange, NSW; BMNH, The Natural History Museum, London, UK; BPB, Bishop Museum, Honolulu, Hawaii; EIHU, Hokkaido University, Sapporo, Japan; EUMJ, Ehime University, Matsuyama, Japan; FMNH, Finnish Museum of Natural History, Helsinki, Finland; IRIPP, Iranian Research Institute of Plant Protection, Teheran, Iran; MNHN, Museum National d'Histoire Naturelle, Paris; NWAFU, Northwest Agriculture and Forestry University Yangling, China; USNM, United States National Museum, Washington DC; WAM, Western Australian Museum, Perth, WA.

DNA barcoding

Adult *Orosius* ($N = 225$) and two nymphs used in DNA barcoding analyses (Supplementary Table S1) included specimens from Australia ($N = 199$) and other countries ($N = 28$). Males comprised $> 60\%$ of the samples. DNA was extracted from specimen digestions using a Corbett Research 1820 X-tractor Gene robotic system with recommended protocols and DNA extraction kit reagents (QIAGEN); final DNA elutions (150 µl) were stored at −20 °C. The COI DNA barcode region was amplified by polymerase chain reaction (PCR) using primer BC1Fm with either JerR2m or Scar-3RDm to provide 646 bp and 667 bp products, respectively (Table 1). PCR preparations, thermal profiles and quality checking followed that reported in Gopurenko *et al.* (2013). We also used PCR procedures reported by Mitchell (2015) to amplify short (<400 bp) overlapping portions of the DNA barcode region. Primer pairs (BC1Fm and

Table 1 Primers used for PCR amplification of partial COI gene products. Degenerate bases in lowercase. Seventeen base pair M13-vector sequence 5′-tails are italicised and underlined in forward and reverse primers, respectively. Primer sources: (1) Cho *et al.* (2008); (2) Mitchell (2015); (3) Bellis *et al.* 2013; (4) Mitchell and Maddox (2010)

Primer	Sequence (5′ – 3′)	Source
BC1Fm	*GTAAAACGACGGCCAGT*TCwACwAAyCAyAArGAyATyGG	1
AMbc3f1m	*GTAAAACGACGGCCAGT*GChCChGAyATAGCnTTyCCnCG	2
miniScarFm	*GTAAAACGACGGCCAGT*TTyCCnCGrmTrAAyAAyATrAG	2
AMbc5r2m	CAGGAAACAGCTATGACGTTCAnCCnGTwCCwGCnCC	2
AMbc5r1m	CAGGAAACAGCTATGACGAdArwGGnGGrTAnACdGTTC	2
AMbc3r1m	CAGGAAACAGCTATGACAryATnGTrATnGCnCCnGC	2
JerR2m	CAGGAAACAGCTATGACCCAAArAAyCArAAyArrTGyTG	3
Scar-3RDm	CAGGAAACAGCTATGACAAAATrTAwACTTCdGGrTGnCC	4

AMbc5r1m) and (AMbc3f1m and AMbc3r1m) were used in PCR amplification of 329 bp and 319 bp portions of the 5′ and 3′ DNA barcode regions, respectively. Serial PCR was used to enrich poorly amplified short PCR products, using prior PCR product (diluted 1:100) as template (1:15) in reactions and employing primer pairs (BC1Fm and AMbc5r2m) and (miniscarFm and AMbc3r1m) to amplify 313 bp and 304 bp of the 5′ and 3′ DNA barcode regions, respectively. All primers (Table 1) were M13 tailed to simplify downstream sequencing. PCR products were purified and bi-directionally sequenced at the Australian Genome Research Facility (Brisbane).

Sequence analyses and integrative taxonomy

Bidirectional AB1 sequence trace files were assembled, quality checked and aligned against a Deltocephaline reference COI sequence [*Nesophrosyne pipturi*; Bennett and O'Grady (2012); GenBank accession # JX433140] using Lasergene SeqMan Pro ver. 8.1.0(3) (DNASTAR Inc., Maddison, WI, USA, http://www.dnastar.com/). Final edits to primer-truncated sequences used BioEdit ver. 7.0.9.0 (Hall 1999). Haplotype sequences (516 bp aligned to positions 52 –567 of accession # JX433140) were identified among specimen sequences using FaBox version 1.35 (Villesen 2007). Haplotype sequence accessions (refer Sup. Table S1) were deposited in GenBank and all DNA barcode records were released as a dataset (10.5883/DS-OROSPUB) at the Barcode of Life Data Systems (Ratnasingham & Hebert 2007).

Phylogenetic relationships among *Orosius* haplotypes and outgroup *Nesophrosyne* COI accessions (JX433118 and JX433140) were estimated by Bayesian inference (BI) as implemented in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003). Sequences were partitioned by codon positions (1, 2, 3) and adjusted using General Time Reversible (GTR) nucleotide substitution parameter rate estimates applied to unlinked partition elements. Two parallel BI searches (each using four chains) were run for 25 million generations and sampled every 1000th generation. Final estimates of the parameter potential scale reduction factor (PSRF = 1) and the average standard deviation of split frequencies (ASDRF < 0.0078) indicated the two searches had adequately converged. Tree log likelihood values stabilised to a narrow range after the initial 2.5 M generations, accordingly we discarded the 1st 2500 samples from each search as 'burn-in'. A 50% majority-rule consensus BI tree reporting clade posterior supports was constructed from 45 002 post burn-in samples.

Summary sequence statistics reporting intra and inter-specific haplotype sequence *p*-distances were generated using MEGA ver. 6 (Tamura *et al.* 2013); missing and or ambiguous nucleotides in sequences were treated using a pair-wise deletion option. An 'approximately maximum likelihood (ML) tree' was inferred using FastTree v.2.1.7 (Price *et al.* 2010) for all sequences. Putative conspecifics split as two or more divergent genetic clades in BI and or ML analyses were re-examined for presence of morphological characters supportive of genetic splitting and potentially indicating novel species presence (Bellis *et al.* 2013, 2014). Specimens were conservatively treated as conspecifics when morphological examinations failed to corroborate genetic splitting.

RESULTS

DNA barcoding

COI DNA barcode sequences > 500 bp were recovered from 202 *Orosius* specimens and partial DNA barcodes ranging in size from 260 to 493 bp were recovered from 25 specimens (Supplementary Table S1). Amino acid frame shifts and other symptoms of pseudogene presence were not observed among sequences. One hundred and three distinct in-group exemplar haplotypes were identified from a 516 bp sequence alignment. Searches of exemplar haplotypes at BOLD and GenBank (last queried 01.ii.2016) did not indicate the presence of any obvious contamination taxa (closest matches were always within Deltocephalinae).

The BI phylogeny resolved 14 strongly supported (100% PP; Fig. 1) monophyletic clades representative of 12 *Orosius* species recognised on the basis of male genitalia, and two indeterminate (and putatively novel) species represented by female specimens only (Table 2). The minimum sequence difference between species (9.75%; *O. argentatus* and *Orosius ryukyuensis*) was more than twice the maximum sequence difference within species confined to Australia (4.65% at *O. argentatus*, Table 2) and more cosmopolitan species sampled from various global

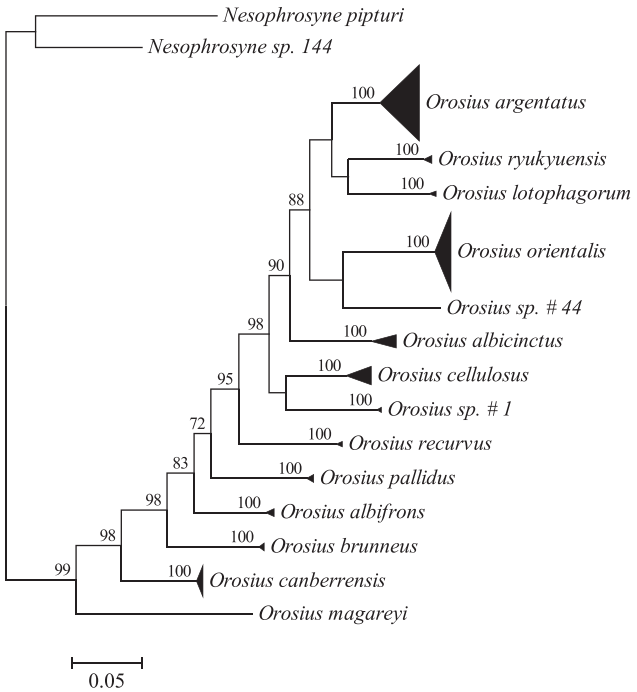


Fig. 1. Bayesian inference (BI) phylogram supporting monophyly of 12 *Orosius* species described here using descriptions of male specimens and two indeterminate *Orosius* (sp. # 1; sp. # 44) represented by females only. Majority-rule consensus BI phylogram included 103 COI *Orosius* haplotypes and two out-group *Nesophrosyne* species. Terminal intra-specific tips and collapsed haplotype clades are labelled to species as detailed in Table 2. BI posterior probabilities reported as percentages above branches; values < 70% not shown. Scale bar indicates number of expected substitutions per site (under GTR nucleotide substitution model).

regions (<3.7% at *O. albicinctus* and *O. orientalis*). Retrospectively identified *O. argentatus* and *O. orientalis* specimens were reciprocally monophyletic and separated by a minimum interspecific difference of 11.47% observed between all specimens and 11.82% between sympatric specimens (data not shown). Subtle but consistent differences in male genitalia were evident between replicates of these two species (see later). Two indeterminate *Orosius* taxa represented solely by female specimens each differed from other species by > 10.53% sequence difference (Table 2). Neither taxon was identified in comparisons to species sequence records in GenBank and BOLD (last queried 01.ii.2016). An approximate ML tree of all sequences (Supplementary Figure S1) sorted all males into clades consistent with the species clade separations identified using BI. Females, nymphs and degraded specimens were also sorted to clades in the ML tree and identified to species based solely on their affiliation in genetic clades containing morphologically identified male specimens.

Morphology

The 11 species initially identified using male genitalia were *O. albicinctus*, *O. orientalis*, *O. lotophagorum*, *O. canberrensis*, *O. ryukyuensis* and *O. cellulosus* plus five new species, two from Barrow Island, Western Australia, one from New South Wales and two from Loxton, South Australia.

DNA barcoding, however, suggested the presence of 14 species which includes the 11 species recognised on the basis of the male genitalia plus three additional species. Two of the three additional species were represented by female specimens only and therefore they cannot be confirmed as separate species using morphology. The third additional species, however, resulted from a clear differentiation between specimens identified morphologically as *O. orientalis*, with most specimens from Western Australia aligning with *O. orientalis* from Japan and China while most specimens from eastern Australia were clearly distinct from *O. orientalis*. This indicated that *O. argentatus*, which was originally described from Victoria, is a valid species distinct from *O. orientalis.* The distribution of

Table 2 Summary of COI sequence diversity in 12 described and two indeterminate *Orosius* species. Indeterminate *Orosius* (# 1 and # 44) represented by females only and identified by genetic analysis. Numbers of examined specimens (N) and haplotypes detected (N_{haps}). Average ($D_{intra\text{-}ave}$) and maximum ($D_{intra\text{-}max}$) percent sequence difference among haplotypes within species reported; minimum interspecific difference ($D_{inter\text{-}min}$) also reported. Specimen sample localities in Australia (1 = New South Wales, 2 = Northern Territory, 3 = Queensland, 4 = South Australia, 5 = Victoria, 6 = Western Australia) and elsewhere (7 = China; 8 = Japan, 9 = India, Israel, Pakistan and Sudan)

Orosius spp.	N	N_{haps}	$D_{intra\text{-}ave}$	$D_{intra\text{-}max}$	$D_{inter\text{-}min}$	Locality
albicinctus	19	5	1.74	2.52	10.22	9
albifrons	20	3	0.33	0.71	10.85	1,6
argentatus	64	29	0.76	4.65	9.75	1,3,4,5,6
brunneus	4	3	0.52	0.78	10.85	1,6
canberrensis	15	12	0.54	1.19	12.22	1,4,6
cellulosus	11	7	1.69	2.99	9.96	1
lotophagorum	6	2	—	0.58	9.96	2,3
magareyi	1	1	—	—	16.76	4
orientalis	67	30	1.63	3.68	10.55	1,2,5,6,7,8
pallidus	4	3	0.26	0.49	11.39	1
recurvus	7	2	—	0.30	11.86	1
ryukyuensis	6	3	0.39	1.56	9.75	1,2,3
# 1	2	2	—	0.15	11.04	6
# 44	1	1	—	—	10.53	6

both species is not allopatric, with a few records of *O. orientalis* from both NSW and Victoria and records of *O. argentatus* from Western Australia indicating some sympatric overlap.

Also of note was that specimens identified morphologically as *O. albicinctus* from India, North Africa and the Middle East were confirmed as conspecific. No specimens from these regions aligned with *O. orientalis* and recent literature on *O. orientalis*, from the Middle East in particular, almost certainly refers to *O. albicinctus.*

O. minuicus was examined morphologically only and not included in the DNA analysis. This species is quite distinctive in male genitalia and is excluded from the genus *Orosius* below. The presence of accessory processes on the aedeagal shaft clearly differentiates it from species of *Orosius*.

The five undescribed species recognised in the Australian fauna, and for which males are available, are described below as *Orosius magareyi* Fletcher and Löcker sp. nov. (South Australia), *Orosius albifrons* Fletcher and Löcker sp. nov. (Western Australia), *Orosius pallidus* Fletcher and Löcker sp. nov. (South Australia), *Orosius recurvus* Fletcher and Löcker, sp. nov. (New South Wales) and *Orosius brunneus* Fletcher and Löcker, sp. nov. (Western Australia).

TAXONOMY

Genus *Orosius* Distant

Orosius Distant, 1918: 85. Type species: *Orosius albicinctus* Distant, by original designation

Nesaloha Oman, 1943: 33 synonymised by Evans 1947: 236. Type species: *Nesaloha cantonis* Oman, by original designation.

Description

Face shagreen. Anteclypeus parallel-sided, slightly narrower towards apex. Postclypeus broad, convex. Vertex about as wide as a single eye, shorter in midline than wide between eyes, anteriorly roundly angulate, slightly longer in midline than long against eyes, bearing occipital suture on basal half. Pronotum shagreen or obscurely transversely ridged, short at sides. Tegmina about four times as long as broad, their apices broadly rounded, venation mainly obscured by markings. Fore femur with two short apical spines dorsally, intercalary setae present as row of fine hairs, AM1 not differentiated, AV row present as short sharp curved setae. Posterior femora with apical setal formula 2 + 2 + 1. Lateral setae with row PV long and strong with short seta at base of each main seta and intercalaries present between proximal setae. Row AV decreasing in length from apex to base. Row PD with alternating long and short setae. Male genitalia: Pygofer narrow laterally with clear basal cleft and apical lobe rounded and bearing numerous long macrosetae except along narrow marginal echinate band. Subgenital plates narrow triangular, rounded laterally at base with an annulated linear process apically, line of macrosetae along lateral margin, plate and usually process with marginal long fine hairs. Paramere with well developed preapical process; apical process straight, directed posterolaterad, apically acute, often slightly inturned. Connective with arms curving towards each other, well separated apically, longer than short quadrate body. Aedeagus with two shafts, each with gonopore, lacking accessory processes. Female: pregenital sternite more or less quadrate with posterior margin curved posteriorly, medially prominent or undulate, median emargination present.

Diagnosis

The species of *Orosius* can usually be recognised by the markings on the wings which comprise a filigree brown pattern with oval pale spots which are largely independent of the cells. The vertex is also marked with distinctive fine brown markings. The male genitalia are characterised by an aedeagus with two gonopores subapically on the dorsal surface of separate shafts which lack accessory processes and subgenital plates which are extended apically to form an annulated finger-like process.

Remarks

Some species of the genus, and some individuals of other species, are almost completely pallid, although some indication of the distinctive filigree colour markings can usually be recognised. The presence of two aedeagal shafts with a gonopore on each places the genus in the Tribe Opsiini Emeljanov (Zahniser & Dietrich 2013). The various species have been defined primarily by the structure of the aedeagus but, as other features of the male genitalia provide little diagnostic information, there are some limitations using the male genitalia to identify some species which might need DNA barcoding for confirmation of identity. Identification of females of some species may be possible using the shape of the hind margin of the pregenital sternite.

Key to species of *Orosius* males

1 Aedeagus shafts apically divergent or parallel in ventral view (Figs 15, 25, 35)……2
Aedeagus shafts curved inwards in ventral view, sometimes only over the apical portion (*O. pallidus* may be slightly incurved at the very apex, see Figure 35)……4

2 Aedeagus shafts apically divergent in ventral view (Fig. 15) (Africa, Middle East, India)……*O. albicinctus*
Aedeagus shafts more or less parallel throughout (Australia)……3

3 Aedeagus shafts more than twice as long as maximum distance between the shafts (Fig. 35)……*O. pallidus* sp. nov.
Aedeagus shafts less than twice as long as maximum distance between the shafts (Fig. 25)……*O. canberrensis*

4 Aedeagal shafts, in ventral view, strongly inwardly arcuate throughout (e.g. Fig. 33)……*O.* magareyi sp. nov.
Aedeagal shafts, in ventral view, not strongly arcuate…5

5 Aedeagus shafts, in lateral view, strongly sinuate (Fig. 26) (Africa, India?, Australia)……*O. cellulosus*
Aedeagus shafts, in lateral view, not strongly sinuate……6

6 Aedeagus shafts, in lateral view, abruptly narrowed on apical portion (Figs 20, 22, 30)……7
Aedeagus shafts, in lateral view, gradually narrowing throughout……9

7 Aedeagus shafts, in ventral view, abruptly incurved at apex (Fig. 31) (Australia)……*O. recurvus* sp. nov.
Aedeagus shafts, in ventral view, incurved throughout length, not abruptly incurved at apex……8

8 Aedeagus shafts, in lateral view, parallel-sided from base before apical narrowed section (Fig. 22) (Japan, Australia)……*O. ryukyuensis*
Aedeagus shafts, in lateral view, expanded before apical narrowed section (Fig. 20) (Australia)……*O. lotophagorum*

9 Face pale, in contrast to remainder of body; aedeagal shafts, in lateral view (Fig. 28), curved dorsally throughout, more strongly so beyond gonopore (Australia) ……..*O. albifrons* sp. nov.
Face with extensive dark markings, if pale, then remainder of body also pale; aedeagal shafts, in lateral view, straight or slightly sinuate……10

10 Small brown insects with obscure pale stripe along centre of forewing (Fig. 13) (Australia)……*O. brunneus* sp. nov.
Insects not brown, whitish with dark brown filigree pattern, particularly on forewing, which lacks a stripe……11

11 Aedeagal shafts, in lateral view (Fig. 18), straight except for apical portion; in ventral view (Fig. 19), shafts more or less parallel (Australia)……*O. argentatus*2–4
Aedeagal shafts, in lateral view (Fig. 16), slightly sinuate; in ventral view (Fig. 17), shafts slightly divergent (Australia, Asia)……*O. orientalis*

Orosius albicinctus Distant

(Figs 2, 14–15)

Orosius albicinctus Distant 1918: 85
Thamnotettix filigranus Haupt 1927: 30, synonymised by Ghauri 1966: 236
Orosius aegypticus Ghauri 1966: 250. syn. nov.
Orosius aegyptius [*sic*] Ghauri, Linnavuori 1969: 206

Types and Material Examined

Holotype

female of *O. albicinctus* (not examined), Kodaikanal, South India (BMNH).

Other material

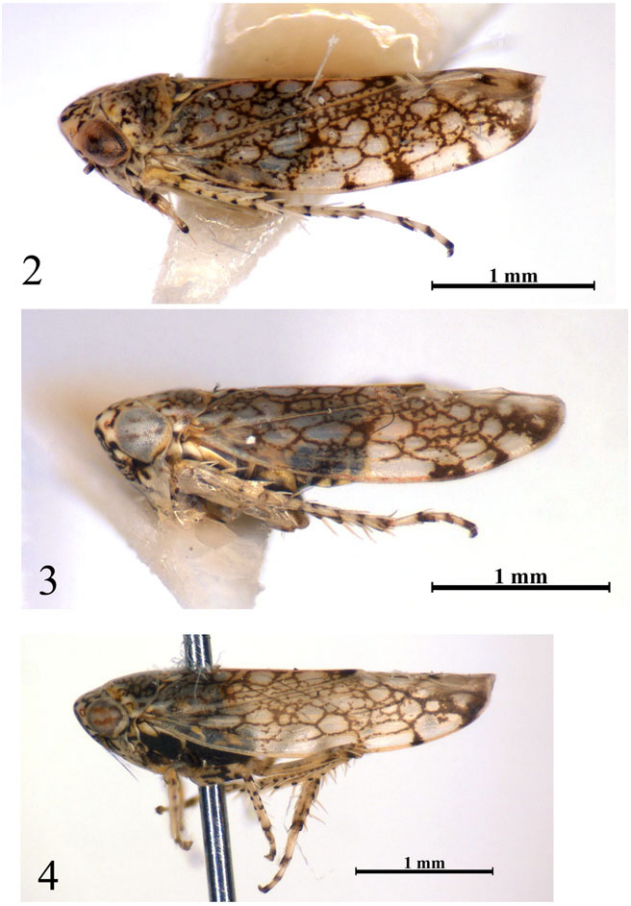
ISRAEL: 1 male, 2 females, Bet-Dagan, on *Tribulus* sp., M. Klein, viii.1999; 2 males, 1 female, Moshav Hatzvah, northern Arava Valley, tarragon greenhouse, vacuum sampler, P. Weintraub, 26.ix.2002.

PALESTINE: 1 female (holotype of *T. filigranus*) (not examined), Ben-Shemen, Palestine, 7–8.v.1926 (Quedlinburg, Germany).

INDIA: 3 males, Bangalore, Karnataka, ex *Crotolaria* sp., S. Rani, 30.x.2006.

AFRICA: 4 males, 4 females, North Africa, ex. culture, B. Gronenborn, 30.vi.2008; 1 male (holotype of *O. aegypticus*) (not examined), Egypt, Siwa, 12.v.1965, J. Omer-Cooper, Armstrong College Expedition BM 1935-354 (BMNH).

PAKISTAN: 5 females, Sindh Province, Tharparkar district, 12.xi.2007, I. Khatri (all in ASCU).



Figs 2–4. *habitus.* (**2**) *O. albicinctus;* (**3**) *O. orientalis;* (**4**) *O. argentatus.*

Description

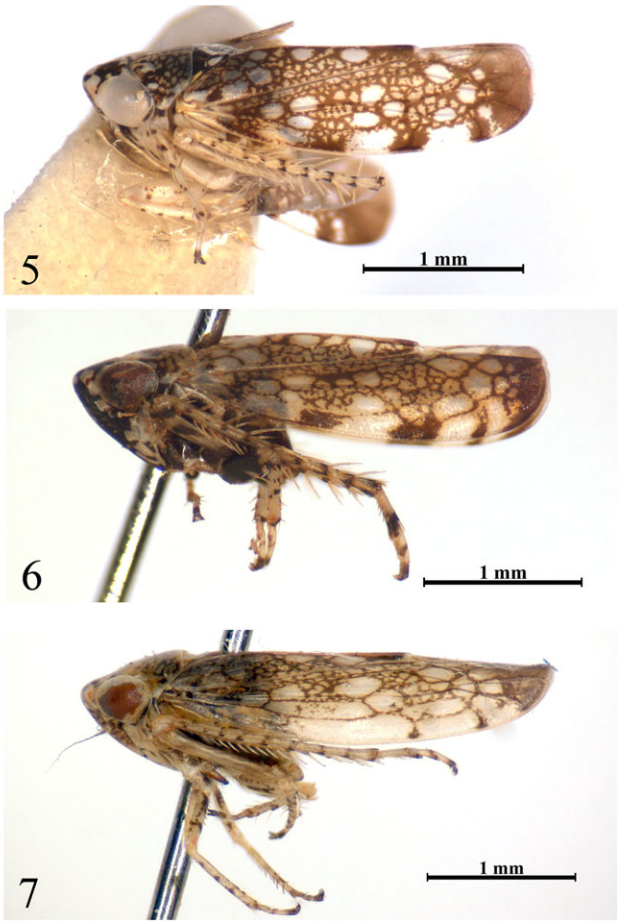
Habitus picture lateral see Figure 2.

Length: males ($N=9$) 2.75–3.21 mm; females ($N=8$) 2.87–3.35 mm.

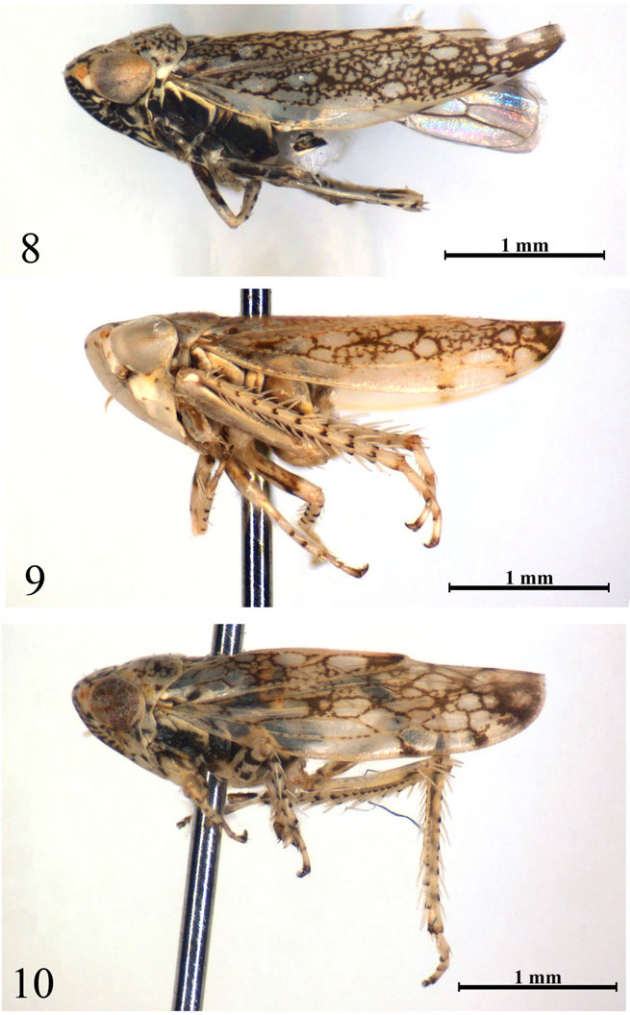
Colour ivory white on head and body with brown lacy pattern. Tegmen milky white with filigree brown markings delineating ovate areas lacking brown as in Figure 2.

Male genitalia. Pygofer broadly rounded with 11–12 macrosetae. Subgenital plate bearing six marginal macrosetae. Paramere with preapical lobe roundly acute, relatively short; apical process with inner margin straight with a few small denticles, outer margin slightly convex, narrowing to inwardly turned apex. Aedeagus, in lateral view (Fig. 14) with large atrium and arms narrow, tapered and slightly dorsally curved from base to apex; in ventral view (Fig. 15), with lateral shafts diverging from base to apex, tapered from base to apex.

Female. Posterior margin of pregenital sternite lightly undulate.



Figs 5–7. *habitus.* (**5**) *O. lotophagorum;* (**6**) *O. ryukyuensis;* (**7**) *O. canberrensis.*



Figs 8–10. *habitus.* (**8**) *O. cellulosus;* (**9**) *O. albifrons;* (**10**) *O. recurvus.*

Diagnosis

Males of this species are easily recognised by examination of the ventral view of the aedeagus in which the shafts diverge throughout. The species is recorded from North Africa through the Middle East to India. This species is remarkably stable in COI sequence diversity and in the structure of the male genitalia despite its wide distribution.

Remarks

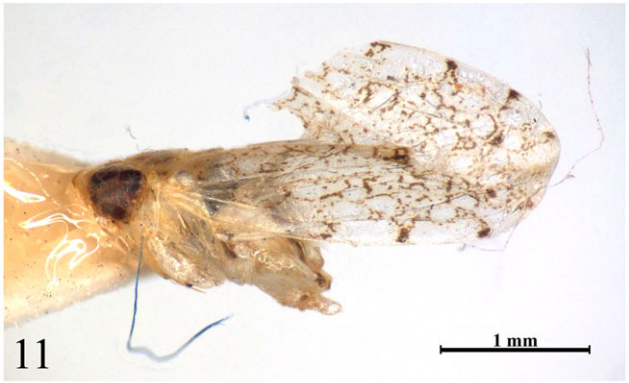
The species has appeared as *O. orientalis* in the economic literature from India (Horn *et al.* 1993), Israel (Klein *et al.* 2001; Orenstein *et al.* 2003; Weintraub *et al.* 2004) and Turkey (Baspinar *et al.* 1993; Kersting *et al.* 1997) but our work has shown that *O. orientalis* is restricted to the Oriental, eastern Palaearctic and Australian regions. Other recent publications have correctly used *O. albicinctus*, (e.g. Akhtar *et al.* 2013)

Ghauri (1966) created *O. aegypticus* based on a single male and an unspecified number of females with the same collection data. The holotype has a determination label by W. Wagner identifying it as *O. cellulosus*. Ghauri (1966) stated that its status should be regarded as tentative because it was possible that the specimen was malformed. Specimens which have been identified as this species were reported from Pakistan (Khatri *et al.* 2011). Only one male has been collected in Pakistan and this was not available for our study but a series of females found in association with the male was available and COI sequences were acquired from them. The females aligned with *O. albicinctus*. It appears that Ghauri (1966) was correct in suspecting that the male form which he described as *O. aegypticus* is an aberrant form of *O. albicinctus* and that the male from Pakistan is a similar aberration. It is significant that the enlarged basal chamber of the aedeagus described and illustrated by Khatri *et al.* (2011) differs from the same structure described and illustrated by Ghauri (1966) and the name is here considered a new synonym of *O. albicinctus.*

Orosius orientalis (Matsumura)

(Figs 3, 16–17)

Eutettix orientalis Matsumura 1914: 192
Nesaloha cantonis Oman 1943: 33. syn. nov.
Nesophrosyne orientalis (Matsumura), Ishihara 1963: 121
Orosius argentatus novaebrittaniae Ghauri 1966: 245. syn. nov.
Orosius orientalis (Matsumura), Kwon & Lee 1979: 92



Figs 11–13. *habitus.* (**11**) *O. magareyi;* (**12**) *O. pallidus;* (**13**) *O. brunneus.*

Types and Material Examined

Types

Lectotype, female (not examined), Honshu (Akashi), designated by Ishihara (1982)

Paralectotypes (not examined), five males, Honshu (Akashi), one male, three females, Taiwan (Ako), unknown specimens, Taiwan (Shirin, Banshoryo) (EIHU);

Other material

AUSTRALIA, Western Australia: 4 males, 1 female, Barrow Island [N01], 20°49′47″S 115°26′39″E, mounted ex ethanol, 6.v.2006, S. Callan & R. Graham; 1 male, 2 females, same as previous but [N02], 20°47′47″S 115°21′01″E; 2 females, same as previous but [N03], 20°49′26″S 115°19′48″E; 1 male, same as previous but [N11], 20°48′51″S 115°22′32″E; 3 males, 2 females, same as previous but [N16], 20°47′47″S 115°21′10″E; 11 males, 7 females, same as previous but [N22], 20°49′55″S 115°26′35″E; 2 males, 1 female, same as previous but [N26], 20°49′01″S 115°26′06″E, (ASCU); 6 males, Barrow Island, R1 DMP. SUC. AL., 20°47′30″ S 115°20′33″ E, 24.iv.2005, S. Callan; 5 males, R2 DMP SUC AL, 20°47′30″ S 115°20′33″ E, 17.v.2005, S. Callan. New South Wales: 1 male, Griffith, sticky trap 482, 20.x.2005, G. Ali; 1 male, Forbes, sticky trap 519, 18.v.2005, G. Ali; 1 female, Forbes, ex lucerne & canopy of weeds, 10.vi.2005, G. Ali; 1 male, Forbes, ex *Chenopodium murale*, 25.i.2005, G. Ali. Victoria: 4 males, 1 female, specimens ex culture from Darwin (Northern Territory) and Rutherglen (Victoria), 21.xi.2008, P. Trébicki (all in ASCU).

NEW GUINEA: 1 male (holotype of *Orosius argentatus novaebrittaniae*) (examined), New Britain, Rabaul, Keravat, 31.viii.1959, A.J. von Velsen, ex *Crotolaria goreensis* (BMNH).

CHINA: 1 male (holotype of *N. cantonis*) (not examined), Canton Island, 1.viii.1940, R.H. Van Zwaluwenburg, on foliage of *Boerhaavia diffusa* L. (USNM); 1 female, Mount Jianfeng Ling, Hainan Prov., 5.vi.2007, Duan Yani; 1 sex unknown (abdomen missing), Yangling, Shaanxi Prov., 9.x.2002, Zhu Yumei; 1 male, Yangling, Shaanxi Prov., ex light trap, 13.viii.2002; 1 male, Wugong, Shaanxi Prov., ex light trap, 20.vii.1987 (all in NWAFU).

JAPAN: 2 males, Ryukyu Islands, Kabira, Ishigaki Island, 27.xi.2006, M. Hayashi; 1 male, Kita-Okinosu, Tokushima City, Tokushima Pref., Shikoku, 20.x.2006, M. Hayashi; 1 male, Shimizu-Miho, Shizuoka City, Shizuoka Pref., Honshu, 27.x.2006, M. Hayashi *et al.*; 2 males, Kinkai, Oku-Setouchi, Okayama Pref., Honshu, 29.x.2006, M. Hayashi *et al.* (all in ASCU).

INDONESIA: 5 males, West Java, 250 m, Bogor, vii.1954, *Arachis hypogea*, leg. B.H.H. Beigmah (BMNH).

Description

Habitus picture lateral see Figure 3.

Length: males ($N=47$) 2.54–3.26 mm; females ($N=19$) 2.79–3.31 mm

Colour of head and body creamy white with brown lacy markings, these sometimes darker and heavier on face. Thoracic sternites dark brown to blackish. Tegmen milky white with brown filigree markings delineating oval areas lacking brown as in Figure 3.

Male genitalia. Pygofer with posterior lobe oblique, roundly acute distally with 12–15 macrosetae, those proximally shorter than those distally. Subgenital plate with 6–7 marginal macrosetae, relatively short. Paramere with preapical process roundly acute, short. Apical process with inner margin very slightly incurved at tip, outer margin lightly convex throughout and slightly denticulate. Aedeagus, in lateral view (Fig. 16) with shafts slightly sinuate, apically curved ventrally; in ventral view (Fig. 17), with apodeme triangular, rounded at base, shafts diverging slightly from base to near apex with apical portion incurved.

Female. Posterior margin of pregenital sternite prominent medially with very shallow median emargination.

Diagnosis

This species is difficult to differentiate from *O. argentatus* which has a similar aedeagus with the shafts lacking distinctive features other than the apices being inturned, in ventral view. In *O. orientalis* the shafts in lateral view are slightly sinuate while in *O. argentatus* they are more or less straight. In ventral view, the shafts of *O. orientalis* are more or less parallel while in *O. argentatus* they are slightly divergent. These differences are slight and, for significant identifications such as those associated with identification of disease vectors, examination of a COI barcode may be required.

Remarks

Ishihara (1982) noted that the single female from Akashi had been designated as lectotype, apparently by Prof. S. Tagachi of Hokkaido University, although no publication appears to have formalised this. It is accepted here that Ishihara's (1982) reference to a lectotype indicating unambiguously which specimen was the lectotype constitutes designation of the female from Akashi as the lectotype. It is unfortunate that this is so because the type series includes six males, any one of which would have made a more appropriate name-bearing type.

Ishihara (1982) proposed a synonymy between this species and *O. albicinctus* on the basis of comprehensive measurements of the holotype female of *O. albicinctus* and the Japanese lectotype female of *O. orientalis.* Although the measurements of these females show close similarity, the size and coloration of the females has little bearing on recognition of the species in this genus. Our work has shown that even male genitalia can be misleading, although it is certainly easy to differentiate what is accepted as *O. albicinctus* based on its characterisation by Ghauri (1966) and *O. orientalis* based on its characterisation by Ishihara (1963) using the structure of the aedeagus.

Ishihara (1963) figured the species as *Nesophrosyne orientalis*. Ghauri (1966) rejected Ishihara's (1963) identification on the basis that the specimens figured were from 'Formosa' (= Taiwan) and not from the type locality. He referred Ishihara's specimens to *O. cantonis* (Oman). However, as pointed out by Ishihara (1982), Ghauri (1966) had neglected the fact that the type series of *O. orientalis* was not restricted to Japan but included material from Taiwan, including Ako from which locality Ishihara's (1963) specimens had been acquired. Interestingly, *O. cantonis* was subsequently synonymised with *O. argentatus* by Linnavuori (1975) and *O. argentatus* was synonymised with *O. orientalis* by Kwon and Lee (1979). The results of our work, however, clearly demonstrate that *O. orientalis* and *O. argentatus* are both valid species separated clearly by COI sequencing and with minor but consistent differences in the male genitalia not always reflected in published illustrations.

If Ghauri's (1966) illustration of *O. cantonis* were accurate, then Linnavuori's (1975) proposed synonymy of *O. cantonis* with *O. argentatus* would be supported here. However, Oman's (1943) original illustrations of the aedeagus of *N. cantonis* indicate that, in lateral view, the shafts are slightly sinuate which matches the shafts of *O. orientalis* although the ventral view, as illustrated by Oman (1943) is not clearly one species or the other. The specimens from China examined above proved to be *O. orientalis* although none of these originated from Canton Island, the type locality of *O. cantonis*. However, the wide distribution of *O. orientalis* and lack of records of *O. argentatus* in the Oriental region supports the view that *N. cantonis* is a synonym of *O. orientalis*, rather than of *O. argentatus.* This view was first suggested by Ghauri (1966) who referred *O. orientalis* to *N. cantonis* without formalising the names because *O. orientalis* has priority and Ghauri (1966) would have then treated the species as *O. orientalis* rather than *O. cantonis*. The transfer of the synonymy from *O. argentatus* to *O. orientalis* is formally proposed here.

Ghauri (1966) described a form of *O. argentatus* from Java in which the coloration of the head included some reddish markings (which can also be found in some specimens of *O. canberrensis*) and some minor variation in the male genitalia. In particular, the shafts of the aedeagus are described as 'slightly diverging' although Ghauri's figure (Ghauri 1966: Fig. 7J) does not show any great difference in this feature compared to his figure (Ghauri 1966: Fig. 6J) of *O. argentatus* from Australia. An examination of five males in BMNH which were examined by Ghauri showed that the colouration is within the range seen in both *O. argentatus* and *O. orientalis.* Examination of the dissected genitalia of one of the males showed that the shafts of the aedeagus are slightly divergent in ventral view and slightly sinuate in lateral view, which are features of *O. orientalis*. The specimens are therefore considered to be *O. orientalis.*

The subspecies *novaebrittaniae* was described by Ghauri (1966) as a colour variant of *O. argentatus* which also differed from *O. argentatus* in the disposition of the aedeagal shafts. An examination of the holotype has shown that the aedeagal shafts are slightly divergent in ventral view and slightly sinuate in lateral view, as described by Ghauri (1966), and these are features of *O. orientalis* rather than *O. argentatus.* Ghauri's (1966) indication that this form differed from *O. argentatus* in colour was not supported by our examination and the subspecies is here synonymised with *O. orientalis.*

Orosius argentatus (Evans) stat. rev.

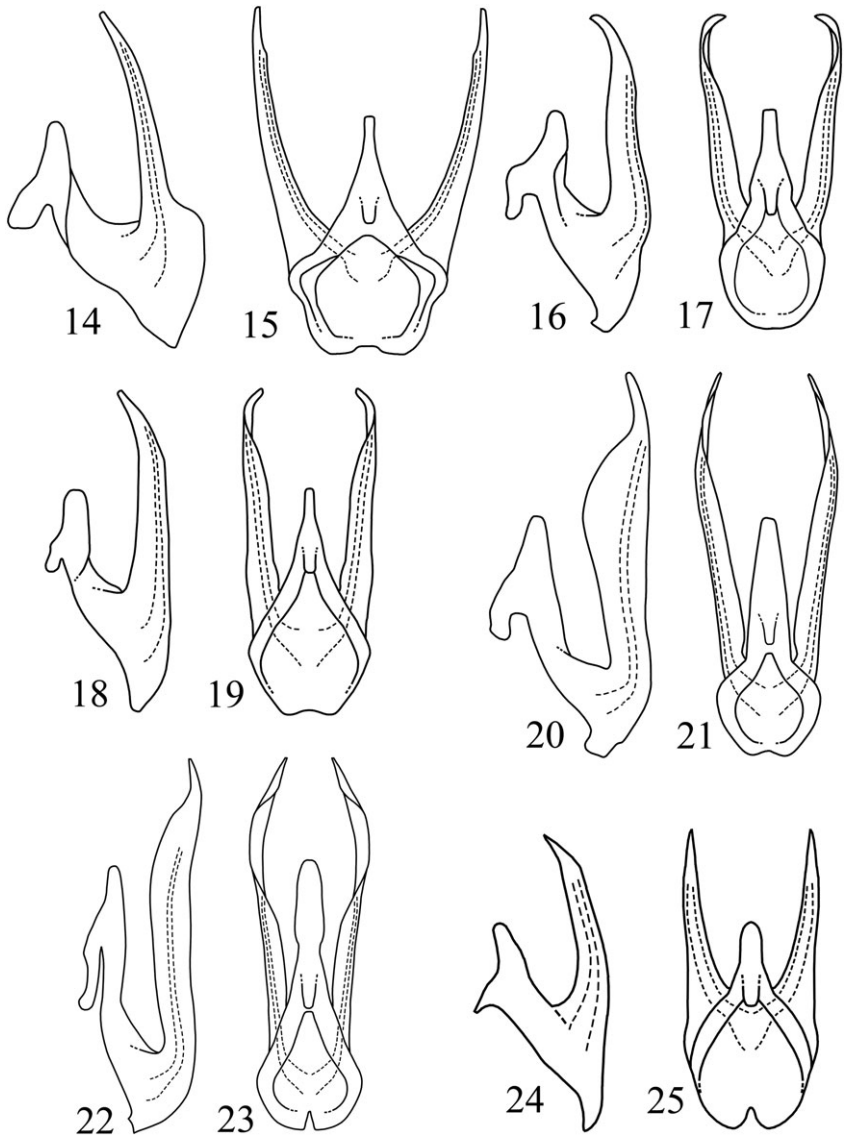
(Figs 4, 18–19)

Thamnotettix argentatus Evans 1938: 15
Orosius argentatus (Evans), Oman 1949: 11

Types and Material Examined

Holotype

male, Burnley, Victoria, J.W. Evans (AM)



Figs 14–25. *Orosius spp*. Male aedeagus. (**14–15**) *O. albicinctus,* (**14**) lateral view; (**15**) ventral view; (**16–17**) *O. orientalis,* (**16**) lateral view; (**17**) ventral view; (**18–19**) *O. argentatus*, (**18**) lateral view; (**19**) ventral view; (**20–21**) *O. lotophagorum,* (**20**) lateral view; (**21**) ventral view; (**22–23**) *O. ryukyuensis*, (**22**) lateral view; (**23**) ventral view; (**24–25**) *O. canberrensis,* (**24**) lateral view; (**25**) ventral view.

Other material

AUSTRALIA, New South Wales: 1 male, 12 km W of Balranald, ex eucalypt mallee, 6.iv.1997, M.J. Fletcher & J.S. Mann; 1 female, Breeza via Tamworth, ex faba beans, 2. x.2002, A. Tomkins; 1 male, Forbes, ex sticky trap 527, lucerne, 18.v.2005, G. Ali; 1 male, Forbes, ex sticky trap 530, lucerne, 18.v.2005, G. Ali; 1 male, Forbes, ex sticky trap 539, cottonbush, 18.v.2005, G. Ali; 1 male, Forbes, ex sticky trap 549, nitre goosefoot, 18.v.2005, G. Ali; 1 male, Forbes, ID 568, ex cottonbush, sweep net, 26.v.2005, G. Ali; 2 males, 5 females, Forbes, ex lucerne & canopy of weeds, 10. vi.2005, G. Ali; 1 male, Forbes, ex lucerne, 15.vi.2005, G. Ali; 6 males, 5 females, Forbes, ex. *Chenopodium murale*, 25.i.2005, G. Ali; 3 males, 1 female, Gilgandra, Breelong NP, ex. malaise trap MT1, 31°47′18″ S 148°44′59″ E, 20–22.iii.2009, M.J. Fletcher; 1 male, 1 female, same as previous but ex malaise trap MT2, 31°51′22″ S 148°44′59″ E; 1 male, Griffith, ex sticky trap 479, 20.x.2005, G. Ali; 1 male, same as previous but ex sticky trap 478; 1 male, same as previous but ex sticky trap 488; 1 female, Griffith, ex yanga bush, sweep net, 18.x.2005, G. Ali; 1 male, Orange, ex golden diosma, 20.ii.1998, B.C. McNeil; 1 male, Orange, Paling Yards Reserve, 33°21′ S 148°54′ E, 16.ii.2003, M.J. Fletcher, m.v. light. South Australia: 1 female, Loxton, 34° 27′00″ S 140°34′01″ E, P. Magarey. Victoria: 5 males, Rutherglen, ex culture, 21.xi.2008, P. Trébicki; 2 females, Horsham, ex culture, 1.xi.2008, P. Trébicki. Queensland: 2 males, Stanthorpe, ex strawberry Queen Rosa block, iii.2009, J. Smith; 3 males, 2 females, Stanthorpe, ex lucerne Red Jewel, 11.x.2006, G. Waite. Western Australia: 2 females, Barrow Island, [N03], 20°49′26″S 115°19′48″E, 6.

v.2006, S. Callan & R. Graham, mounted ex ethanol; 2 males, same as previous but [N06], 20°47′51″S 115°25′57″ E; 2 males, same as previous but [N26], 20°49′01″S 115° 26′06″E; 1 male, same as previous but [N27], 20°52′22″S 115°26′35″E; 1 male, 1 female, Kununurra tip, ex *Cenchrus*, 20.i.1999, R. Blanche & L. Tran-Nguyen; 1 female, Moorine Rocks, 11.7 km N of Great Eastern Highway on Noongar Rd., ex Santalaceae *Exocarpos aphyllus*, 31°13′42″ S 118°58′44″ E, 345 m, 4.xii.1997, Schuh, Cassis, Brailovsky & Asquith [97-01] (all in ASCU).

Description

Habitus picture lateral see Figure 4.

Length: males ($N = 39$) 2.67–3.29 mm; females ($N = 23$) 2.82–3.30 mm

Colour of head and body creamy white with brown lacy markings, these sometimes darker and heavier on face. Thoracic sternites dark brown to blackish. Tegmen milky white with brown filigree markings delineating oval areas lacking brown as in Figure 4.

Male genitalia. Pygofer with 12 long macrosetae. Subgenital plate with 5–6 marginal macrosetae. Paramere with preapical process roundly acute. Apical process with inner and outer margins straight, tapering to apex which is slightly inturned. Inner margin of apical process diverging slightly from outer margin of preapical process. Aedeagus, in lateral view (Fig. 18) shafts straight or slightly curved from base to near apex which is curved ventrally; in ventral view (Fig. 19), with apodeme roughly diamond-shaped, truncate or slightly emarginate at base, shafts more or less parallel from base to near apex, apices curved medially.

Female. Posterior margin of pregenital sternite undulate with 3 shallow convexities on either side of shallow v-shaped median emargination.

Diagnosis

This species is most closely similar to *O. orientalis* (see Diagnosis of that species for details).

Remarks

This species was synonymised with *O. orientalis* by Kwon and Lee (1979) based on material from Korea following the suggestion by Linnavuori (1975) that the two names might be synonymous. The synonymy was not widely accepted because no comparison had been made with specimens from Japan, the type locality of *O. orientalis*. Examination of specimens from several locations in Japan by the senior author confirmed the close similarity of the male genitalia between *O. orientalis* from Japan and *O. argentatus* from Australia and the external morphology is also closely similar. Subsequent use of the name *O. orientalis* in the Australian literature followed (e.g. Trébicki *et al.* 2009). Our results, however, show >11% COI sequence divergence between the species where they are in sympatry and this demonstrates that both species are valid and both are present in Australia. *Orosius orientalis* is the more common species in Western Australia and *O. argentatus* is more common in eastern Australia, although records of each are known from opposite sides of the continent. The structures of the male genitalia are very similar between the two species. They can be separated by the slight sinuosity of the aedeagal shafts in lateral view and their slight divergence in ventral view in *O. orientalis* while, in *O. argentatus*, the shafts are not sinuous in lateral view and are more parallel in ventral view.

The significance of this discovery has implications for studies of disease transmission in Australia. *O. argentatus* has been cited as a vector for several phytoplasma-associated diseases in Australia including tomato big bud (Hill 1941; Bowyer 1974; but see Pilkington *et al.* 2004a), tobacco yellow dwarf (Hill 1941; Helson 1942, 1950), lucerne witches broom (Helson 1951), legume little leaf (Hutton & Grylls 1956), potato purple top wilt and pawpaw yellow crinkle (Grylls 1979; Padovan & Gibb 2001), Australian lucerne yellows (Pilkington *et al.* 2004a) and equivocally for Australian grapevine yellows (Beanland *et al.* 1999) and strawberry lethal yellows (Streten *et al.* 2005). It could be expected that vector studies in eastern Australia are focussed on *O. argentatus* although care needs to be taken to confirm the identity of any vectors, using COI gene sequencing if necessary, particularly because additional species, three described as new in this paper, are known from southern and eastern Australia.

Orosius lotophagorum (Kirkaldy)

(Figs 5, 20–21)

Allygus lotophagorum Kirkaldy 1907: 62
Nesophrosyne (*Orosius*) *lotophagorum* (Kirkaldy), Linnavuori 1960a: 57
Nesophrosyne argentatus distans Linnavuori 1960b: 322, synonymised by Ghauri 1966: 241

Types and Material Examined

Types

Syntypes, male, female, quantity unknown, FIJI: Viti Levu, Rewa (iii–iv.1905, Muir), Ba (i.1905, Muir) (BPB).

Other material

AUSTRALIA, Northern Territory: 1 male, 1 female, Tipperary Stn, on *Sida cordifolia*, 16.iii.1987, C. Wilson; 1 female, Tipperary Stn, on *Sida cordifolia*, 23.iii.1987, C. Wilson; 2 females (+1? without abdomen), Fogg Dam, on *Sida cordifolia*, 9.ii.1987, C. Wilson (all in ASCU). Queensland:); 2 males, 1 female, Doomadgee, 18.iv.1983, J.F. Donaldson, D-vac; 1 male, 31 km W of Dimbulah, 31.i.1982, J.F. Donaldson, D-vac; 1 male, 17 km W of Gamboola, 23.iv.1983, J.F. Donaldson & J. F. Grimshaw, D-vac. in grass (all in QDPI)

MICRONESIA, 1 male (not examined) (holotype of *Nesophrosyne argentatus distans*), Wake Atoll, 30.vii.1923, E. H. Bryan Jr (BPB: 2235).

Description

Habitus picture lateral see Figure 5.

Length: males ($N = 6$) 2.78–3.02 mm; females ($N = 10$) 3.06–3.41 mm

Head pale cream with heavy brown markings coalescing into patches. Tegmen milky white with dense network of brown markings clearly delineating pale oval patches (Fig. 5).

Male genitalia. Pygofer with posterior lobe oblique, bearing numerous long macrosetae. Subgenital plate long triangular, tapering into apical process, with five marginal macrosetae. Paramere with preapical process acutely rounded, short. Apical process 2.5 x preapical process, tapered base to apex which is distinctly inturned, echinate in some views. Aedeagus with basal apodeme broad and long, triangular, base rounded with medial emargination. Shafts, in lateral view (Fig. 20), slightly sinuate, widening from base to about two thirds length then sharply narrowed to short narrow apical process beyond gonopore; in ventral view (Fig. 21), diverging from base to about two thirds length then curving inwards.

Female. Posterior margin of pregenital sternite distinctly undulate with four convex sections, the central two on either side of v-shaped emargination.

Diagnosis

O. lotophagorum is one of three species in which the aedeagal shafts are abruptly narrowed towards the apex. It can be differentiated from *O. recurvus* in ventral view by the aedeagal shafts being incurved throughout rather than abruptly incurved near the apex as in *O. recurvus.* From *O. ryukyuensis*, it can be differentiated by the increasing width of the shafts in lateral view in *O. lotophagorum* while in *O. ryukyuensis* the shafts are more or less parallel sided from the base. Specimens of *O. lotophagorum* also usually have the brown patterning on the tegmina more closely netlike so that the ovate markings are clearer than in other species of the genus, including *O. ryukyuensis.*

Remarks

Linnavuori (1960a) synonymised *Thamnotettix argentatus* Evans with this species but clearly misidentified the Evans species. Linnavuori (1960b: 321) provided illustrations of the male genitalia of *O. lotophagorum* under the name *Nesophrosyne argentatus* and described a new variety 'var. *distans*' from Wake Atoll based on larger size and paler colouring. The genitalia were described as being similar to the nominal form and the illustrations match the genitalia of *O. lotophagorum*. Because the name *distans* was published prior to 1961 as a variety of *N. argentatus*, it is deemed to be subspecific under Article 45.6.4 of the Code (ICZN 1999).

The key provided by Ghauri (1966) has all specimens with the aedeagal shafts abruptly narrowing towards the apex with a short narrow apical process beyond the gonopore identified as *O. lotophagorum*. However, the COI sequences of specimens matching this distinctive aedeagal type show that there are two clades. Both clades contain specimens from Queensland and the Northern Territory so the clades do not represent geographical variants.

Examination of a single male specimen in the JW Evans collection of *O. ryukyuensis* from the Ryukyu Islands, Japan, revealed that the aedeagal shafts of this species also narrow abruptly preapically. However, there is a clear difference in shafts between *O. ryukyuensis* and *O. lotophagorum* as detailed in the diagnosis above and shown in Figures 20 and 22. Closer examination of the male genitalia of the specimens in the two clades identified by COI sequences revealed that one of the clades is *O. ryukyuensis* while the other matches *O. lotophagorum*.

The species has been collected on *Sida cordifolia* (Malvaceae) on three occasions in two separate localities in the Northern Territory.

Orosius ryukyuensis (Ishihara)

(Figs 6, 22–23)

Nesophrosyne ryukyuensis Ishihara 1965a: 19
Orosius ryukyuensis (Ishihara), Linnavuori 1975: 628.

Types and Material Examined

Holotype

male (not examined), Miyako Is., Ryukyus, 9.vi.1964, A. Shinkai (EUMJ)

Other material

JAPAN: 1 male, same data as holotype (JWE), 3 males, 3 females, Ikei-jima, Okinawa, 4.x.1963, A. Shinkai (ASCU).

AUSTRALIA, New South Wales: 1 female, Moree, ex water trap, 27.vi–8.vii, 1994, C. Coll (ASCU). Northern Territory: 1 female, Alyangula Port, ex sweeping *Ipomoea pes-caprae*, 13° 51′33″ S 136°25′12″ E, 17.ii.1998, G. Bellis [GAB98141] (ASCU); 2 females, Darwin, ex sugarcane/weeds, Berrimah Farm, 11.vi.1999, R. Blanche (ASCU). Queensland: 1 female, bred ex colony Brisbane, vii.1977 (QDPI); 1 male, Bundaberg, pitfall trap, ex. tomatoes (70% ethanol), 16.v.1994, I. Kay (QDPI); 1 male, Gatton, iii.1977, G.M. Behncken; 5 females, 1 nymph, bred ex colony Brisbane, vii.1977 (QDPI); 1 male (genitalia mounted separately as 'Homoptera terminalia 16'), same data as previous (QDPI).

Description

Habitus picture lateral see Figure 6.

Length: males ($N = 1$) 3.03 mm; females ($N = 5$) 3.21–3.53 mm

Head and body pale cream with extensive dark brown to black markings, particularly on face and vertex. Tegmen milky white with brown filigree markings delineating oval areas lacking brown as in Figure 6.

Male genitalia. Pygofer with posterior lobe bearing about 20 macrosetae on most of surface. Subgenital plate bearing 5–7 marginal macrosetae. Paramere with preapical process acutely rounded and apical process almost 3 x length of preapical process, measured from base of notch between processes. Apical process tapering throughout, slight angular prominence on outer margin near apex which appears slightly inturned; denticulate on outer surface. Angle between inner margin of apical process and outer margin of subapical process approximately 45°. Aedeagus with basal apodeme long, triangular (Fig. 23). Shafts in lateral view (Fig. 22), slightly sinuate, width more or less even from base to level of gonopore, then narrowed to short narrow process beyond gonopore; in ventral view (Fig. 23) diverging slightly from base to about two thirds length, then evenly curved medially.

Female. Posterior margin of pregenital sternite rounded on either side of median shallow v-shaped emargination,

Diagnosis

O. ryukyuensis is one of three species in which the aedeagal shafts are abruptly narrowed towards the apex. It can be differentiated from *O. recurvus* in ventral view by the aedeagal shafts being incurved throughout rather than abruptly incurved near the apex as in *O. recurvus.* From *O. lotophagorum*, it can be differentiated by the increasing width of the shafts in lateral view in *O. lotophagorum* while in *O. ryukyuensis* the shafts are more or less parallel sided from the base.

Remarks

Initial recognition of this species was based on the male genitalia of a specimen from the JW Evans collection (property of AM, currently at ASCU) with the same collection data as the holotype but attempts to extract DNA were unsuccessful, presumably because of the age of the specimen. Specimens were subsequently sourced from Japan and male genitalia matched to those of the JW Evans specimen. Subsequently, specimens from Australia were also found to match these specimens in male genitalia and the COI sequences of these specimens form a clade close to, but distinct from, *O. lotophagorum* (Fig. 1). We have concluded that *O. ryukyuensis* is present in Australia (Qld, NT, NSW) as well as in Japan and can be differentiated from *O. lotophagorum* on the basis of male genitalia and COI sequences.

Specimens from SE Queensland which were identified as *O. lotophagorum ryukyuensis* (Ishihara) by M.S.K. Ghauri in 1977 were included in this study. These specimens (now in QDPI) have the aedeagal shafts parallel-sided from base to the apical narrowing, a characteristic of *O. ryukyuensis.* Ghauri's use of the name *ryukyuensis* as a subspecies of *O. lotophagorum* indicates that he recognised the similarity between the two species demonstrated here both in the structure of the male genitalia and in the relative closeness of the two clades in the COI sequence analysis (Fig. 1). However, the two are here regarded as valid species because of the consistent differences in the male genitalia and the minimum 10.36% sequence difference between the two haplotype clades.

This species was reported from Australia, as *O. lotophagorum ryukyuensis*, by Behncken (1984) as the vector of little leaf and phyllody disease of bellvine (*Ipomaea plebeia* R.Br., Convolvulaceae).

Orosius canberrensis (Evans)

(Figs 7, 24–25)

Thamnotettix canberrensis Evans 1938: 15
Orosius canberrensis (Evans), Ghauri 1966: 247

Types and Material Examined

Holotype

female (not examined), Canberra, ACT, J.W. Evans (AM)

Other material

AUSTRALIA, New South Wales: 1 male, 12 km E of Balranald, 34°38′00″ S 143°41′60″ E, 6.iv.1997, M.J. Fletcher & J.S. Mann; 3 males, 1 female, Gilgandra, Breelong NP, ex malaise trap MT1, 31°47′18″ S 148°44′59″ E, 20–22.iii.2009, M.J. Fletcher; 2 females, same as previous but ex malaise trap MT2, 31°51′22″ S 148°44′59″ E; 1 female, same as previous but ex malaise trap MT3, 31°53′20″ S 148°46′30″ E; 1 male, Griffith, ex sticky trap 475, 20.x.2005, G. Ali; 1 male, same as previous but ex sticky trap 484; 1 male, same as previous but ex sticky trap 485; 1 male, Forbes, sticky trap 540, cottonbush, 18.v.2005, G. Ali; 1 female, Forbes, ex lucerne & canopy of weeds, 10.vi.2005, G. Ali. Northern Territory: 1 female, 20 km S of Katherine, vacuum swept from ground covers in papaya plantation, 14°38′60″ S 132°16′01″ E, 1998, J. McMahon. South Australia: 1 male, Loxton, sticky trap 3.3.15.1.3, 34°27′00″ S 140°34′01″ E, 17–29.ix.2004, P. Magarey. Western Australia: 1 male, Barrow Island [N20], 20°44′59″S 115°26′50″E, mounted ex ethanol, 6.v.2006, S. Callan & R. Graham (all in ASCU).

Description

Habitus picture lateral see Figure 7.

Length: males ($N = 10$) 3.01–3.33 mm; females ($N = 6$) 3.00–3.33 mm

Colour of head and body creamy white with brown lacy markings, these sometimes pale and indistinct and often with a reddish tinge. Tegmen milky white with brown filigree markings delineating oval areas lacking brown as in Figure 7.

Male genitalia. Pygofer bearing 10–12 macrosetae on basal half of lobe with scattered smaller setae on marginal echinate band. Subgenital plates with 4–6 marginal macrosetae. Paramere with preapical process almost rightangled. Apical process slightly inwardly curved on inner and outer margins, tapering to slightly inturned apex. Aedeagus, in lateral view (Fig. 24), shafts curve slightly ventrally; in ventral view (Fig. 25) with shafts short, parallel.

Female. Posterior margin of pregenital sternite rounded with narrow notch medially.

Diagnosis

This species can be differentiated from all other species of the genus by the short, parallel shafts of the aedeagus in ventral view. *O. pallidus* also has parallel shafts but they are much longer in *O. pallidus* (more than twice as long as the distance between the shafts) than in *O. canberrensis* which is also slightly larger than the other species occurring in Australia. Both species are often quite pale with the distinctive filigree markings on the tegmina rather obscure. *O. canberrensis* also often has reddish markings on the head in association with the brown pattern. This species is consistent in male genitalia and in COI sequence data across Australia.

Orosius cellulosus (Lindberg)

(Figs 8, 26–27)

Thamnotettix cellulosus Lindberg 1927: 90
Nesophrosyne cellulosus (Lindberg), 1958: 176
Orosius cellulosus (Lindberg), Ghauri 1966: 239

Types and Material Examined

Holotype

female (examined), Khartoum, Sudan, 17.x.1924, H.B. Johnston, sucking human blood (FMNH).

Other material

AFRICA, 1 male (abdomen encased in glue), Sudan, Blue Nile, Umm Banein, 14.xi.1962, R. Linnavuori, *ad lucem*, 1 female, Sudan, Atbara, 22.x.1962, S. Panelius, *ad lucem* (FMNH). AUSTRALIA, New South Wales: 1 male, Griffith, sticky trap 485, nightshade, 19.v.2006, G. Ali; 1 male, Griffith, sticky trap 485, Yanga bush, 18.x.2006, G. Ali; 1 female, Griffith, ex yanga bush, sweep net, 18.x.2005, G. Ali; 5 females, Griffith, ex nightshade, sweep net, 19.v.2006, G. Ali; 1 male, Forbes, sticky trap 546, cottonbush, 18.v.2005, G. Ali; 1 male, Forbes, sticky trap 543, cottonbush, 18.v.2005, G. Ali; 1 male, Forbes, sticky trap 569, cottonbush, sweep net, 26.v.2005, G. Ali (all in ASCU).

Description

Habitus picture lateral see Figure 8.

Length: males ($N=4$) 2.99–3.13 mm; females ($N=7$) 3.12–3.46 mm

Head and thorax pale ivory white with dark brown to black lacy markings becoming heavier on ventral surfaces which can be almost entirely black. Tegmen milky white with dark brown filigree markings delineating ovate areas lacking brown as in Figure 8.

Male genitalia. Pygofer with posterior lobe oblique, bearing 20 + macrosetae, those more proximal and distal shorter than those across middle of lobe. Subgenital plate with 5–6 marginal macrosetae. Paramere with preapical process broadly acute, apical process with inner margin slightly convex and very apex slightly inturned with indications of a preapical angle on outer margin; small denticles on apical half of both margins. Aedeagal shafts, in lateral view (Fig. 26), strongly sinuate and narrowing more or less evenly from base to apex which is narrow and curved downwards; in ventral view (Fig. 27), evenly and shallowly curved inwards from base to apex.

Female. Posterior margin of pregenital sternite convex with broad shallow emargination medially.

Diagnosis

This species differs from all other species of the genus in the strongly sinuate shafts of the aedeagus in lateral view.

Remarks

Lindberg (1927) stated that, in addition to the holotype female, he possessed another female plus a male, with the female differing in coloration from the holotype. Ghauri (1966) examined a male and a female specimens in BMNH which he termed paratypes and which would therefore appear to be the other two specimens to which Lindberg (1927) referred.

Although no specimens from North Africa of this species were available for our barcoding analysis, there are a number of specimens in Australia which have been identified as this species based on the strongly sinuate shafts of the aedeagus in lateral view. Supporting evidence is provided by the pregenital sternite of the female. Examination of the female holotype has revealed no significant difference in this feature from those of the females from Australia, despite there being distinct differences between most species of the genus.

This Australian material represents the first record of the species from Australia. Specimens which may also be *O. cellulosus* have been reported from North India (CA Viraktamath pers. comm. 2007).

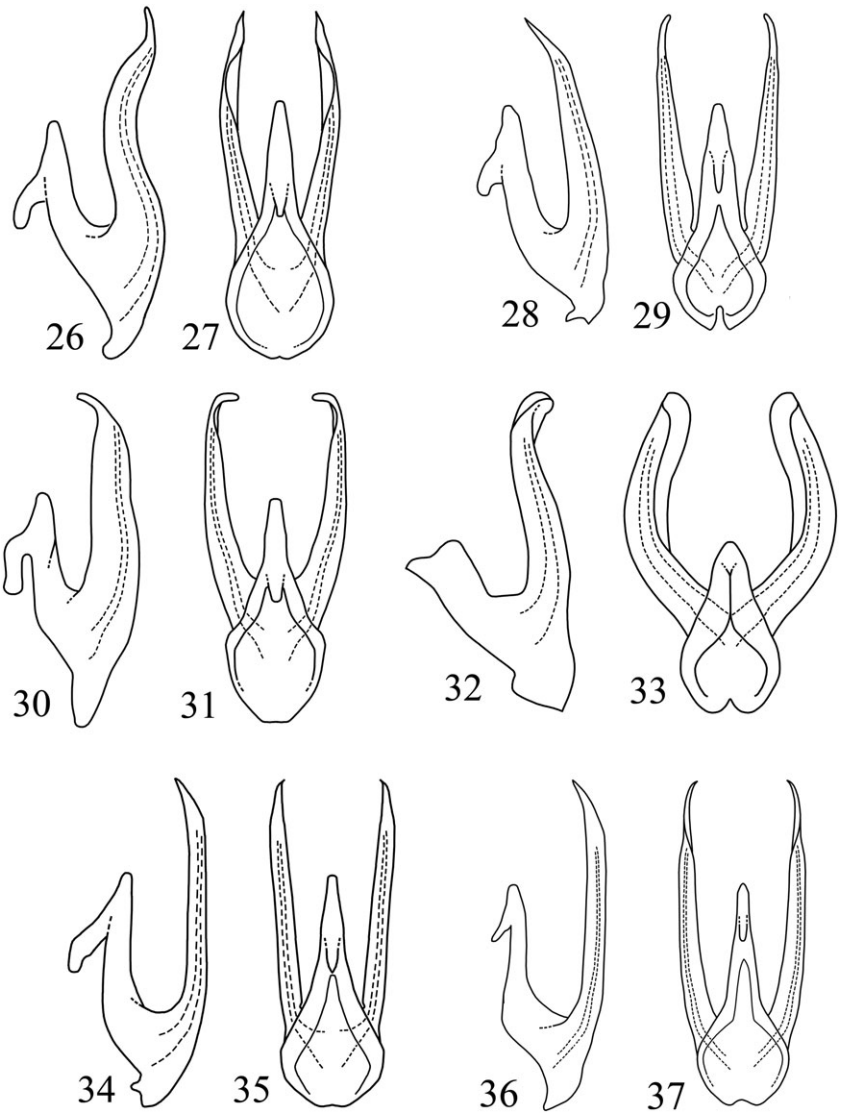
Orosius albifrons Fletcher & Löcker sp. nov.

(Figs 9, 28–29)
http://zoobank.org/urn:lsid:zoobank.org:act:F5D9A5E4-8835-46D1-ABB6-73AE4D0709D7

Types and Material Examined

Holotype

male, Australia, Barrow Island, Western Australia [N27], 20°52′22″S 115°26′35″E, 6.v.2006, S. Callan and R. Graham, mounted ex ethanol (WAM).



Figs 26–37. *Orosius* spp. Male aedeagus. (**26–27**) O. cellulosus, (**26**) lateral view; (**27**) ventral view; (**28–29**) *O. albifrons,* (**28**) lateral view; (**29**) ventral view; (**30–31**) *O. recurvus,* (**30**) lateral view; (**31**) ventral view; (**32–33**) *O. magareyi,* (**32**) lateral view; (**33**) ventral view; (**34–35**) *O. pallidus,* (**34**) lateral view; (**35**) ventral view; (**36–37**) O. brunneus, (**36**) lateral view; (**37**) ventral view.

Paratypes

AUSTRALIA, Western Australia: 9 males, 4 females, same data as holotype; 1 male, same data as holotype but [N04], 20°43′29″ S 115°28′19″E (all in ASCU).

Other material

2 nymphs, same data as holotype (ASCU).

Description

Habitus picture lateral see Figure 9.

Length: males ($N = 13$) 2.56–2.65 mm; females ($N = 6$) 2.78–3.05 mm

Head and thorax pale whitish cream with pale brown lacy markings on vertex but these almost entirely absent from face. Tegmen milky white with filigree markings pale brown, tending to reddish on clavus, and delineating ovate areas lacking brown as in Figure 9.

Male genitalia. Pygofer bearing up to 20 macrosetae, those proximal shorter than those more distal. Subgenital plate with 6 marginal macrosetae. Paramere with preapical process almost rightangled, short. Apical process with inner margin slightly convex, outer margin straight to slightly concave. Apex obliquely truncate to slightly incurved apex. Surface of apical process denticulate. Aedeagus in lateral view (Fig. 28), shafts slightly curved dorsally throughout, slightly bent upwards at gonopore and tapering to acuminate apex; in ventral view (Fig. 29) with shafts slightly divergent from base to level with gonopore, then incurved to apex.

Female. Posterior margin of pregenital sternite straight-oblique from lateral corners to two shallow rounded prominences on either side of broadly v-shaped median emargination.

Diagnosis

This species is distinctive in its white face which contrasts to the rest of the body and tegmina which have the dark brown filigree patterning typical of most species of the genus. The male aedeagus is similar to that of *O. pallidus* sp.nov. but the shafts converge slightly at the very apex, while in *O. pallidus* they do not converge apically.

Distribution

All known specimens are from Barrow Island, Western Australia, and were collected as part of an invertebrate survey of the island associated with the Gorgon Gas development project (Majer *et al.* 2008a,2008b).

Etymology

The species name refers to the distinctive white face found in this species.

Orosius recurvus Fletcher & Löcker sp. nov.

(Figs 10, 30–31)
http://zoobank.org/urn:lsid:zoobank.org:act:74E7F192-11A3-4B0A-B042-0F30D43E9206

Types and Material Examined

Holotype

male, Australia, Forbes, New South Wales, sticky trap 554, nitre goosefoot, 18.v.2005, G. Ali (ASCU: ASCT00174398).

Paratypes

AUSTRALIA, New South Wales: 1 male, same data as holotype but sticky trap 548; 2 males, 3 females, Forbes, ex lucerne, sweep net, 15.vi.2005, G. Ali (all in ASCU).

Description

Habitus picture lateral see Figure 10.

Length: males ($N=4$) 2.94–3.04 mm; females ($N=3$) 3.31–3.48 mm

Colour of head and body creamy white with brown lacy markings. Thoracic sternites dark brown to blackish. Tegmen milky white with brown filigree markings delineating oval areas lacking brown as in Figure 10.

Male genitalia. Pygofer with around 15 macrosetae. Subgenital plate with 5–6 marginal macrosetae with some finer setae scattered between. Paramere with preapical process rounded acute, short. Apical process obliquely acute at apex, forming a slight angular protrusion on outer margin. Inner margin very slightly sinuate. Outer surface with some denticles in some views. Aedeagus, in lateral view (Fig. 30), shafts broad, width more or less even from base to level of gonopore, then narrowed to short narrow process beyond gonopore, apically curved dorsally; in ventral view (Fig. 31) shafts curving slightly from base to near apex, abruptly incurved at apex.

Female. Posterior margin of pregenital sternite laterally more or less straight-oblique laterally with wide shallow concavity medially.

Diagnosis

O. recurvus is one of three species in which the aedeagal shafts are abruptly narrowed towards the apex. It can be differentiated from both *O. lotophagorum* and *O. ryukyuensis* in ventral view by the aedeagal shafts being abruptly incurved near the apex rather than incurved throughout as in the other two species.

Biology

Some of the specimens were collected from lucerne, *Medicago sativa* (Fabaceae) and others from nitre goosefoot, *Chenopodium nitrariaceum* (Chenopodiaceae).

Etymology

The species name refers to the abrupt incurving of the apices of the aedeagal shafts.

Orosius magareyi Fletcher & Löcker sp. nov.

(Figs 11, 32–33)
http://zoobank.org/urn:lsid:zoobank.org:act:83C1F405-D079-4630-A10E-37D04349B182

Types and Material Examined

Holotype

male, Australia, Loxton, South Australia, sticky trap 3.3.15.1.3, 17–29.ix.2004, P. Magarey (ASCU: ASCTHE030135).

Paratypes

4 males, Australia, Loxton, South Australia, sticky trap 4.1.6.1, 10.v.–14.ix.2005, P. Magarey (ASCU).

Description

Habitus picture lateral see Figure 11.

Length: males ($N=5$) 3.07–3.32 mm; females unknown.

Colour of head and body creamy white with brown lacy markings. Thoracic sternites pale brown. Tegmen milky white with brown filigree markings delineating oval areas lacking brown as in Figure 11.

Male genitalia. Pygofer with posterior lobe oblique with 11–12 long macrosetae distributed across the lobe except on marginal band. Subgenital plate with apical process almost equal in length to inner margin of plate. Paramere with preapical process

roundly rightangled. Apical process with inner margin straight, outer margin slightly convex, more so in some views, with minute denticles on surface. Apex not noticeably incurved. Aedeagus, in lateral view (Fig. 32), with shafts slightly curved or almost straight and tapering from base, apex curved ventrally; in ventral view (Fig. 33) with shafts strongly arcuate (horse shoe-like).

Female. Unknown.

Diagnosis

This species has the most distinctive aedeagus of any of the known species of the genus with the shafts strongly incurved in ventral view to form a horseshoe shape.

Comment

All specimens of *O. magareyi* have been collected in sticky traps and are in relatively poor condition.

Etymology

This species is named after Peter Magarey, Plant Pathologist of Loxton, South Australia who collected the type series.

Orosius pallidus Fletcher & Löcker sp. nov.

(Figs 12, 34–35)
http://zoobank.org/urn:lsid:zoobank.org:act:E98D8B55-8FF6-4EC0-AEDC-1D09E60A56D3

Types and Material Examined

Holotype

male, Australia, Loxton, South Australia, sticky trap 3.5.2.1.3, 29.ix.2004, P. Magarey (ASCU: ASCT00174407).

Paratypes

AUSTRALIA, South Australia: 1 male, Loxton, sticky trap 2.1.4.1, 8–18.xi.2004, P. Magarey; 1 male, Loxton, sticky trap 3.8.2.1, 10–24.xi.2004, P. Magarey; 1 female, Loxton, sticky trap 2.1.1.1, 8–18.xi.2004, P. Magarey (all in ASCU).

Description

Habitus picture lateral see Figure 12.

Length: males ($N=3$) 2.88–3.05 mm; females ($N=1$) 3.03 mm

Head and thorax pale creamy brown with faint lacy markings. Tegmen milky white with indistinct pale brown filigree markings delineating unmarked oval areas as in Figure 12.

Male genitalia. Pygofer with 12–14 macrosetae. Subgenital plates bearing 4–5 marginal macrosetae. Paramere with preapical process short, acutely rounded apically. Apical process straight, tapering, inner margin straight, outer margin slightly sinuate to preapical angle before slightly inturned apex. Outer surface of apical process minutely denticulate. Aedeagus, in lateral view (Fig. 34), shafts straight parallel sided with apex tapering and slightly curved dorsally beyond gonopore; in ventral view (Fig. 35) with shafts straight, slightly divergent from base, length more than double maximum distance between the shafts, apices tapering and incurved slightly.

Female. Posterior margin of pregenital sternite convex with additional convex prominence on either side of v-shaped median emargination.

Diagnosis

This species is similar to *O. canberrensis* in having more or less parallel aedeagal shafts with minimal apical convergence. It can be differentiated from *O. canberrensis* by the length of the shafts which, in *O. pallidus*, are more than twice as long as the distance between the shafts. The known specimens of *O. pallidus* have the brown reticulate markings pale and reduced but specimens of *O. canberrensis* may also have these markings reduced or unclear.

Etymology

The species name comes from the pale colouring of the known specimens of this species.

Orosius brunneus Fletcher & Löcker sp. nov.

(Figs 13, 36–37)
http://zoobank.org/urn:lsid:zoobank.org:act:148F80FE-3C29-49CB-9CD4-63EB733498AF

Types and Material Examined

Holotype

male, Australia, Barrow Island, Western Australia [N27], 20°52′22″S 115°19′48″E, 6.v.2006, S. Callan & R. Graham, mounted ex ethanol (WAM).

Paratypes

1 male, 2 females, same data as holotype (ASCU).

Description

Habitus. Small brown insects with obscure pale stripe along centre of forewing (Fig. 13).

Length: males ($N=2$) 2.17–2.52 mm; females ($N=2$) 2.38–2.47 mm.

Colour of head and body creamy white with extensive brown lacy markings. Tegmen pale brown with brown markings delineating oval areas lacking brown, with elongate pale marking from base to about half length of claval suture and another in centre of tegmen extending along middle third of tegmen as in Figure 13.

Male genitalia. Pygofer with 11–12 macrosetae, narrow marginal echinate band with a few short macrosetae. Subgenital plate with 4 macrosetae and some finer setae along outer margin.

Paramere with preapical lobe short, acutely rounded. Apical process long with inner margin convex over most of length, then concave to inwardly turned acute apex. Outer margin straight to apex which curves inward, echinate throughout. Aedeagus, in lateral view (Fig. 36), shafts straight, almost parallel-sided and dorsally tapering at apex; in ventral view (Fig. 37) with shafts slightly curved outwards, then incurved to apex.

Female. Posterior margin of pregenital sternite extending more or less straight to convex prominences on either side of v-shaped median emargination.

Diagnosis

This species is easily recognisable because of its small size and distinctive colouration, particularly the irregular pale stripe along the tegmen. The novelty of this species is well supported by COI sequence data. The specimens currently available are semimacropterous, a feature otherwise not found in species of *Orosius*.

Etymology

The species name refers to the general brownish colouring of this species.

Orosius minuicus Dlabola

Orosius minuicus Dlabola 1979: 255

Types and Material Examined

Holotype

male (not examined), Iran, Khuzestan, Minu-Insel, 29.iv.1976, leg. Pazuki & Abai (Type series not present in IRIPP Plant Protection Institute, Teheran)

Other material

IRAN: 1 male, paratype (examined), same data as holotype (MNHN). SAUDI ARABIA: 1 male, Al Hunayy, 22.x.1978, W. Büttiker (MNHN); 1 female, Wadi Khumra, 12.v.1978, W. Büttiker (MNHN)

Remarks

Dlabola (1979) stated that the type series was deposited in IRIPP but a request to borrow these specimens from IRIPP was unsuccessful, because the type material was never actually deposited there (Fariba Mozaffarian, pers. comm. 24.viii.2010). The Dlabola collection is now in MNHN and a male specimen labelled by Dlabola as paratype was located in that collection and was examined morphologically along with the other material listed above.

The aedeagus of this species is quite distinctive and indicates that the species is not a true *Orosius*, although clearly related. The presence of a subapical lateral tooth on each shaft of the aedeagus and the strongly incurved shafts in ventral view are unlike any other known species, although *O. magareyi* also has strongly incurved shafts. The species is also relatively large, 3.5–3.6 mm in length. Because of these differences, the species is here excluded from the genus and a new genus is being created for its reception (MR Wilson pers. comm. 2015).

DISCUSSION

This study provides diagnostic species identification tools to be used as a standard for future investigations of species of the genus *Orosius*. Species discrimination is critical for investigation of leafhoppers implicated in transmission of plant pathogens, particularly in a biosecurity sense where exotic incursion of a leafhopper has the potential to represent incursion of a new pathogen. With a genus such as *Orosius* containing species with extreme external similarity, reliable diagnostic techniques not only provide an accurate diagnostic capability but also provide the ability to identify species based on life stages which lack the diagnostic morphological features of the male genitalia. We recommend that future studies of pathogen transmission by *Orosius* species include DNA barcode data to confirm species identity. Evidence here of a clear two-fold gap between inter- and intra-specific COI sequence differences at both regional and cosmopolitan sampling scales indicates DNA barcoding will likely remain effective for *Orosius* identifications at increased geographic scales of sampling, as reported for other cosmopolitan insect groups (deWaard *et al*. 2010; Huemer *et al*. 2014; Čandek & Kuntner 2015).

This paper brings the number of described species in the genus *Orosius* to 12, all but one of which have been recognised in the Australian fauna. The non-Australian species is *O. albicinctus*, which is widespread in the central and western Asiatic regions, the Middle East and North Africa. It is possible that our recognition of *O. cellulosus* in the Australian fauna may prove to be incorrect once barcoding of material of this species from North Africa has been undertaken, although the available morphological evidence provided by male and female features supports our recognition of the Australian material listed here as *O. cellulosus*. The recognition of two additional (putative and indeterminate) species based solely on females and delimited by DNA barcoding highlights the utility of this technique and may indicate that additional cryptic species occur in Australia. The status of the colour form recognised on Wake Atoll, *O. lotophagorum* var. *distans* (Linnavuori 1960b) may also change if COI sequence data can be acquired for this form.

The diversity of the genus in the Australian region implies that its centre of radiation is in this region and it has subsequently spread to other parts of the world. This is supported by the uniformity of *O. albicinctus* and *O. orientalis* across their wide geographic ranges.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Table S1 Summary of *Orosius* specimens (N = 227) used in genetic analyses.

Figure S1 Approximately maximum likelihood tree (derived using FastTree 2) of all *Orosius* COI sequences ($N = 227$) including sequences < 500 bp ($N = 25$), rooted at two outgroup *Nesophrosyne* species. Tree tip labels indicate specimen ID (refer Supp. Table S1), a letter indicating the specimen's sex (M = male, F = female, A = indeterminate adult, i.e. samples with damaged abdomens, N = indeterminate nymph), sequence length in nucleotides, the number or ambiguous nucleotides (*N*'s) and species identifications. Indeterminate *Orosius* species labelled as per Table 2. Scale bar indicates 5% sequence difference.