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Nine new species of *Dasineura* (Diptera: Cecidomyiidae) from flowers of Australian *Acacia* (Mimosaceae)

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Abstract. Thirteen species of Australian acacias are invasive plants in agricultural and native vegetation areas of South Africa. Biological control programmes for Australian acacias in South Africa have been implemented and are aimed at suppressing reproductive vigour and, in some cases, vegetative growth of these weeds. Gall-forming midges are under consideration as potential biological control agents for invasive acacias in South Africa. Entomological surveys in southern Australia found a diverse cecidomyiid fauna associated with the buds, flowers and fruits of *Acacia* species. Nine new *Dasineura* species are described and two species, *D. acaciaelongifoliae* (Skuse) and *D. dielsi* Rübsaamen, are redescribed. The newly described taxa are *D. fistulosa* **sp.n.**, *D. furcata* **sp.n.**, *D. glauca* **sp.n.**, *D. glomerata* **sp.n.**, *D. oldfieldii* **sp.n.**, *D. oshanesii* **sp.n.**, *D. pilifera* **sp.n.**, *D. rubiformis* **sp.n.** and *D. sulcata* **sp.n.** All eleven species induce galls on ovaries and prevent the formation of fruit. Two general types of gall are caused. Type A comprises woody, tubular galls with larvae living inside ovaries (*D. acaciaelongifoliae*, *D. dielsi*, *D. fistulosa*, *D. furcata*, *D. glauca*, *D. glomerata*, *D. oldfieldii*). Type B includes soft-tissued, globose galls that belong to four subtypes: inflated, baglike, hairy galls with larvae living between ovaries (*D. pilifera*); pyriform, pubescent swellings with larvae living inside ovaries (*D. rubiformis*); globose, hairy, swellings with larvae living superficially on ovaries in ovoid chambers (*D. oshanesii*); and inconspicuous, glabrous swellings with larvae living superficially on ovaries in shallow groovelike chambers (*D. sulcata*). The gall types are associated with a particular pupation pattern. In type A galls, larvae pupate within larval chambers in galls, whereas in type B galls pupation takes place between ovaries in galls or in the soil beneath the host tree. Gall midges responsible for the same general gall type are morphologically related and differ from species causing the other gall type. Phylogenetic analysis of a 410 bp fragment of the mitochondrial cytochrome *b* gene supports the division of the gall midge species into two groups except for *D. sulcata*, which appears as a subgroup of the group causing type A galls. The interspecific divergence values in group A species were between 0.5 and 3.9% with intraspecific divergence estimates of 0–0.2%. Gall midges causing type B galls had interspecific divergence values of 4.6–7.3% and intraspecific divergence values of 0–3.7%. Closely related biology and morphology together with low cytochrome *b* divergence estimates suggest a more recent speciation in group A when compared with species of group B. *Dasineura rubiformis* and *D. dielsi* are proposed as potential biological control agents for *Acacia mearnsii* De Wild. and *Acacia cyclops* A. Cunn. ex G. Don, respectively, in South Africa due to their narrow host range and ability to form high population densities that reduce seed formation. Both species produce galls with low biomass, which makes them compatible with commercial exploitation of their host species in Africa.

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Introduction

Thirteen species of Australian *Acacia* (*A. baileyana* F. Muell., *A. cyclops* A. Cunn. ex G. Don, *A. dealbata*

Link, *A. decurrens* Willd., *A. elata* A. Cunn. ex Benth., *A. implexa* Benth., *A. longifolia* (Andrews) Willd., *A. mearnsii* De Wild., *A. melanoxylon* R. Br., *A. paradoxa* DC., *A. podalyriifolia* A. Cunn. ex G. Don, *A. pycnantha* Benth., *A. saligna* (Labill.) H. L. Wendl.) are invasive in South Africa (Henderson, 2001) and cause harm to natural and agricultural ecosystems. Most have been utilized in South Africa since the 1820s for stabilization of drift sands, timber and pulp production, tannin extraction, and as garden ornamentals (Shaughnessy, 1980). Naturalization has been widespread and most now form a conspicuous part of the South African landscape. *Acacia mearnsii* (black wattle), *A. melanoxylon* (black wood) and, to a lesser extent, *A. decurrens* (green wattle) are utilized in South Africa for timber, tannin or pulp, whereas *A. cyclops* and *A. saligna* are collected from naturalized populations and widely utilized as a source of domestic firewood. However, all five species are invasive weeds and are responsible for reduced water production, loss of biodiversity, altered ecosystem functions and reduced agricultural productivity (Versfeld & van Wilgen, 1986; Versfeld *et al.*, 1998). Other invasive Australian acacias in South Africa have no recognized commercial value and are generally regarded as undesirable species.

Widespread, problematic infestations of Australian acacias in South Africa necessitated the development of biological suppression methods that commenced in the 1970s with explorations that concentrated initially on host-specific seed-reducing biological agents (Neser & Annecke, 1973). In the case of acacias with commercial value, organisms capable of causing damage to vegetative growth are not considered as appropriate biological control agents because of their potential to cause a conflict of interest with wattle-based industries in the region.

Australia has a rich, but still poorly described Cecidomyiidae fauna (Kolesik, 1998a, b; Bugledich, 1999; Gagné & Law, 1999; Kolesik *et al.*, 2002), including a large assemblage of gall-forming *Dasineura* Rondani and *Asphondylia* Loew from the reproductive organs of acacias (Adair *et al.*, 2000). Surveys of gall-forming cecidomyiids from acacias in southern Australia were undertaken to assess the potential of this guild for biological suppression of invasive acacias in South Africa. Cecidomyiids restricted to *A. mearnsii* and *A. cyclops* or their close relatives are under consideration as potential biological control agents for these plants in South Africa (Adair, 2001, 2002). In 2003, *D. dielsi* was approved for release in South Africa as a biological control agent for *A. cyclops*.

This paper describes nine new species of *Dasineura* discovered in the inflorescences of acacias in Australia and redescribes *D. acaciaelongifoliae* (Skuse) and *D. dielsi* Rübsaamen. Gall morphology, life history and sequence data from the mitochondrial cytochrome *b* gene are used to validate the segregation of these cecidomyiids.

Materials and methods

Collecting and preparation of galls and insects

Between 1998 and 2003, 141 Australian *Acacia* species were surveyed for cecidomyiid galls on inflorescences at 500 field sites in Western Australia, South Australia, Victoria, Tasmania, New South Wales, Australian Capital Territory and Queensland. Field sites were located south of 25° S as this latitude corresponds approximately to the northern distribution limit of invasive Australian acacias in South Africa (Henderson, 2001). Additionally, three African species (*A. karroo* Hayne, *A. xanthophloea* Benth., *Acacia* sp.) and one Central American species (*A. caven* (Mol.) Mol.) found in horticultural plantings at Melbourne, Canberra and Perth in the presence of Australian acacias were surveyed for gall-forming cecidomyiids. No cecidomyiids were found on these non-native acacias in Australia. Sites were selected where at least three sexually mature trees of the same acacia species were present. Selected plants were visually searched for up to 10 min, depending on size, for the presence of galls. The searched plants were identified, counted and their phenological stage recorded. If cecidomyiid galls were located, the plant organs affected, gall condition and stage of insect development were recorded. Insect voucher specimens were collected where the cecidomyiid could not be identified. At selected sites, representative samples of fresh, mature galls were used to annotate gall size, shape, colour, external and internal morphology, and number of individual flower galls forming gall clusters on flower heads. The position and colour of immature cecidomyiid stages (larvae, pupae) within galls were recorded and representative samples were either preserved in 70% ethanol or reared to adults for mounting and identification. Adults were reared in one of two ways, depending on the species. Species pupating within galls were reared by holding mature galls in clear, ventilated plastic containers until emergence of adults. Galls of cecidomyiids that pupate in soil were placed on the surface of pulverized garden peat held in open plastic containers within emergence cages. Newly emerged adults were collected and preserved in 70% ethanol. Pupal skins were collected and stored dry in gelatine capsules. Permanent microscope slides were prepared for type series and other material. For each accession, five or six specimens each of larvae, pupae, pupal skins, females and males were macerated in 20% KOH (larvae and pupae were perforated laterally with a thin needle to improve maceration), washed in 20% acetic acid, 70% and then 99% ethanol, cleared in Histoclear and mounted in Canada balsam on glass slides under round glass cover slips 10 mm in diameter. Whole larvae and pupae were mounted dorsoventrally. Adults were dissected into four pieces with the particular body parts mounted separately: wings and head frontally, thorax laterally, abdomen dorsoventrally or for half of the female specimens laterally. Pinned type specimens of *D. acaciaelongifoliae* (Skuse) were dipped in 70% ethanol for about 10 s, immersed in distilled water for 5 min to dissolve the glue

used to attach the insects to the paper label cards, immersed in 99% ethanol and then processed following ethanol-stored specimens. Type specimens of *D. dielsi* Rübsaamen were not examined as their glycerol mounts, stored in the Museum für Naturkunde der Humboldt-Universität zu Berlin, Germany, are currently not accessible due to high fragility (M. Kotrba, pers. comm.). The type series are deposited in the South Australian Museum, Adelaide (SAMA) and Australian National Insect Collection, Canberra (ANIC). Dried galls of *D. acaciaelongifoliae* are deposited in the Macleay Museum, Sydney (MLMS). Plant vouchers are deposited in the State Herbarium either in Perth, Melbourne or Sydney.

Morphology

Insect morphology was investigated with bright field and phase contrast microscopy. Length measurements were made with a digital imaging system. Measurements refer to type series unless stated otherwise. Drawings were made with the aid of a drawing tube. The terminology of adults and pupae follows Gagné (1981) and of larvae follows Gagné (1989). States of five larval, three pupal, three female and nine male morphological characters were used to distinguish between the eleven species described here (Table 1). Of these twenty characters, all but two uninformative characters (M3 and M6) were used to reconstruct the phylogenetic relationships. Binary coding was employed for ten of the eighteen informative characters, and the remaining eight characters were treated as unordered multistate characters. Phylogenetic analyses were executed in PAUP*4 version beta 10 (Swofford, 2002) using maximum parsimony (MP). Trees were generated using the heuristic search option with tree-bisection-reconnection (TBR) branch swapping and stepwise addition using 1000 random sequence addition replicates. Nodal support was assessed from the MP analysis of 10 000 nonparametric bootstrap replicates (full heuristic search; 'as is' stepwise addition of taxa). Additionally, Bremer support values (Bremer, 1994) were calculated with TREEROT version 2 (Sorenson, 1999) using default parameter settings, as the use of the bootstrap for assessing nodal support in morphological analyses, where the number of characters relative to the number of taxa is small, has been questioned (Sanderson, 1989). Trees were rooted using as an outgroup *D. wahlenbergiae* Kolesik & Skuhravá, an Australian species that induces soft-tissued shoot bud galls on *Wahlenbergia stricta* (R. Br.) Sweet (Campanulaceae) (Kolesik & Skuhravá, 1997). As the larvae of *D. oldfieldii* **sp.n.** were not collected, for the purpose of the analysis, larval characters of this species were assumed to be identical to those of the other species in group A.

DNA extraction, amplification, sequencing and phylogenetic analysis

A fragment of the mitochondrial cytochrome *b* gene was amplified, sequenced and aligned for representative accessions of the eleven *Dasineura* species described in this study. Total DNA was extracted from immature stages or

Table 1. Matrix of morphological characters.

	Character																			
	L1	L2	L3	L4	L5	P1	P2	P3	F1	F2	F3	M1	M2	M3	M4	M5	M6	M7	M8	M9
D. pilifera	0	1	2	0	1	1	1	1	0	1	2	1	0	0	1	1	1	0	1	1
D. rubiformis	0	1	1	0	1	1	1	1	0	1	1	1	0	1	0	0	0	0	1	0
D. oshanesii	0	1	1	0	1	1	1	1	0	1	1	1	0	1	0	1	0	0	1	1
D. sulcata	0	1	0	1	1	1	1	1	0	1	1	1	0	1	0	1	0	0	1	0
D. glomerata	1	0	0	2	0	0	0	0	1	0	2	1	1	1	1	0	0	1	2	0
D. fistulosa	1	0	0	2	0	0	0	0	1	0	0	2	1	1	1	1	0	2	2	0
D. dielsi	1	0	0	2	0	2	0	0	1	0	2	0	1	1	0	1	0	3	3	0
D. oldfieldii	?	?	?	?	?	2	0	0	1	0	2	0	1	1	1	1	0	3	0	0
D. glauca	1	0	0	2	0	0	0	0	1	0	2	1	1	1	0	0	0	0	3	0
D. furcata	1	0	0	2	0	2	0	0	1	0	1	1	1	1	2	1	0	3	3	0
D. acaciaelongifoliae	1	0	0	2	0	2	0	0	1	0	1	1	1	1	2	1	0	0	3	0
D. wahlenbergiae	0	1	2	0	0	1	1	1	0	1	0	2	1	1	0	1	0	0	0	0

Characters in larva (L), pupa (P), female (F) and male (M): L1, antennae obtuse (0) or tapered (1); L2, spatula present (1) or absent (0); L3, number of inner lateral papillae none (0), one (1), three (2); L4, integument covered with smooth round (0), irregular wide (1) or spiky (2) plates; L5, head with posterolateral apodemes longer than (1) or as long as head capsule (0); P1, antennal horns minute (0), medium-sized (1) or large (2); P2, tracheal horns on first thoracic segment longer than wide at base by a factor of ten (1) or four (0); P3, tracheal horns on first thoracic segment tapered (1) or blunt (0); F1, first flagellomere with (1) or without (0) mesal bulge; F2, sclerotization of tergites 1–6 entire (1) or interrupted mesally (0); F3, tergite 7 with constriction less than half (0), half (1) or more than half (2) width of posterior edge; M1, gonostyle tapered distally strongly (0), slightly (1) or not (2); M2, dorsal setulation on gonostyle one-tenth (0) or greater than one-fifth (1) gonostyle length; M3, cerci triangular (1) or subglobular (0); M4, lobes on hypoproct thin (0), medium wide (1) or wide (2); M5, hypoproct terminally wider than or as wide as (1) or narrower (0) than medially; M6, hypoproct's incision with an angle (1) or straight (0); M7, lobe on gonocoxite: none (0), large round (1), small round (3) or small acute (2); M8, aedeagus apically: cylindrical blunt to concave (1), cylindrical rounded (2), conical thin (3) or conical robust (0); M9, sclerotization of tergites 2–7 uninterrupted (1) or weakened mesally (0); ?, character not known.

adults using a standard phenol–chloroform extraction protocol (Sambrook *et al*., 1989). For very small specimens for which phenol–chloroform extraction was considered unfeasible, the entire pupa or larva was added to the polymerase chain reaction (PCR) reaction tube. DNA pellets were resuspended in 150 µl of molecular grade water. Cytochrome *b* insect-specific primers CB1 and CB2 (Simon *et al*., 1994) were used to amplify a 486 bp fragment of cytochrome *b* using PCR. PCR was performed in 0.5 ml thin-walled Eppendorf tubes using 25 µl reaction volumes overlaid with mineral oil. The reaction mixture consisted of each dNTP at 0.2 mM, Biotaq DNA polymerase (Bioline, Whitehead Scientific, USA) at 0.02 U μl^{-1}, 0.2 µM forward and reverse primer, 2.5 mM $MgCl_2$, and magnesium-free 10× reaction buffer (Whitehead Scientific, South Africa) was added to a 1× final concentration. Two microlitres of template DNA was added to each reaction. PCR was performed with the following cycling conditions: one cycle for 3 min at 94 °C, followed by thirty-five cycles of 45 s at 94 °C, 45 s at 40 °C and 1 min at 72 °C, and a final extension step at 72 °C for 10 min. Five microlitres of the resulting PCR product was electrophoresed on a 1.5% agarose gel containing 0.5 µg ml^{-1} ethidium bromide and visualized under ultraviolet light. If amplification was successful, the remaining 20 µl from each PCR reaction was purified using Qiagen QIAquick PCR purification kit columns (Qiagen, Germany). Purified PCR product was eluted in 30 µl of sterile distilled water, pH 8.0. Sequencing reactions were carried out directly on purified PCR products using BigDye terminator chemistry according to the recommendations of the manufacturer (Perkin-Elmer Applied Biosystems, USA). The reaction products were electrophoresed on an ABI 3100 capillary sequencer and manually checked and edited in CHROMAS 2.23 (Technelysium Pty Ltd, Australia). Sequences were aligned using CLUSTAL X (Thompson *et al*., 1997). *Mayetiola destructor* (Say) (Cecidomyiidae: Oligotrophini, GenBank accession number AF488423) and *Contarinia loti* (De Geer) (Cecidomyiidae: Cecidomyiidi, GenBank accession number AY01735) were designated as outgroups to the *Dasineura* ingroup species. Sequences were deposited in GenBank under the accession numbers stated below.

Phylogenetic analyses of an unambiguously alignable fragment consisting of 410 bp were executed in PAUP*4 version beta 10 (Swofford, 2002) using MP and maximum likelihood (ML) optimality criteria. Base frequency stationarity was evaluated using a χ^2 test implemented in PAUP with uninformative characters excluded (Waddell *et al*., 1999). For MP analyses, all parsimony-uninformative characters were removed prior to the analyses. The remaining parsimony-informative characters were given equal weights in the analysis. Trees were generated using the heuristic search option with TBR branch swapping and stepwise addition using 1000 random sequence addition replicates. For the ML analysis, the appropriate nucleotide substitution model was estimated using MODELTEST version 3.06 (Posada & Crandall, 1998) using the Akaike information criterion following Buckley *et al*. (2001). The general time reversible model with variable sites assumed to follow a discrete gamma distribution (GTR + Γ model) (Yang, 1994) was selected as the best-fit model for the sequence data by MODELTEST with the following parameters: base frequency of A = 0.35, C = 0.13, G = 0.10, T = 0.42; the rate matrix was $R_{[A-C]} = 4.76$, $R_{[A-G]} = 19.13$, $R_{[A-T]} = 9.98$, $R_{[C-G]} = 0.00$, $R_{[C-T]} = 32.12$, $R_{[G-T]} = 1.00$, gamma correction $\alpha = 0.38$. A heuristic ML analysis was implemented in PAUP with the starting tree obtained using ten random sequence addition replicates and TBR branch swapping under the optimal model suggested by MODELTEST. For both datasets, nodal support for the MP and ML analyses was assessed from 1000 and 100 nonparametric bootstrap replicates, respectively (full heuristic search; 'as is' stepwise addition of taxa). In this study, bootstrap values < 50% are considered as not supported, bootstrap values (BS) between 50 and 70% as weakly supported, and bootstrap values > 70% as strongly supported (Daniels *et al*., 2002).

Combined phylogenetic analysis

Possible incongruence between the morphological and molecular datasets was evaluated using the incongruence length difference (ILD) test (Farris *et al*., 1994) implemented as the 'Partition Homogeneity Test' in PAUP*4 version beta 10. The test was implemented under parsimony with ten random addition sequences of taxa and 500 replicates to generate the null distribution. Uninformative characters were excluded prior to the analysis (Lee, 2001). For the combined analysis, only ingroup taxa were included due to different outgroups used for the morphological and molecular analyses. The combined dataset therefore consisted of 430 characters in total (410 bp plus twenty morphological characters), forty-five of which were parsimony informative. A heuristic MP search was implemented using 1000 random sequence addition replicates. Nodal support was evaluated from 1000 nonparametric bootstrap replicates with ten random stepwise addition of taxa.

Results

Galls

Dasineura females oviposit in open acacia flowers and deposit eggs within the perianth tube, usually around the ovary. Larvae cause the ovary to evaginate and form developmental chambers around the larvae that vary from shallow depressions to deeply infolded sacks or elongated tubes. Infested flower heads are transformed into clusters of galls, which may vary in number of individual galls depending on the number of flowers infested. The individual galls of species described here are mostly polythalamous, the number of larval chambers varies depending on the number of eggs deposited within the flower. Each larval chamber is occupied by one larva, only rarely two larvae can be found in a single chamber. Two general gall types induced by *Dasineura* were found on the flower heads of Australian

acacias. Type A comprises woody tubular galls, with elongate larval chambers that have exit holes positioned distally on the tubular lobes and larvae living inside malformed ovaries. This type was induced by *D. acaciaelongifoliae* (Figs 1H, 2M, N), *D. dielsi* (Figs 1J, 2R), *D. fistulosa* (Figs 1F, 2I, J), *D. furcata* (Figs 1I, 2O, P), *D. glauca* (Figs 1G, 2K, L), *D. glomerata* (Figs 1E, 2G, H) and *D. oldfieldii* (Fig. 2S, T). Type B includes soft-tissued, galls that can be further divided into four subtypes: inflated, baglike, hairy galls with larvae living between ovaries

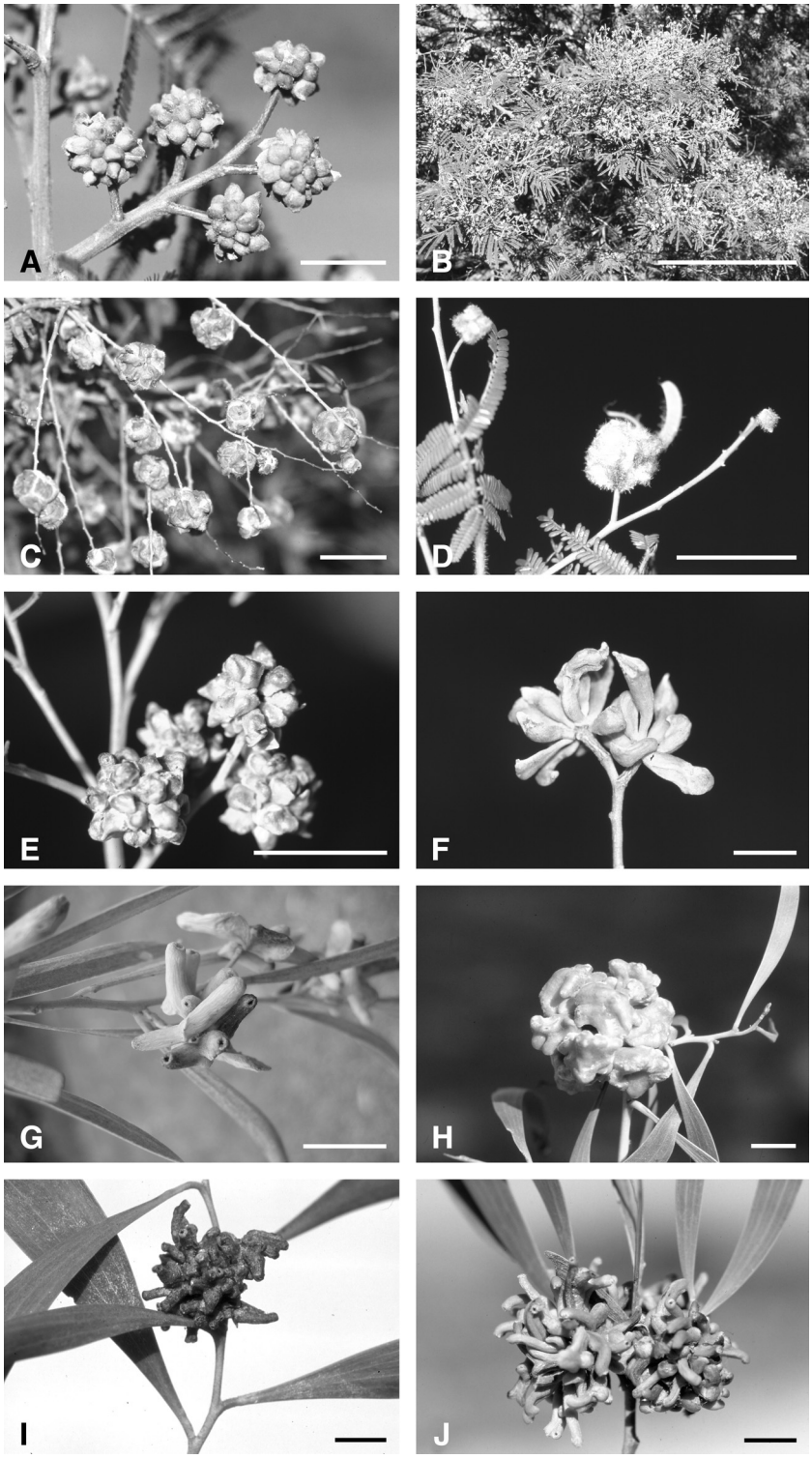

Fig. 1. Galls of *Dasineura* spp. on *Acacia* spp., surface view. A, B, *Dasineura rubiformis* on *A. mearnsii*; C, *D. pilifera* on *A. baileyana*; D, *D. oshanesii* on *A. oshanesii* (one pod not galled); E, *D. glomerata* on *A. mearnsii*; F, *D. fistulosa* on *A. mearnsii*; G, *D. glauca* on *A. pendula*; H, *D. acaciaelongifoliae* on *A. implexa*; I, *D. furcata* on *A. melanoxylon*; J, *D. dielsi* on *A. cyclops*. Scale bars represent 10 mm in A, C–J and 1 m in B.

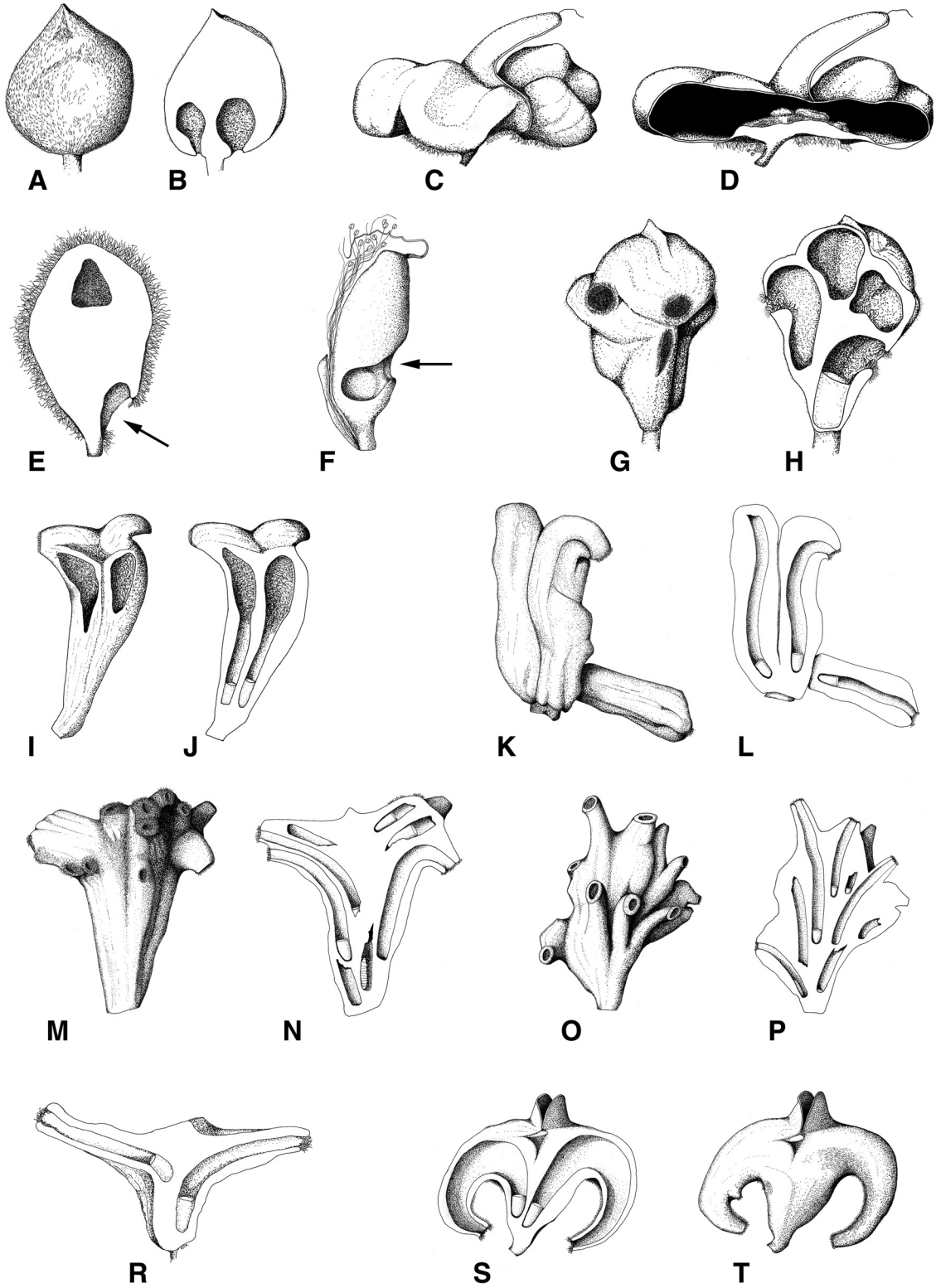

Fig. 2. Galls of *Dasineura* spp. on *Acacia* spp., internal morphology. A, B, *Dasineura rubiformis* on *A. mearnsii*; C, D, *D. pilifera* on *A. baileyana*; E, *D. oshanesii* on *A. oshanesii*; F, *D. sulcata* on *A. saligna*; G, H, *D. glomerata* on *A. mearnsii*; I, J, *D. fistulosa* on *A. mearnsii*; K, L, *D. glauca* on *A. pendula*; M, N, *D. acaciaelongifoliae* on *A. implexa*; O, P, *D. furcata* on *A. melanoxylon*; R, *D. dielsi* on *A. cyclops*; S, T, *D. oldfieldii* on *A. oldfieldii*. Arrows indicate larval chambers.

in shallow depressions (*D. pilifera*; Figs 1C, 2C, D); pyriform, pubescent swellings with larvae living inside ovaries (*D. rubiformis*; Figs 1A, B, 2A, B); globose, hairy, swellings with larvae living outside ovaries in semiopen chambers (*D. oshanesii*; Figs 1D, 2E); and inconspicuous, glabrous swellings with larvae living outside ovaries in shallow groovelike chambers (*D. sulcata*; Fig. 2F). The two general gall types were found to be associated with a particular pupation pattern. In type A, larvae pupate within larval chambers in galls, whereas in type B pupation takes place either in the soil (*D. rubiformis*) or partially between galls in gall clusters and partially in the soil beneath the host tree (*D. oshanesii*, *D. pilifera*, *D. sulcata*).

Descriptions of species

Genus Dasineura Rondani

Dasineura Rondani, 1840: 12, 17
Citation list after 1840 in Gagné (2004)

Type species. *Tipula sisymbrii* Schrank, 1803 (ICZN 1998).

Diagnosis. *Dasineura* is a large, cosmopolitan genus containing species of the supertribe Lasiopteridi with toothed tarsal claws, R5 wing vein that meets C anteriorly to the wing apex, and female tergite 8 divided into two longitudinal sclerites. Most species are gall inducers, some live inside flowers without causing tissue deformities and several are inquilines inside galls induced by other gall midges.

Characters shared by all eleven species treated here

Mature larva. Thoracic segments on each side: three dorsal, two pleural papillae, all setose; one sternal, one ventral papilla, both asetose. First seven abdominal segments on each side: three dorsal, two pleural, one ventral papilla, all setose; two sternal papillae, both asetose. Abdominal segment 8 on each side: one dorsal, two pleural, two ventral papillae, all setose. Abdominal segment 9 with six to eight terminal papillae, setose. Transverse rows of spiculae on anterior half of ventral side of thoracic and abdominal segments 1–7. Colour: pale orange.

Pupa. Cephalic papilla with long seta. Antenna at vertex with angular, strongly sclerotized horns. Prothoracic spiracle with trachea ending at apex. Frons on each side: two frontal and three lateral facial papillae, one of each set setose, remaining asetose. Integument of abdominal segments covered with spiculae, segments 2–8 each with field of large dorsal spines on anterior half. Abdominal segments 2–7 on each side with one outer dorsal papilla, setose; two pairs of inner dorsal papillae, each pair consisting of closely situated papillae: distal asetose, proximal setose, in some species number of papillae reduced in some specimens. Abdominal segment 8 with one outer dorsal papilla, setose. Colour: brown, thorax becoming black shortly before adult emergence.

Adults. *Head*. Antenna: flagellomeres progressively smaller, each consisting of node and neck; nodes with closely appressed circumfila comprising two transverse and two longitudinal bands; necks progressively longer, quarter node length basally to half node length distally in male and very short in female, first and second flagellomeres fused fully in females and partially in males. Apical flagellomeres fused in some specimens in either sex. Palpus four-segmented, segments 2 and 3 longer than 1 and shorter than 4. Palpiger half length and as narrow as first palpal segment. Labella pointed distally. Eye bridge five to six ocelli long in male, two to three long in female.

Thorax. Wing: C with break at juncture with R5, RS reduced to bend on R5; R1, C anteriorly to juncture with R1 and entire Sc cell pigmented, all covered with scales. Tarsal claws toothed, empodia about as long as tarsal claw, pulvilli.

Abdomen. Sclerites with pair of sensory setae anteriorly, long setae posteriorly, sparsely covered with scales. Sternites 2–8 each consisting of two transverse sclerites, covered with long setae. Tergites rectangular, 1–7 with posterior band of setae associated with stronger sclerotization. Tergites 1–6 with sclerotization slightly weakened mesally in male in all species and female in some species. In female, tergite 7 constricted medially, tergite 8 consisting of two longitudinal sclerites. Male genitalia: gonocoxite cylindrical, setose and setulose; gonostyle setose, setulose basally and striate distally, bearing distal comblike claw; cerci covered with regular patches of microtrichia, each with several setae apically; hypoproct bilobed, uniformly covered with microtrichia, one to three setae on each lobe; parameres sheathing aedeagus slightly shorter than aeadeagus, densely covered with long setulae, one to several setose papillae on each lobe apically. Female genitalia: ovipositor long, protractile; cerci large, fused into single terminal lamella, covered with regular patches of microtrichia, several thick sensory hairs apically; hypoproct small, trapezoid in ventral view, with pair of setae apically.

Colour. Eyes, setae and scales black; unsclerotized parts of thorax and abdomen orange, sclerotized parts brown; head, legs, antennae, palpi and halteres grey.

Dasineura rubiformis Kolesik sp.n. (Figs 1A, B, 2A, B, 3A–P)

Types. Western Australia. Holotype male, 5 km east of Albany [34° 97444′S, 117°88861′E], ex flower gall on *A. mearnsii* collected 29.vii.1999 (RJA2901), I21573 (SAMA). Paratypes: two males, three females, three pupal skins, three larvae (SAMA, I21574–I21584), two males, two females, three pupal skins, three larvae (ANIC), collected with holotype.

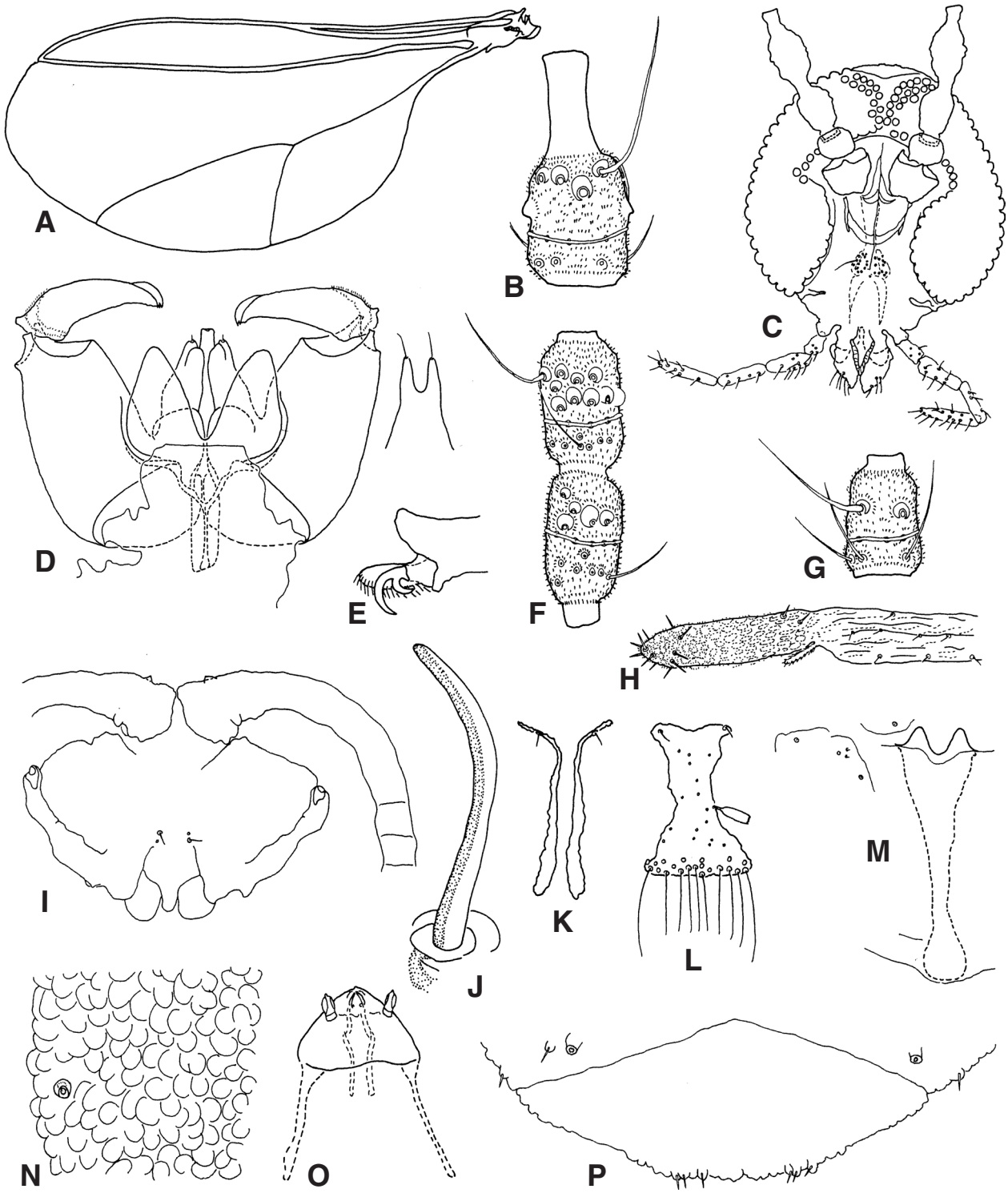

Fig. 3. *Dasineura rubiformis*. A–E, Male; F–H, K, L, female; I, J, pupa; M–P, larva. A, Wing; B, flagellomere 6; C, head frontally; D, genitalia dorsally; E, tarsal claw and empodium; F, flagellomeres 1–2; G, flagellomere 6; H, end of ovipositor laterally; I, anterior part ventrally; J, prothoracic spiracle; K, tergite 8; L, sternite 7; M, sternal spatula; N, integument; O, head ventrally; P, terminal segment dorsally.

Other material examined. RJA2609: Western Australia, 5 km east of Albany [34°97444′S, 117°88861′E], ex flower gall on *A. mearnsii* collected 3.xi.1998, five males, five females.

DNA analysis. Cytochrome b sequenced for RJA2609 (GenBank accession number AY278712) (Western Australia, 5 km east of Albany [34°97444′S, 117°88861′E] ex flower gall on *A. mearnsii* collected 3.xi.1998) and RJA3011 (GenBank accession number AY278737) (Victoria, Keilor, Brimbank Park [37°43491′S, 144°49601′E], ex flower gall on *A. mearnsii* collected 5.vii.2000).

Description

Larva (Fig. 3M–P). Length 1.95 mm (1.67–2.41 mm, n = 6). Integument smooth except for field of transverse rows of spiculae on anterior half of ventral side of each thoracic and first to seventh abdominal segments; covered with large, round, regularly shaped plates. Head: antennae obtuse; posterolateral apodemes 1.5× longer than head capsule. Thoracic segment 1 sternal spatula bilobed, long shafted; on each side: one inner pleural papilla, asetose; triplet of outer lateral papillae, two of them with setulae; one inner lateral papilla, asetose. Setation on thoracic segments 2 and 3 as on segment 1 except inner pleural papilla setose. Terminal papillae six in number.

Pupa (Fig. 3I, J). Length 1.67 mm (1.45–1.82 mm, n = 6). Antennal horns of medium size. Prothoracic spiracle tapered distally, 10× longer than wide at base. Number of inner dorsal papillae on abdominal segments in some specimens reduced to two asetose papillae.

Male (Fig. 3A–E). Wing length 1.59 mm (1.45–1.68 mm, n = 5). Antenna: scape and pedicel wider than long; flagellomeres sixteen (fifteen to sixteen) in number, node of first one globular, remaining ones cylindrical, 1.5× longer than wide. Tergites 1–7 with sclerotization slightly weakened mesally. Genitalia: gonostyle slightly tapered distally, covered with setulae to one-tenth length dorsally and two-thirds ventrally; gonocoxite bearing no protuberance; aedeagus cylindrical, blunt to concave apically; cerci triangular; hypoproct medially and distally narrower than basally, incision U-shaped with depth one-third hypoproct length.

Female (Fig. 3F–H, K–L). Wing length 1.74 mm (1.62–1.88 mm, n = 5). Flagellomeres: fifteen (fourteen to fifteen) in number; flagellomere 1 barrel-shaped, symmetrical, 2× longer than wide, flagellomere 2 shaped evenly, cylindrical, remaining ones conical. Hypoproct one-fifth to one-quarter length of cerci. Tergites 1–7 with uninterrupted sclerotization. Constriction of tergite 7 half width of posterior edge. Sclerites of tergite 8: posterior three-quarters parallel, strongly widening towards posterior ends; anterior one-quarter strongly divergent, uniformly narrow; sensory setae placed at midlength of anterior quarter.

Etymology

The name refers to the resemblance of the gall cluster to a young fruit of blackberry, *Rubus*. Common name: ‘tiny flower galler’.

Biology and distribution (Figs 1A, B, 2A, B)

Dasineura rubiformis induces a soft-tissued flower gall on several species of the *Acacia* section Botrycephalae in southern Australia. The gall is obovate-pyriform to irregularly globose, usually with an asymmetrical bulge in the basal half. The apex is broadly acute, often tapered with a short, flattened keel. Between one and thirty-six individual flower galls make up a gall cluster that is 3–13 mm long and 6–16 mm wide ($n = 160$). Individual galls are 2–6 mm long and 2–5 mm wide ($n = 125$). The outer gall surface is green with appressed, sparsely pubescent white hairs, becoming pale yellow–orange basally and around the perimeter of the exit hole. There are one to five ovoid larval chambers per individual gall with their respective exit holes positioned basally. One or occasionally two larvae occupy each larval chamber. Galls mature in June to July. The larvae exit the gall and drop to the soil beneath the host tree to pupate. Adults emerge in September to December. *Dasineura rubiformis* is univoltine and widespread in eastern Australia. In Western Australia, it is restricted to naturalized *A. mearnsii* in the higher rainfall region of the southwest region, where gall densities can be extremely high, impeding fruit and seed production. In eastern Australia, gall densities of *D. rubiformis* are highly variable. Galls similar to those of *D. rubiformis* on *A. mearnsii* with similar larvae and closely matched DNA sequences (divergence 2.2–2.9% compared with *D. rubiformis* from the type locality) were found on the Botrycephalae acacias: *A. deanei* (R. Baker) Welch, Coombs & McGlynn (RJA3007), *A. irrorata* Sieber ex Sprengel (RJA3201), *A. parramattensis* Trind. (RJA3185, 3183), *A. leucoclada* Trind. (RJA2725) and *A. constablei* Trind. (not DNA tested) in New South Wales and the Australian Capital Territory. Adults need to be reared and examined to confirm these hosts of *D. rubiformis*.

Dasineura pilifera **Kolesik sp.n. (Figs 1C, 2C, D, 4A–D)**

Types. Victoria. Holotype male, Lilydale [37°7661′S, 145°37028′E], ex flower gall on *A. baileyana* collected 29.xi.1998 (RJA2602), I21585 (SAMA). Paratypes: two males, three females, three pupae, three larvae (SAMA, I21586–I21596), two males, two females, three pupal skins, three larvae (ANIC), collected with holotype.

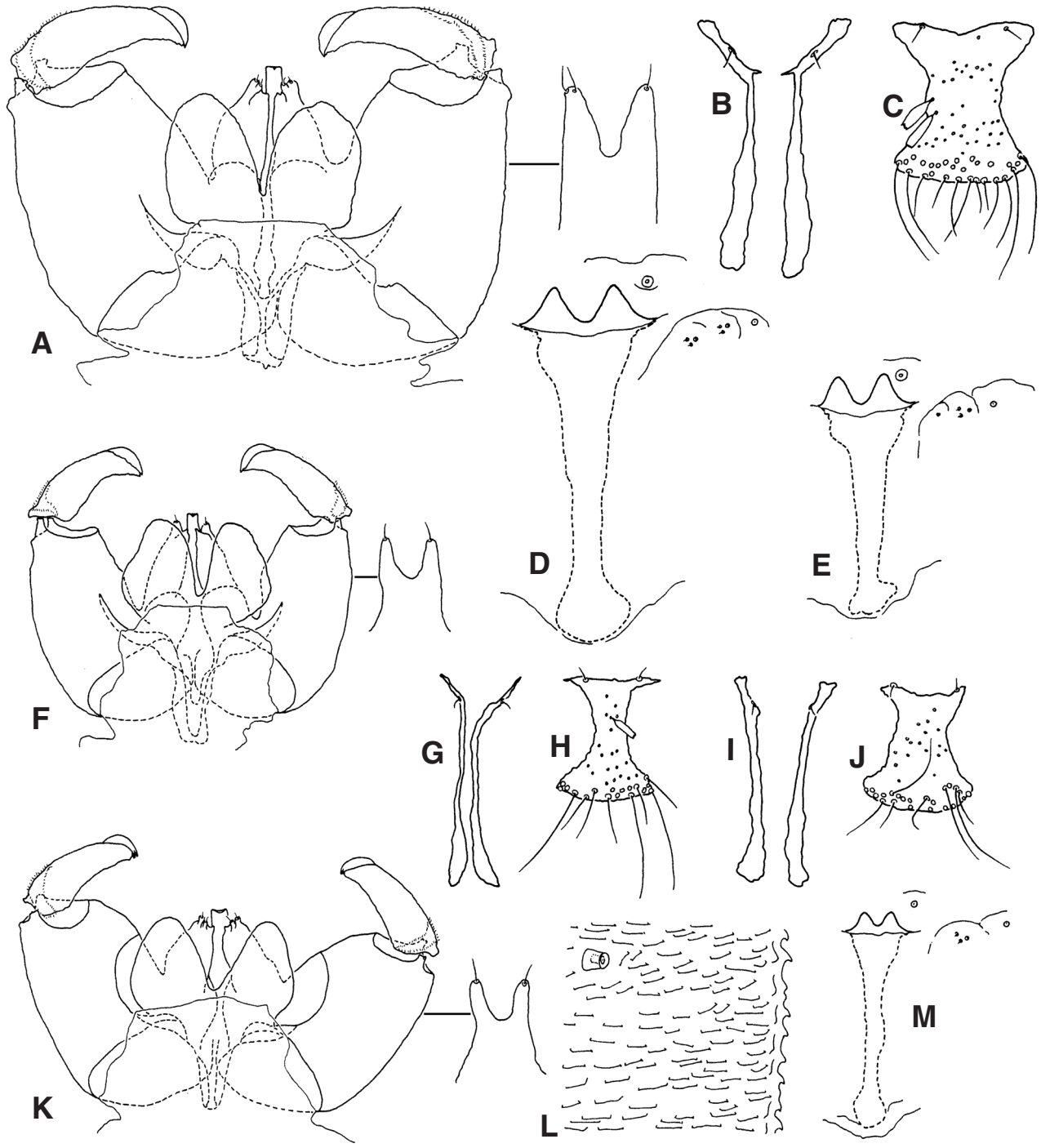

Fig. 4. A–D, *Dasineura pilifera*; E–H, *D. oshanesii*; I–M, *D. sulcata*. A, Male genitalia dorsally; B, female tergite 8; C, female tergite 7; D, E, larval sternal spatula; F, male genitalia dorsally; G, female tergite 8; H, female tergite 7; I, female tergite 8; J, female tergite 7; K, male genitalia dorsally; L, larval integument; M, larval sternal spatula.

Other material examined. RJA2607: Victoria, Merricks North [38° 21321′S, 145° 04609′E], ex flower gall on *A. baileyana* collected 21.x.1998, five larvae; RJA2624: ACT, Canberra North [35° 22222′S, 149°17639′E], ex flower gall on *A. baileyana* collected 25.viii.1998, one pupa; RJA2761: Victoria, Warrandyte [37°74139′S, 145°22139′E], ex flower gall on *A. baileyana* collected 11.iv.1999, five males, five females, six pupae, four pupal skins, three larvae; RJA2599: Western Australia, Northcliffe [34°63694′S, 116° 11111′E], ex flower gall on *A. decurrens* collected 2.xi.1998, five

males, one female, twelve pupal skins, six larvae; RJA2663: Victoria, Swanpool [36° 75750′S, 146° 00194′E], ex flower gall on *A. decurrens* collected 30.vii.1998, one larva, two pupae, three pupal skins; RJA2661: Victoria, Myrtleford [36° 33′S, 146° 43′E], ex flower gall on *A. decurrens* collected 31.vii.1998, three males, four pupae, two pupal skins, four larvae; RJA2668: ACT, Canberra [35° 16′S, 149° 09′E], ex flower gall on *A. dealbata* collected 22.xii.1998, six larvae; RJA2736: ACT, Madew, Federal Highway 20 km northeast of Canberra [34°99278′S, 149°38250′E], ex flower gall on *A. dealbata* collected 19.xii.1998, five males, one female, two pupae, six pupal skins.

DNA analysis. Cytochrome b sequenced for RJA2602 (GenBank accession number AY278727), RJA2663 (GenBank accession number AY278735), RJA2884 (GenBank accession number AY278726) (Western Australia, Jarrahdale 32°20174′S, 116°03572′E], ex flower gall on *A. decurrens* collected 15.x.1999) and RJA3148 (GenBank accession number AY278718) (New South Wales, Baldry 32°52038′S, 148°29558′E], ex flower gall on *A. baileyana* collected 25.x.2001).

Description

Characters other than below are as in *D. rubiformis*.

Larva (Fig. 4D). Length 2.68 mm (2.52–2.76 mm, $n = 6$). Triplets of inner and outer lateral papillae, two of each set with setulae. Terminal papillae eight in number.

Pupa. Length 2.23 mm (1.95–2.44 mm, $n = 6$).

Male (Fig. 4A). Wing length 2.30 mm (2.15–2.41 mm, $n = 5$). Flagellomeres fifteen (fourteen to fifteen) in number. Sclerotization of tergite 1 weakened mesally, tergites 2–7 with uninterrupted sclerotization. Genitalia: cerci subglobular; hypoproct evenly wide, with keyhole-shaped incision of depth two- to three-fifths hypoproct length.

Female (Fig. 4B, C). Wing length 2.58 mm (2.50–2.62 mm, $n = 5$). Flagellomeres fourteen (thirteen to fifteen) in number. Constriction of tergite 7 more than half width of posterior edge. Hypoproct one-quarter to one-third length of cerci. Sclerites of tergite 8: posterior two-thirds parallel, strongly widening towards posterior ends; anterior one-third strongly divergent, slightly widening towards anterior ends; sensory setae placed at midlength of anterior third.

Etymology

Pilifera is Latin for 'hairy' and refers to conspicuous indumentum of the gall. Common name: 'hairy inflated galler'.

Biology and distribution (Figs 1C, 2C, D)

Larvae of *D. pilifera* cause the ovary of a host plant to swell into a soft-tissued, thin-walled, inflated, baglike gall that is irregularly convoluted, often bullate, and compressed dorsoventrally. A short flattened keel often occurs on the apex. The outer gall surface is villose-woolly, to sparsely pilose-pubescent with the indumentum most developed in the basal half of the gall. Larvae develop in a shallow, glabrous depression outside the ovary near the junction with the pedicel. Individual galls are 3–13 mm long and 6–13 mm wide ($n = 34$) and form a compact, globose cluster, 12–21 mm long and 13–25 mm wide ($n = 20$). Up to fifty larvae live gregariously within a gall cluster. Some of the larvae pupate within the gall clusters, others in the soil beneath the host tree. Adults emerge in May to August and are univoltine. *Dasineura pilifera* is endemic to eastern Australia where it is polyphagous on acacias from several sections and although widespread, gall densities are mostly sparse. The species is naturalized in Western Australia where it develops on *A. baileyana*, *A. decurrens* and *A. dealbata* and can be abundant. In eastern Australia, accessions with identical gall morphology and developmental biology to populations from *A. baileyana*, *A. decurrens* and *A. dealbata* were found on *A. filicifolia* Cheel & Welch, *A. fimbriata* A. Cunn. ex G. Don, *A. perangusta* (C. White) Pedley, *A. leucoclada* (RJA2788), *A. leptoclada* Cunn. ex Benth., *A. linearifolia* Cunn. ex Maiden & Blakely, *A. prominens* A. Cunn. ex G. Don, *A. polybotrya* Benth. and *A. podalyriifolia* A. Cunn. ex G. Don (RJA2758). Adults need to be reared from these hosts to confirm that the gall inducer is *D. pilifera*.

Dasineura oshanesii **Kolesik sp.n. (Figs 1D, 2E, 4E–H)**

Types. Queensland. Holotype male, Beerwah [26°84401′S, 152°89317′E], ex flower gall on *A. oshanesii* F. Muell. & Maiden collected 26.iv.1999 (RJA2729), I21597 (SAMA). Paratypes: two males, three females, three pupal skins, three larvae (SAMA, I21598–I21608), two males, two females, three pupal skins, two larvae (ANIC), collected with holotype.

DNA analysis. Cytochrome b sequenced for RJA3155 (GenBank accession number AY278709).

Description

Characters other than below are as in *D. rubiformis*.

Larva (Fig. 4E). Length 1.53 mm (1.14–1.89 mm, $n = 5$). Terminal papillae eight in number.

Pupa. Length 1.60 mm (1.55–1.81 mm, $n = 6$).

Male (Fig. 4F). Wing length 1.41 mm (1.32–1.50 mm, $n = 5$). Flagellomeres fifteen (fourteen to fifteen) in number. Sclerotization of tergite 1 weakened mesally, tergites 2–7 with uninterrupted sclerotization. Genitalia: hypoproct distally as wide as basally, narrower medially, incision one-quarter to one-third hypoproct length.

Female (Fig. 4G, H). Wing length 1.60 mm (1.50–1.63 mm, $n = 5$). Flagellomeres fourteen (thirteen to fourteen) in number. Constriction of tergite 7 half width of posterior edge. Sclerites of tergite 8: anterior third slightly less divergent than in *D. rubiformis*.

Etymology

This gall midge is named after its host plant species. Common name: 'small inflated galler'.

Biology and distribution (Figs 1D, 2E)

Larvae of *D. oshanesii* induce flower galls on *A. oshanesii*, its only known host. Galls are obovate-globose with a dense pillose-woolly indumentum. When immature, hairs are white, but become pale ferruginous at a later stage. A short, laterally compressed apical keel resembling a vestigial pod is common. Individual galls are 3–4 mm long and 4–6 mm wide ($n = 10$), tightly appressed into a globose cluster that is 5–10 mm long and 6–14 mm wide ($n = 10$). Larvae develop in deeply concave depressions on the outside surface of the malformed ovary, at the base of the gall. Each of these primitive larval chambers is occupied by one larva. Pupation takes place in the gall cluster, usually among the indumentum surrounding the larval chamber. Adults emerge most months and appear to be multivoltine, utilizing the near year-round flower production of *A. oshanesii*. *Dasineura oshanesii* occurs in southern Queensland and northern New South Wales and can be locally abundant.

Dasineura sulcata Kolesik sp.n. (Figs 2F, 4I–M)

Types. New South Wales. Holotype male, Wollongong [34°23547′S, 150°53839′E], ex flower gall on *A. saligna* collected 28.ix.1999 (RJA2774), I21609 (SAMA). Paratypes: two males, three females, three pupal skins, one pupa, three larvae (SAMA, I21610–I21621), two males, two females, three pupal skins, three larvae (ANIC), collected with holotype.

DNA analysis. Cytochrome b sequenced for RJA2774 (GenBank accession number AY278707).

Description

Characters other than below are as in *D. rubiformis*.

Larva (Fig. 4L, M). Length 1.84 mm (1.76–2.06 mm, $n = 6$). Integument covered with short, wide irregularly shaped plates. Triplet of outer lateral papillae, two of them with setulae; no inner lateral papillae; terminal papillae eight in number.

Pupa. Length 1.72 mm (1.60–1.83 mm, $n = 7$).

Male (Fig. 4K). Wing length 1.44 mm (1.34–1.50 mm, $n = 5$). Flagellomeres twelve (eleven to twelve) in number. Tergites 1–7 with sclerotization slightly weakened mesally. Hypoproct constricted medially, incision depth one-third hypoproct length.

Female (Fig. 4I, J). Wing length 1.46 mm (1.32–1.55 mm, $n = 5$). Flagellomeres twelve (eleven to twelve) in number. Sclerites of tergite 8: posterior third slightly divergent, posterior ends widened; anterior two-thirds slightly divergent, anterior quarter strongly divergent, widened anteriorly; sensory setae placed in posterior part of anterior quarter.

Etymology

Sulcata is Latin for 'grooved' and refers to the shape of the larval chamber situated at the base of a malformed ovary. Common name: 'groove galler'.

Biology and distribution (Fig. 2F)

Larvae of *D. sulcata* induce galls on *A. saligna* in eastern Australia. Individual flower galls are small (2.5–2.9 mm long, 0.5–1.3 mm wide, $n = 23$) and concealed among perianth remains of the flower heads. The malformed ovary is glabrous, elongate, thickened and slightly swollen basally, containing a larval chamber in the form of a transverse groove situated externally at the base of the ovary near the junction with the pedicel. Larvae are solitary in the larval chambers and emerge from the flower heads from late winter to early spring to pupate in the soil beneath the host plant. Adults emerge 1–2 weeks later. *Dasineura sulcata* was abundant at the type locality, and although only recorded from this site, it may have a wider distribution as galls are easily overlooked.

Dasineura-induced galls with similar morphology were found on *A. genistifolia* Link (RJA3191) and *A. longifolia* (RJA3139) in eastern Australia with a low DNA sequence divergence (1.0–2.2%) compared with *D. sulcata* collected from *A. saligna*, indicating that *D. sulcata* has a host range broader than *A. saligna*. Adults need to be reared to confirm these host plants. Accessions of groove galls from *A. extensa* (RJA2814), *A. myrtifolia* (Sm.) Willd. (RJA3086) and *A. urophylla* Benth. ex Lindley (RJA3084) from Western Australia had similar gall morphology and biology to *D. sulcata*, with DNA sequence divergences of 4.1, 2.7 and 3.7%, respectively, when compared with *D. sulcata* type series. Although relatively high, these DNA divergences are below the interspecific threshold of 4.6% for group B,

suggesting that these Western Australian populations might belong to *D. sulcata*.

Dasineura glomerata **Kolesik sp.n. (Figs 1E, 2G, H, 5A–J)**

Types. Victoria. Holotype male, Keilor [37°43491′S, 144°49601′E], ex flower gall on *A. mearnsii* collected 10.vii.1998 (RJA2658), I21622 (SAMA). Paratypes: two males, three females, three pupal skins, three larvae (SAMA, I21623–I21633), two males, two females, three pupal skins, three larvae (ANIC), collected with holotype.

Other material examined. RJA2659: Victoria, Bittern [38°21195′S, 145°10914′E], ex flower gall on *A. mearnsii* collected 28.vi.1998, one male, five females, eleven pupal skins, one larva; RJA2660: Victoria, Mornington [38°14571′S, 145°01696′E], ex flower gall on *A. mearnsii* collected 19.vii.1998, five males, five females, six pupal skins, five larvae; RJA2739: Victoria, Hastings [30°17165′S, 145°08909′E], ex flower gall on *A. mearnsii* collected 18.vii.1998, five males, five females, five pupal skins, three larvae; RJA2579: Victoria, Lara [38°02′S, 144°25′E], ex flower gall on *A. pycnantha* collected 18.xi.1998, five males, five females, five pupal skins, five larvae; RJA2604: Victoria, Somers Koala Reserve [38°23437′S, 145°09064′E], ex flower gall on *A. melanoxylon* collected 29.xi.1998, seven males, one pupa, five larvae; RJA2610: Victoria, Keilor [37°43491′S, 144°49601′E], ex flower gall on *A. retinoides* collected 10.viii.1998, one male, three pupae, five larvae; RJA2741: Victoria, Keilor [37°43491′S, 144°49601′E], ex flower gall on *A. retinoides* collected 10.viii.1998, one female, two pupae, three larvae; RJA2743: Victoria, Somerville [38°33754′S, 145°18834′E], ex flower gall on *A. retinoides* collected 29.i.1999, one pupa, five larvae; RJA2652: Victoria, Baxter [38°19300′S, 145°17364′E], ex flower gall on *A. schinoides* Benth. collected 17.viii.1998, four males, three females, six pupae, six larvae.

DNA analysis. Cytochrome b sequenced for RJA2576 (GenBank accession number AY278732) (Victoria, Somers [38°23437′S, 145°09064′E], ex flower gall on *A. pycnantha* collected 16.xi.1998), RJA3142 (GenBank accession number AY278684) (New South Wales, Wellington [32°32′S, 148°56′E], ex flower gall on *A. deanei* collected 25.x.2001).

Description

Larva (Fig. 5F–H). Length 2.21 mm (1.36–2.61 mm, n = 6). Integument entirely covered with spiculae. Head: antennae tapered, posterolateral apodemes as long as head capsule. Thoracic segments on each side with ventral papilla, setose; triplet of outer lateral papillae, two of them with setulae; no inner lateral papillae evident. Terminal papillae six to eight in number, with long setae.

Pupa (Fig. 5D, E). Length 2.89 mm (2.69–3.08 mm, n = 6). Antennal horns minute. Prothoracic spiracle of uniform width, 4× longer than wide at base.

Male (Fig. 5A). Wing length 2.51 mm (2.35–2.57 mm, n = 5). Antenna: scape and pedicel wider than long; flagellomeres sixteen (sixteen to seventeen) in number, node of first globular, remaining ones cylindrical, 2× longer than wide. Tergites 1–7 with sclerotization weakened mesally. Genitalia: gonostyle slightly tapered distally, covered with setulae to one-quarter length dorsally and two-thirds ventrally; gonocoxite with large round ventrodistal lobe; aedeagus cylindrical, rounded apically; cerci triangular; hypoproct basally slightly wider than medially and distally, incision U-shaped, one-third hypoproct length.

Female (Fig. 5B, C, I, J). Wing length 2.65 mm (2.51–2.77 mm, n = 5). Flagellomeres fifteen in number; flagellomere 1 as long as wide, with ventrodistal bulge; remaining flagellomeres barrel-shaped, 1.5× longer than wide. Hypoproct one-quarter to one-third length of cerci. Tergites 1–6 with weakened sclerotization mesally. Constriction of tergite 7 more than half width of posterior edge. Sclerites of tergite 8: wide, anterior two-thirds divergent, posterior third slightly divergent, sensory setae placed close to anterior ends.

Etymology

The Latin word *glomerata* means 'formed into a ball' and refers to the shape of the gall cluster induced by this species. Common name: 'common flower galler', as this is one of the most frequently encountered cecidomyiids on acacias.

Biology and distribution (Figs 1E, 2G, H)

The flower gall of *D. glomerata* is semiwoody, obovate and irregularly lobed, often bearing a short, laterally compressed apical keel. The gall contains one to twenty-seven larval chambers, each inhabited by a single larva. The lobes are short and obtuse, with a terminal exit hole. Individual galls are 5–15 mm long and 3–10 mm wide ($n = 90$). Larval chambers are arched, with short simple hairs lining the surface near the throat. The outer gall surface is pubescent to woolly-villose with a dense fringe of hairs plugging the entrance of the exit hole. Gall clusters are 3–25 mm long and 3–31 mm wide ($n = 184$). Pupation occurs within the larval chamber. Adults are bivoltine and emerge in winter to early summer.

Dasineura glomerata is polyphagous and recorded from *A. deanei* (RJA3142, DNA divergence to RJA2576 of 0.02%), *A. elata* (RJA3221, DNA divergence to RJA2576 of 0.7%), *A. hakeoides* Cunn. ex Benth. (RJA3154, not DNA tested), *A. mearnsii*, *A. melanoxylon*, *A. pycnantha*, *A. retinoides* Schltdl. and *A. schinoides*. Gall morphology on all hosts is similar. Adults that emerge early in the season utilize alternative hosts later in the year with *A. mearnsii*–

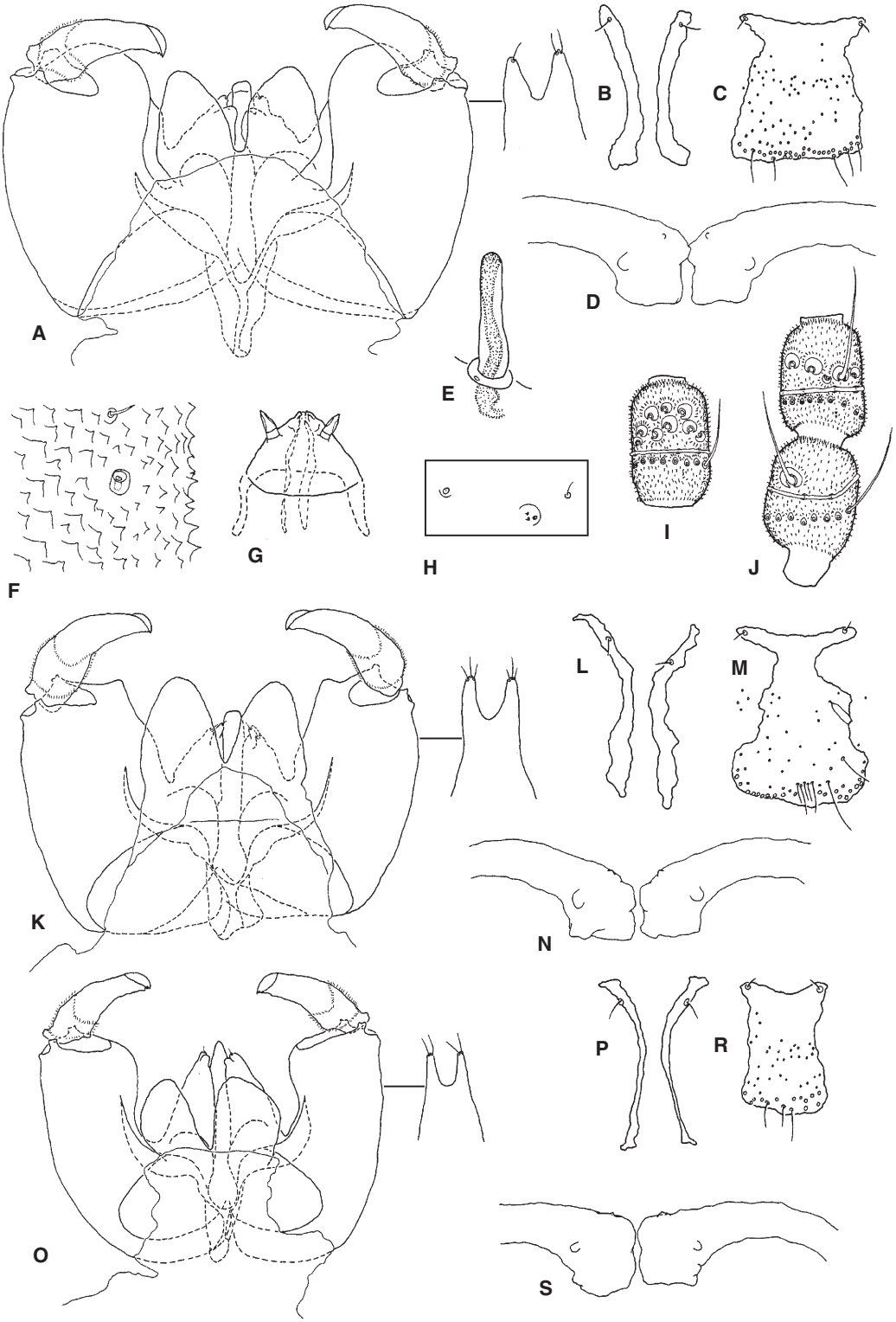

Fig. 5. A–J, *Dasineura glomerata*; K–N, *D. fistulosa*; O–S, *D. glauca*. A, Male genitalia dorsally; B, female tergite 8; C, female tergite 7; D, base of pupal antennae ventrally; E, pupal prothoracic spiracle; F, larval integument; G, larval head ventrally; H, sternal and lateral papillae of larval thoracic segment 1; I, female flagellomere 6; J, female flagellomeres 1 and 2; K, male genitalia dorsally; L, female tergite 8; M, female tergite 7; N, base of pupal antennae ventrally; O, male genitalia dorsally; P, female tergite 8; R, female tergite 7; S, base of pupal antennae ventrally.

A. pycnantha–A. mearnsii and *A. mearnsii–A. melanoxylon–A. mearnsii* as common successive host combinations. *Dasineura glomerata* is widely distributed in southeastern Australia, commonly encountered and able to reach outbreak population densities in some seasons.

Dasineura fistulosa **Kolesik sp.n. (Figs 1F, 2I, J, 5K–N)**

Types. Victoria. Holotype male, Bundoora [37°71833′S, 145°04667′E], ex flower gall on *A. mearnsii* collected 16.x.1998 (RJA2581), I21634 (SAMA). Paratypes: two males, three females, three pupae, two larvae (SAMA, I21635–I21644), two males, two females, two pupal skins, one larva (ANIC), collected with holotype.

Other material examined. RJA2600: Victoria, Tyabb [38°15736′S, 145°09932′E], ex flower gall on *A. mearnsii* collected 8.xi.1998, one male, five females, six pupal skins; RJA2646: New South Wales, Ulladulla [35°53944′S, 150°268333′E], ex flower gall on *A. irrorata* collected 21.xii.1998, four males, three females, four pupal skins.

DNA analysis. Cytochrome b sequenced for RJA2581 (GenBank accession number AY278685), RJA3198 (GenBank accession number AY278693) (New South Wales, Drake [28°55697′S, 152°22721′E], ex flower gall on *A. irrorata* collected 10.iv.2002).

Description

Characters other than below are as in *D. glomerata*.

Larva. Length 2.30 mm (1.95–2.62 mm, $n = 3$).

Pupa (Fig. 5N). Length 2.83 mm (2.65–3.25 mm, $n = 5$).

Male (Fig. 5K). Wing length 2.19 mm (2.05–2.25 mm, $n = 5$). Flagellomeres sixteen in number, in some specimens one of antennae with seventeen, node of first flagellomere globular, of remaining ones cylindrical, 1.5× longer than wide. Genitalia: gonostyle strongly tapered distally, covered with setulae to one-third length dorsally and two-thirds ventrally; gonocoxite with small acute ventrodistal lobe; aedeagus cylindrical, rounded apically; cerci triangular; hypoproct basally slightly wider than medially and distally, incision one-third hypoproct length.

Female (Fig. 5L, M). Wing length 2.40 mm (2.29–2.52 mm, $n = 5$). Flagellomeres sixteen in number. Constriction of tergite 7 less than half width of posterior edge. Sclerites of tergite 8: anterior third divergent; posterior two-thirds parallel; sensory setae placed at midlength of anterior third.

Etymology

Fistulosa is Latin for 'made up of pipes' and refers to the anatomy of the gall cluster. Common name: 'elongate fluted galler'.

Biology and distribution (Figs 1F, 2I, J)

The galls are woody, each containing one to six larval chambers that are elongate and mostly parallel. Exit holes are located terminally on short irregular apical lobes, within shallow rims or longitudinal furrows. Individual galls are 4–20 mm long and 2–9 mm wide ($n = 132$). The indumentum consists of appressed, hoary, whitish hairs, but becomes densely pubescent-cobwebby around the entrance of the exit hole. The inner surface of the rim that surrounds the exit hole is densely pubescent. Larval chambers contain solitary larvae that pupate in the basal end of the chamber. Up to twenty-five individual flower galls form a gall cluster that is 10–48 mm long and 4–43 mm wide ($n = 80$). Adults emerge in September to October and are univoltine. *Dasineura fistulosa* occurs sporadically on *A. mearnsii* and the closely related *A. irrorata* in eastern Australia, often co-occurring with *D. glomerata* and *D. rubiformis*.

Dasineura glauca **Kolesik sp.n. (Figs 1G, 2K, L, 5O–S)**

Types. South Australia. Holotype male, Adelaide – Goodwood [34°57′S, 138°35′E], ex flower gall on *A. pendula* A. Cunn. ex G. Don collected 5.i.1994 (Peter Kolesik), I21645 (SAMA). Paratypes: two males, three females, three pupal skins, three larvae (SAMA, I21646–I21656), two males, two females, two pupal skins, two larvae (ANIC), collected with holotype.

Other material examined. RJA2710: New South Wales, Gunnedah [31°09455′S, 149°94798′E], ex flower gall on *A. pendula* collected 21.iv.1999, five males, five females, four pupae.

DNA analysis. Cytochrome b sequenced for RJA3144 (GenBank accession number AY278786) (New South Wales, Gilgandra [32°01267′S, 148°34479′E], ex flower gall on *A. pendula* collected 25.x.2001).

Description

Characters other than below are as in *D. glomerata*.

Larva. Length 3.25 mm (2.90–3.55 mm, $n = 5$).

Pupa (Fig. 5S). Length 2.85 mm (2.51–2.98 mm, $n = 5$).

Male (Fig. 5O). Wing length 2.19 mm (1.91–2.28 mm, $n = 5$). Flagellomeres sixteen (sixteen to seventeen) in

number, node of first globular, remaining nodes cylindrical, 1.5× longer than wide. Genitalia: gonostyle slightly tapered distally, covered with setulae to one-quarter to one-third length dorsally and three-quarters to entirely ventrally; gonocoxite with no lobe; aedeagus conical, thin; cerci triangular; hypoproct basally slightly wider than medially and distally, lobes thin, incision one-third hypoproct length.

Female (Fig. 5P–R). Wing length 2.15 mm (1.96–2.34 mm, $n = 5$). Flagellomeres sixteen (fifteen to eighteen) in number. Constriction of tergite 7 more than half width of posterior edge. Sclerites of tergite 8: narrow, anterior third divergent, posterior two-thirds divergent, sensory setae placed at midlength of anterior third.

Etymology

Glauca is Latin for 'grey' and refers to the colour of the gall. Common name: 'grey fluted galler'.

Biology and distribution (Figs 1G, 2K–L)

The galls of *D. glauca* form woody tubes 10–16 mm long and 3–13 mm wide ($n = 10$), greyish green and sparsely pubescent, becoming densely pubescent around the rim of the exit hole. Hairs are white-ferruginous. The tubular lobes of flower galls are often curved and may be fused or diverge up to 90° to the main body of the gall. Exit holes open terminally on the tubular arms. An individual gall contains one to twelve larval chambers each occupied by a solitary larva. Gall clusters are 9–22 mm long and 15–22 mm wide ($n = 10$). Larvae pupate at the base of the larval chambers. Adults emerge most months suggesting that *D. glauca* is multivoltine. It occurs commonly on *A. pendula* in New South Wales and South Australia, often at high population densities that prevent seed production on whole trees. Galls similar to those on *A. pendula* occur on *A. omalophylla* A. Cunn. ex G. Don (RJA3158), a close relative of *A. pendula*, in eastern Australia. Larvae from *A. omalophylla* galls had a DNA sequence closely matching *D. glauca* (0.2% divergence), indicating that *A. omalophylla* is a host of *D. glauca*.

Cecidomyiid larvae in galls similar to *D. glauca* also occur on *A. ramulosa* W. Fitzg. (RJA2886, RJA3211) in Western Australia and on *A. aneura* F. Muell. ex Benth. (RJA3147) in New South Wales, but appear distantly related as their sequence divergences vary between 9 and 9.5% to *D. glauca*, respectively. Adults have to be reared to clarify the taxonomic position of these gall makers that diverge strongly in their sequences from any other species described here.

***Dasineura acaciaelongifoliae* (Skuse) (Figs 1H, 2M, N, 6A–D)**

Cecidomyia acaciae-longifoliae Skuse (1890: 374, plate XVI, figs 1, 1b).
Dasineura acaciaelongifoliae (Skuse): Gagné & Marohasy (1993: 80).
Cecidomyia acaciaelongifoliae Skuse: Gagné (2004: 274).

Types. New South Wales. Sydney, ex flower gall on *A. longifolia* (date of collection not known). Syntypes, five males, nine females, seven pupal skins (ANIC). Gall, collected with syntypes (MLMS).

Other material examined. RJA2574: Australian Capital Territory, Canberra [35°16730′S, 149°06414′E], ex flower gall on *A. maidenii* F. Muell. collected 9.xi.1998, two males, one female, nine pupal skins; RJA2595: Victoria, Braeside [37°99306′S, 145°12750′E], ex flower gall on *A. implexa* collected 13.ix.1998, five males, five females, five pupal skins, five larvae; RJA2719: Queensland, Toowoomba [27°47302′S, 151°94281′E], ex flower gall on *A. implexa*, collected 25.iv.1999, three males, five females, five pupal skins; RJA2626: Victoria, Lilydale [37°7661′S, 145°37028′E], ex flower gall on *A. stricta* (Andrews) Willd. collected 10.i.1999, five males, five females, seven pupal skins, five larvae; RJA2738: Victoria, Braeside [37°99306′S, 145°12750′E], ex flower gall on *A. sophorae* (Labill.) R. Br. × *A. oxycedrus* Sieber ex DC. collected 2.xii.1998, five males, five females, six pupae, six pupal skins, six larvae.

DNA analysis. Cytochrome b sequenced for RJA2574 (GenBank accession number AY278680), RJA2626 and RJA3157 (New South Wales, Uranga [30°27746′S, 153°00202′E], ex flower gall on *A. sophorae* collected 27.x.2001).

Description

Characters other than below are as in *D. glomerata*. Measurements refer to RJA2595 due to the poor condition of Skuse's types.

Larva. Length 2.55 mm (2.14–2.85 mm, $n = 5$).

Pupa (Fig. 6D). Length 2.64 mm (2.43–3.01 mm, $n = 5$). Antennal horns large. Distance between asetose and setose inner dorsal papillae of same pair varies between specimens from same host and locality.

Male (Fig. 6A). Wing length 1.98 mm (1.65–2.34 mm, $n = 5$). Flagellomeres sixteen (fifteen to eighteen) in number, node of first one globular, remaining ones cylindrical, 1.5× longer than wide. Genitalia: gonostyle slightly tapered distally, covered with setulae to one-fifth to one-quarter length dorsally and two-thirds to three-quarters ventrally; gonocoxite with no lobe; aedeagus conical, thin; cerci triangular; hypoproct of uniform width, lobes wide, incision depth one-quarter to one-third hypoproct length.

Female (Fig. 6B, C). Wing length 2.40 mm (2.25–2.52 mm, $n = 5$). Flagellomeres sixteen (sixteen to seventeen) in number. Constriction of tergite 7 half width of posterior

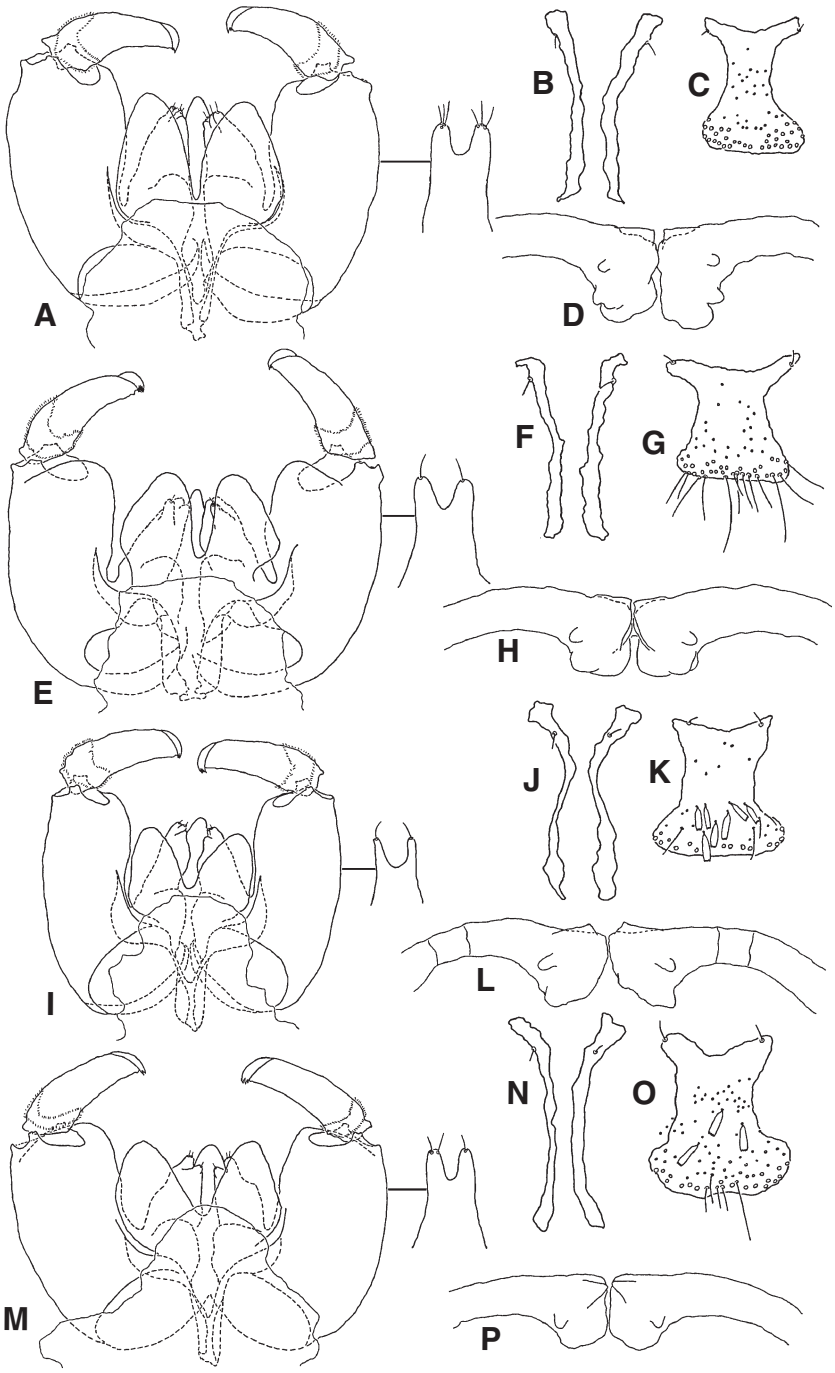

Fig. 6. A–D, *Dasineura acaciaelongifoliae*; E–H, *D. furcata*; I–L, *D. dielsi*; M–P, *D. oldfieldii*. A, Male genitalia dorsally; B, female tergite 8; C, female tergite 7; D, base of pupal antennae ventrally; E, male genitalia dorsally; F, female tergite 8; G, female tergite 7; H, base of pupal antennae ventrally; I, male genitalia dorsally; J, female tergite 8; K, female tergite 7; L, base of pupal antennae ventrally; M, male genitalia dorsally; N, female tergite 8; O, female tergite 7; P, base of pupal antennae ventrally.

edge. Sclerites of tergite 8: anterior half divergent, posterior half parallel, sensory setae placed close to anterior ends.

Etymology

Common name: 'obtuse fluted galler'. In the past this species has been referred to as 'acacia gnat' (Brewster *et al*., 1920).

Biology and distribution (Figs 1H, 2M, N)

Dasineura acaciaelongifoliae forms conspicuous globose clusters of tightly packed flower galls 15–41 mm long and 13–43 mm wide ($n = 15$). Individual galls are woody, obovate, 6–19 mm long and 3–25 mm wide ($n = 36$) with short evenly wide lobes. One to thirty-six individual galls occur in a gall cluster. Larval chambers are elongate and contain a solitary larva. Dense hairs plug the entrance and

throat of the larval chamber. The outer gall surface is sparsely pubescent, becoming densely pubescent around the entrance of the exit hole. Larvae pupate in the base of the chambers. *Dasineura acaciaelongifolia* occurs on *A. longifolia*, *A. sophorae*, *A. sophorae* × *A. oxycedrus*, *A. implexa*, *A. stricta* and *A. maidenii* in southeastern Australia, where it can be locally abundant. Skuse (1890) reported this species on *A. longifolia* as bivoltine, with galls of the first generation maturing in August, those of the second generation in December to January.

Dasineura furcata **Kolesik sp.n. (Figs 1I, 2O, P, 6E–H)**

Types. Victoria. Holotype male, Braeside [37°99306′S, 145°12750′E], ex flower gall on *A. melanoxylon* collected 19.vii.1998 (RJA2662), I21657 (SAMA). Paratypes: one male, three females, three pupal skins, three larvae (SAMA, I21658–I21667), two females, three pupal skins, two larvae (ANIC), collected with holotype.

DNA analysis. Cytochrome b sequenced for RJA2662 (GenBank accession number AY278681), RJA3207 (GenBank accession number AY278692) (Victoria, Knox [37°54212′S, 145°14153′E], ex flower gall on *A. melanoxylon* collected 13.iv.2002).

Description

Characters other than below are as in *D. acaciaelongifoliae*.

Larva. Length 2.66 mm (2.23–2.97 mm, $n = 5$).

Pupa (Fig. 6H). Length 2.60 mm (2.22–2.74 mm, $n = 5$).

Male (Fig. 6E). Wing length 2.34 mm (2.28–2.40 mm, $n = 2$). Flagellomeres sixteen in number. Gonocoxite with small ventrodistal lobe.

Female (Fig. 6F, G). Wing length 2.53 mm (2.48–2.60 mm, $n = 6$). Flagellomeres sixteen in number. Constriction of tergite 7 half width of posterior edge.

Etymology

Furcata is Latin for 'forked' and refers to the appearance of the gall. Common name: 'forked fluted galler'.

Biology and distribution (Figs 1I, 2O, P)

The flower gall of *D. furcata* is woody and irregularly lobed. Lobes contain larval chambers with terminal exit holes and may be simple or forked, often developing into elongate tubes. Lobes project laterally or vertically and are occasionally recurved. The outer gall surface is glabrous with a dense fringe of short white hairs plugging the entrance of exit holes. There are up to eleven larval chambers per gall, each containing a solitary larva. Pupation occurs in the base of the larval chamber in a white silky larval cocoon. Adults emerge in June to August and are univoltine. Larvae can diapause within cocoons in developmental chambers. *Dasineura furcata* is restricted to *A. melanoxylon*. It occurs widely in southeastern Australia up to 1120 m above sea level, but its population densities are low.

Dasineura dielsi **Rübsaamen (Figs 1J, 2R, 6I–L)**

Dasyneura dielsi Rübsaamen (1916: 478, figs 61–63).

Types. Western Australia. Karocketta, ex flower gall on *A. cyclops* collected i.1891 by Diels and Pritzel. Types, mounted in glycerol and deposited in the Museum für Naturkunde der Humboldt-Universität zu Berlin, Germany, are currently inaccessible due to their fragility (M. Kotrba, pers. comm., 1999). In addition to the type material, Rübsaamen (1916) received galls collected in Victoria in April 1904 and diagnosed them as *D. dielsi* on *A. cyclops*. As *A. cyclops* does not occur naturally in Victoria, this collection was most likely not *D. dielsi* but another eastern Australian *Dasineura* species that forms fluted galls on a related acacia.

Other material examined. RJA2571: Western Australia, D'Entrecasteaux National Park [34°83500′S, 116°03833′E], ex flower gall on *A. cyclops* collected 2.ii.1998, one male, five females, one pupa, six pupal skins, six larvae; RJA2598: Western Australia, Cheyene Beach [34°82333′S, 118°32444′E], ex flower gall on *A. cyclops* collected 3.xi.1998, five males, five females, five pupal skins, five larvae.

DNA analysis. Cytochrome b sequenced for RJA2571 (GenBank accession number AY278688).

Description

Characters other than below are as in *D. acaciaelongifoliae*. Measurements refer to RJA2598.

Larva. Length 2.34 mm (2.33–2.88 mm, $n = 5$).

Pupa (Fig. 6L). Length 2.29 mm (2.05–2.54 mm, $n = 5$). Distance between asetose and setose inner dorsal papillae of same pair varies between specimens from same host and locality.

Male (Fig. 6I). Wing length 1.96 mm (1.88–2.00 mm, $n = 5$). Flagellomeres sixteen in number. Genitalia: gonostyle evenly wide, covered with setulae to one-fifth to one-quarter length dorsally and two-thirds ventrally; gonocoxite with small, round ventrodistal lobe; aedeagus conical, thin;

cerci triangular; hypoproct basally slightly wider than medially and distally, incision slightly deeper than one-third hypoproct length, lobes thin.

Female (Figs 6J, K). Wing length 2.08 mm (1.97–2.15 mm, $n = 5$). Flagellomeres sixteen (fifteen to sixteen) in number. Constriction of tergite 7 more than half width of posterior edge. Sclerites of tergite 8: anterior third divergent, widened anteriorly; medial third divergent posteriorly; posterior third parallel; sensory setae placed at midlength of anterior third.

Etymology

Common name: 'small fluted galler'.

Biology and distribution (Figs 1J, 2R)

The gall of *D. dielsi* is woody, and irregularly lobed. Lobes are straight to gently recurved and often diverge acutely in the distal region of the gall. Gall clusters are 7–32 mm long and 3–42 mm wide ($n = 62$). Individual galls vary in size depending on the number of larval chambers and are 4–19 mm long and 3–14 mm wide ($n = 128$). Occasionally, the apex of a gall may extend into a vestigial pod that can also form a longitudinal keel. Most of the outer gall surface is glabrous, with exit holes plugged with a dense fringe of short white hairs that are absent from the throat of the developmental chamber. Each larval chamber contains a solitary larva. Larvae pupate in white, ovoid larval cocoons at the base of the larval chamber. A paperlike membrane closes the exit hole prior to pupation. Adults emerge throughout the year and are multivoltine.

Dasineura dielsi is usually restricted to *A. cyclops* in coastal regions of Western Australia and South Australia, but secondary galling (spill-over feeding) can occur on other hosts (*A. papyrocarpa* Benth., *A. ligulata* A. Cunn. ex G. Don, *A. sophorae*, *A. oswaldii* F. Muell.) at localities where these species are in close proximity to *A. cyclops* with *D. dielsi*.

Dasineura oldfieldii Kolesik sp.n. (Figs 2S, T, 6M–P)

Types. Western Australia. Holotype male, Kalbarri [27°42921′S, 114°19457′E], ex flower gall on *A. oldfieldii* F. Muell. Author collected 24.vii.1999 (RJA2770), I21668 (SAMA). Paratypes: two males, three females, three pupal skins (SAMA, I21669–I21676), two males, two females, two pupal skins (ANIC), collected with holotype.

DNA analysis. Cytochrome b sequenced for RJA2770 (GenBank accession number AY278731).

Description

Characters other than below are as in *D. acaciaelongifoliae*.

Larva. Not known.

Pupa (Fig. 6P). Length 2.44 mm (2.27–2.53 mm, $n = 6$).

Male (Fig. 6M). Wing length 2.15 mm (2.02–2.19 mm, $n = 5$). Flagellomeres fifteen (fourteen to fifteen) in number. Genitalia: gonostyle evenly wide, covered with setulae to one-fifth length dorsally and two-thirds to three-quarters ventrally; gonocoxite with small round ventrodistal lobe; aedeagus conical, robust; cerci triangular; hypoproct basally slightly wider than medially and distally, incision one-fifth to one-quarter hypoproct length, lobes wide.

Female (Fig. 6N, O). Wing length 2.15 mm (2.04–2.20 mm, $n = 5$). Flagellomeres fifteen (fifteen to sixteen) in number. Constriction of tergite 7 more than half width of posterior edge. Sclerites of tergite 8: posterior two-thirds parallel, slightly widening towards posterior ends; anterior third divergent, slightly widening towards anterior ends; sensory setae placed at midlength of anterior third.

Etymology

This midge is named after its main host plant species. Common name: 'ram's horn galler', due to the curved shape of the gall lobes.

Biology and distribution (Fig. 2S, T)

The larvae of *D. oldfieldii* induce woody galls with irregular lobes, tapered towards the base, often bearing a vestigial pod that forms a lateral or apical keel. Lobes are elongate and mostly retrorsely curved to coiled, a distinguishing characteristic. Individual galls are 6–21 mm long and 4–12 mm wide ($n = 19$). The outer gall surface is sparsely pubescent to glabrous with a dense fringe of simple, white hairs plugging the entrance of the exit hole that is located on the lobe's terminus. Larvae pupate in cocoons at the base of the larval chamber. Adults emerge in winter and are univoltine. *Dasineura oldfieldii* is restricted to Western Australia where it develops on *A. oldfieldii* and, less commonly, *A. neurophylla* W. Fitzg.

Phylogeny

Morphology-based phylogeny

Based on insect morphology, the eleven *Dasineura* species form two distinct groups. The major differences between group A (*D. acaciaelongifoliae*, *D. dielsi*, *D. fistulosa*, *D. furcata*, *D. glauca*, *D. glomerata*, *D. oldfieldii*) and group B (*D. oshanesii*, *D. pilifera*, *D. rubiformis*, *D. sulcata*) are in the shape of the plates covering the integument, the shape of the antennae, the number of inner lateral papillae and the presence of spatula sternalis in the larva, the shape of the prothoracic spiracle in the pupa, the extent of sclerotization of abdominal tergites 1–6, the shape of the first

flagellomere in the female, and the shape of the aedeagus and extension of setulation on gonostyle in the male (Table 1). Species in group A differ from each other in the size of pupal horns, the width of the constriction of female abdominal tergite 7, and the shape of the gonostyle, gonocoxite, hypoproct and aedeagus in the male. In group B, the differences between species are in the number of inner lateral papillae in the larva, the shape of cerci and hypoproct and the strength of tergal sclerotization in the male as well as in the width of the constriction of tergite 7 in the female. Additionally, *D. sulcata* differs from the other three species in group B in the shape of the plates covering the integument in the larva. The single consensus tree recovered by parsimony analysis of morphological characters confirmed the two morphological groups as well as the segregation of group B into four clades based on life cycle and gall morphology (Fig. 7).

Cytochrome b-based phylogeny

Sequences were highly A + T biased (79%), particularly at third codon positions (96%), as is characteristic for mitochondrial DNA of insects (Cameron & Mardulyn, 2001) including Cecidomyiidae (Widenfalk *et al.*, 2002; Yukawa *et al.*, 2003). Base frequencies did not deviate from stationarity across the lineages ($\chi^2 = 40.64$, d.f. = 57, $P = 0.95$). Parsimony analysis of sixty-two parsimony-informative characters (15% of all characters) recovered two equally parsimonious trees with a tree length of 119 steps, consistency index = 0.68, retention index = 0.78. The single tree recovered by ML analysis ($-\ln L = 1561.71$) was fully congruent with the strict consensus of the two MP trees when nodes with ML bootstrap support > 50% were considered (data not shown). More nodes obtained bootstrap support under the optimality criterion of parsimony than ML. Furthermore, ML bootstrap values were generally lower than those recovered by parsimony, perhaps indicating that the GTR + Γ model applied to these sequences may not accurately reflect their evolutionary dynamics.

Two distinct groups were recovered corresponding to those recognized using insect morphological characteristics with the exception of *D. sulcata*, which fell into a clade with group A (Fig. 8), although this relationship was only weakly supported. Morphological group A, comprising *D. acaciaelongifoliae*, *D. dielsi*, *D. fistulosa*, *D. furcata*, *D. glauca*, *D. glomerata* and *D. oldfieldii* received strong parsimony bootstrap support, whereas group B (*D. oshanesii*, *D. pilifera*, *D. rubiformis*) received weak bootstrap support. Relationships within group A were fairly well resolved, with *D. dielsi*, *D. fistulosa* and *D. glomerata* forming a sister group to *D. acaciaelonfoliae*, *D. furcata*, *D. glauca* and *D. oldfieldii*. Within group B, *D. rubiformis* and *D. oshanesii* were sister taxa to *D. pilifera*. Within each group, multiple accessions of each species formed monophyletic groups. Two additional accessions of *D. acaciaelongifoliae* (RJA2626 from *A. sophorae*, RJA3157 from *A. stricta*) had 100% sequence similarity to *D. acaciaelongifoliae* from *A. maidenii* RJA2574 and were therefore not included in the analyses.

Uncorrected cytochrome *b* interspecific divergence values in group A ranged from 0.5 to 3.9%, with intraspecific divergence values of 0–0.2% (Table 2). Group B interspecific divergence values were between 4.6 and 7.3% with intraspecific divergence estimates of 0–3.7%.

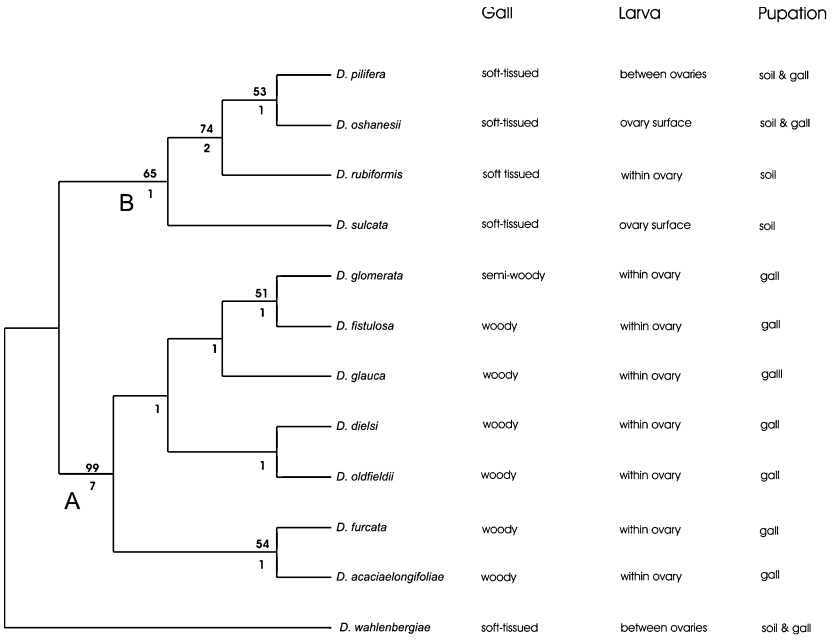

Fig. 7. Single tree recovered from maximum parsimony analysis of morphological characters. *Dasineura wahlenbergiae* was used as the outgroup. The numbers above the branches represent bootstrap values from 10 000 bootstrap replicates. The numbers below the branches represent Bremer support values. Total tree length = 40, consistency index = 0.74, Homoplasy Index = 0.26. Nodes A and B correspond to morphological group A and B, respectively.

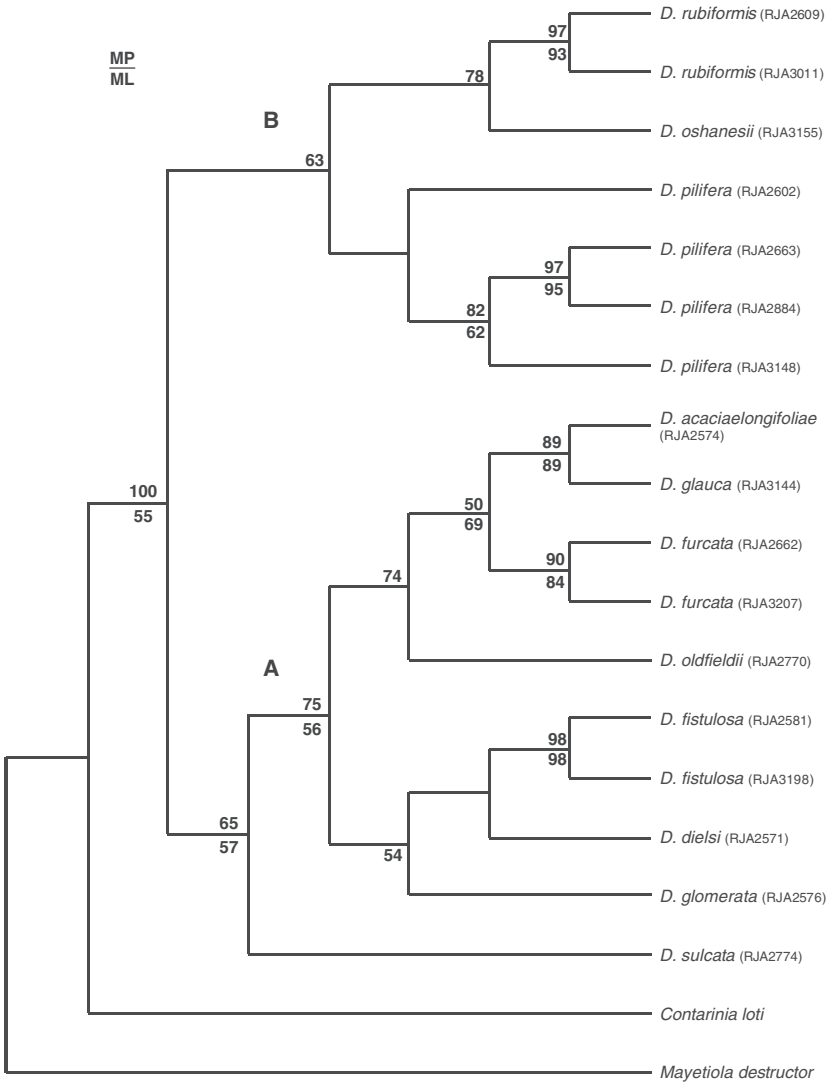

Fig. 8. Cladogram of one of two maximum parsimony trees recovered from a heuristic search of cytochrome *b* sequence data. Tree length = 116, consistency index = 0.68, retention index = 0.78. The numbers above the branches represent maximum parsimony bootstrap values > 50%. The numbers below the branches represent maximum likelihood bootstrap values > 50%. Node A corresponds to morphological group A, node B corresponds to morphological group B. Accession numbers are indicated in parentheses.

Combined phylogeny

The result of the ILD test was not significant ($P = 0.09$), indicating that the null hypothesis of dataset homogeneity could not be rejected. Based on this result, as well as the overall similarity in tree topology between the molecular and morphological datasets, a simultaneous combined analysis was performed under the criterion of parsimony. The strict consensus of the best parsimony trees recovered from a heuristic search of the combined data is presented in Fig. 9. Bootstrap support for species in morphological group A increased to 100% support, indicating congruent signal in the morphological and molecular datasets. Bootstrap support for a clade comprising *D. oshanesii*, *D. rubiformis* and *D. pilifera* also increased markedly in the context of a combined analysis relative to either of the two data partitions on their own. The placement of *D. sulcata* was equivocal in the analysis, reflecting the disagreement between the morphological and molecular partitions as to the placement of this species.

Key to galls of Dasineura spp. from flower galls on Australian Acacia spp.

This key is based on galls found during our study. It includes hosts with galls induced by *Dasineura* with known adults or DNA-tested larvae. Hosts bearing *Dasineura*-induced galls from which neither adults were reared nor larvae DNA tested are listed in 'Biology and distribution' under gall midge species causing the particular gall type.

Table 2. Nucleotide differences (below) and uncorrected divergences (above). Accession numbers are indicated in parentheses.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1. *D. rubiformis* (RJA2609)	*	1.2	4.6	3.7	5.6	5.4	5.4	6.6	6.3	6.8	7.1	6.6	7.3	7.6	7.6	6.9	7.1	14.1	27.2
2. *D. rubiformis* (RJA3011)	5	*	4.4	3.9	5.4	5.1	5.1	6.1	5.9	6.3	6.6	6.1	6.8	7.1	7.1	6.4	6.6	14.1	26.8
3. *D. oshanesii* (RJA3155)	19	18	*	4.9	5.4	5.1	5.6	6.3	6.6	7.1	7.3	6.3	7.1	7.3	8	6.6	7.3	14.4	25.8
4. *D. pilifera* (RJA2602)	15	16	20	*	3.7	3.7	3.7	5.6	5.1	5.6	5.9	5.4	5.6	5.9	6.1	5.1	5.4	14.8	26.3
5. *D. pilifera* (RJA2663)	23	22	22	15	*	0.7	2.7	6.1	5.6	6.1	6.4	5.4	5.6	5.9	6.6	5.2	5.9	14.4	26.2
6. *D. pilifera* (RJA2884)	22	21	21	15	3	*	2.4	5.9	5.4	5.9	6.1	5.1	5.4	5.6	6.3	4.9	5.6	14.6	25.4
7. *D. pilifera* (RJA3148)	22	21	23	15	11	10	*	6.3	5.9	6.3	6.6	5.6	5.9	6.1	6.8	5.4	6.1	15.1	26.3
8. *D. acaciaelongfoliae* (RJA2574)	27	25	26	23	25	24	26	*	0.5	1.7	2.0	1.7	3.4	3.7	3.9	2.9	5.1	15.8	25.7
9. *D. glauca* (RJA3144)	26	24	27	21	23	22	24	2	*	1.3	1.5	1.2	2.9	3.2	3.4	2.4	4.6	15.5	25.2
10. *D. furcata* (RJA2662)	28	26	29	23	25	24	26	7	5	*	0.2	1.2	2.9	3.2	2.9	2.4	5.1	15.7	25.4
11. *D. furcata* (RJA3207)	29	27	30	24	26	25	27	8	6	1	*	1.5	3.2	3.4	3.2	2.7	5.4	16.0	25.2
12. *D. oldfieldii* (RJA2770)	27	25	26	22	22	21	23	7	5	5	6	*	2.7	2.9	3.2	1.7	4.4	15.2	24.6
13. *D. fistulosa* (RJA2581)	30	28	29	23	23	22	24	14	12	12	13	11	*	0.2	2.9	0.2	4.6	15.9	25.6
14. *D. fistulosa* (RJA3198)	31	29	30	24	24	23	25	15	13	13	14	12	1	*	3.2	2.2	4.9	15.6	25.6
15. *D. dielsi* (RJA2571)	31	29	33	25	27	26	28	16	14	12	13	13	12	13	*	2.4	5.1	16.5	26.2
16. *D. glomerata* (RJA2576)	28	26	27	21	21	20	22	12	10	10	11	7	8	9	10	*	3.9	15.0	25.1
17. *D. sulcata* (RJA2774)	29	27	30	22	24	23	25	21	19	21	22	18	19	20	21	16	*	15.2	26.2
18. *Mayetiola destructor*	55	55	56	58	56	57	59	61	60	61	62	59	62	61	64	58	59	*	26.3
19. *Contarinia lotii*	101	100	96	98	97	95	98	96	94	95	94	92	96	96	98	94	98	92	*

1. Larva develops in chamber enclosed in malformed ovary (Fig. 2B, H, J, L, N, P, R, S)..... 2
– Larva develops in depression on exterior surface of malformed ovary (Fig. 2D–F)..... 9
2. Gall soft-tissued, pyriform to globose, without conspicuous lobes. Larval chambers ovoid with exit holes located basally on gall. Pupation in soil. On several Botrycephalae, mainly *A. mearnsii*, *A. deanei*, *A. irrorata*, *A. parramattensis*, *A. leucoclada* (Figs 1A, B, 2A, B)..... *D. rubiformis*
– Gall woody or semiwoody, obovate, usually with conspicuous lobes. Larval chambers narrow and long with exit holes located distally or laterally on gall. Pupation within larval chamber..... 3
3. Gall semiwoody, with short, obovate lobes. Larval chambers arched, with exit holes on distal half of gall. Polyphagous, mainly on *A. deanei*, *A. elata*, *A. mearnsii*, *A. melanoxylon*, *A. pycnantha*, *A. retinoides*, *A. schinoides* (Figs 1E, 2G, H)..... *D. glomerata*
– Gall woody, with long, tubular lobes. Larval chambers with exit holes terminally on tubular lobes..... 4
4. Gall grey when fresh. On *A. pendula*, *A. omalophylla* (Figs 1G, 2K, L)..... *D. glauca*
– Gall green when fresh..... 5
5. Tubular lobes distinctly retrorsely curved to coiled. On *A. oldfieldii* (Fig. 2S, T)..... *D. oldfieldii*
– Tubular lobes straight or only slightly recurved..... 6
6. Tubular lobes short, evenly wide. On *A. longifolia*, *A. sophorae*, *A. sophorae* × *oxycedrus*, *A. implexa*, *A. stricta*, *A. maidenii* (Figs 1H, 2M, N) ..... *D. acaciaelongifoliae* .....
– Tubular lobes long, tapered distally..... 7
7. Tubular lobes often forked. Restricted to *A. melanoxylon* (Figs 1I, 2O, P)..... *D. furcata*
– Tubular lobes mostly straight, sometimes forked..... 8
8. On *A. cyclops* (Figs 1J, 2R)..... *D. dielsi*
– On *A. melanoxylon*, *A. irrorata* (Figs 1F, 2I, J)..... *D. fistulosa*
9. Ovary inflated, baglike. On many hosts, mainly *A. baileyana*, *A. deccurens*, *A. dealbata* (Figs 1C, 2C, D)..... *D. pilifera*
– Ovary swollen, fleshy..... 10
10. Swollen ovary large, globose, densely pilose. Larva in deeply concave chamber. On *A. oshanesii* (Figs 1D, 2E)..... *D. oshanesii*
– Swollen ovary small, cylindrical, glabrous. Larva in shallow groove. On *A. saligna*, *A. genistifolia*, *A. longifolia* (Fig. 2F)..... *D. sulcata*

Key to gall midges of Dasineura spp. from flower galls on Australian Acacia spp.

1. Larva: integument covered with plates (Figs 3N, 4L); antennae obtuse (Fig. 3O); spatula present. Pupal thoracic spiracle tapered distally, 10× longer than wide at base (Fig. 3J). Male genitalia: aedeagus blunt or concave apically, setulation reaching one-tenth gonostyle length dorsally (Fig. 3D). Female: first flagellomere 2× longer than wide, without bulge (Fig. 3F); sclerotization of tergites 1–7 uninterrupted. 2
– Larva: integument covered with spiculae (Fig. 5F); antennae tapered (Fig. 5G); spatula absent. Pupal thoracic spiracle of uniform width, 4× longer than wide at base (Fig. 5E). Male genitalia: aedeagus rounded apically, setulation reaching at least one-fifth gonostyle length dorsally (Fig. 5A). Female: first flagellomere 1.5× longer than wide, with ventrodistal

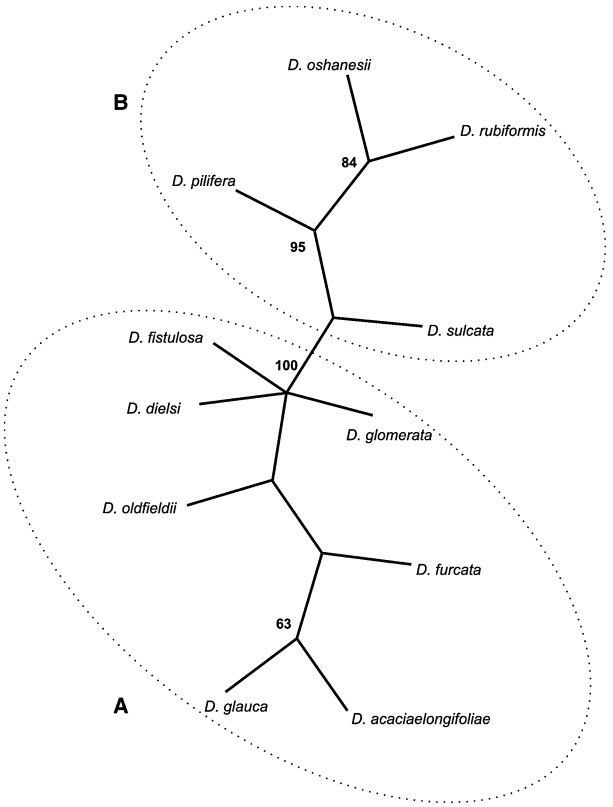

Fig. 9. Unrooted cladogram of the strict consensus of the three best parsimony trees recovered from a heuristic search of combined molecular and morphological character data. Tree length = 133, consistency index = 0.67, retention index = 0.72. The numbers at the nodes represent maximum parsimony bootstrap values > 50%. Groups corresponding to morphological groups A and B are circled.

bulge (Fig. 5J); sclerotization of tergites 1–7 weakened mesally..... 5

2. Larval integumental plates short and wide, irregularly shaped (Fig. 4L); thorax with no inner lateral papillae (Fig. 4M). Adults with twelve or fewer flagellomeres. ..... *D. sulcata*
– Larval integumental plates large, round, regularly shaped (Fig. 3N); inner lateral papillae on thoracic segments present (Figs 3M, 4D). Adults with fourteen or more flagellomeres 3
3. Larval thoracic segments on each side with three inner lateral papillae (Fig. 4D). Male genitalia: cerci subglobular; hypoproct medially as wide as basally, hypoproct incision key hole-shaped (Fig. 4A). Constriction of female tergite 7 more than half width of posterior edge (Fig. 4C). ..... *D. pilifera*
– Larval thoracic segments on each side with one inner lateral papilla (Fig. 2M). Male genitalia: cerci triangular; hypoproct medially narrower than basally, hypoproct incision U-shaped (Fig. 3D). Constriction of female tergite 7 half width of posterior edge (Fig. 3L) 4
4. Male hypoproct narrower distally than medially (Fig. 3D)..... *D. rubiformis*
– Male hypoproct as wide distally as medially (Fig. 4F). ..... *D. oshanesii*
5. Pupal antennae with small horns (Fig. 5D). 6
– Pupal antennae with large horns (Fig. 6D)..... 8
6. Male genitalia: gonocoxite with no lobe, aedeagus conical (Fig. 5O), setulation reaching more than three-quarters gonostyle length ventrally..... *D. glauca*
– Male genitalia: gonocoxite with ventrodistal lobe, aedeagus cylindrical (Fig. 5A), setulation reaching two-thirds gonostyle length ventrally. 7
7. Male genitalia: gonocoxite with large, round, ventrodistal lobe; gonostyle tapered slightly distally, setulation reaching one-quarter gonostyle length dorsally (Fig. 5A). Constriction of female tergite 7 more than half width of posterior edge (Fig. 5C). ..... *D. glomerata*
– Male genitalia: gonocoxite with small, acute, ventrodistal lobe; gonostyle tapered strongly distally, setulation reaching one-third gonostyle length dorsally (Fig. 5K). Constriction of female tergite 7 less than half width of posterior edge (Fig. 5M). .... *D. fistulosa*
8. Gonocoxite with no lobe (Fig. 6A)..... *D. acaciaelongifoliae*
– Gonocoxite with small ventrodistal lobe (Fig. 6E). 9
9. Gonostyle slightly tapered distally (Fig. 6E). Constriction of female tergite 7 half width of posterior edge (Fig. 6G)..... *D. furcata* **sp.n.**
– Gonostyle evenly wide (Fig. 6I). Constriction of female tergite 7 more than half width of posterior edge (Fig. 6K). 10
10. Male genitalia: hypoproct with incision at least one-third its total length, lobes narrow; aedeagus thin (Fig. 6I). ..... *D. dielsi*
– Male genitalia: hypoproct with incision shallower than one-quarter its total length, lobes wide; aedeagus robust (Fig. 6M). ..... *D. oldfieldii*

Discussion

Acacia is Australia's largest plant genus with around 960 species (Maslin, 2001). Extensive species radiation, together with physiological and ecological diversification, has fostered the reciprocal development of a rich phytophagous fauna (New, 1984) including gall-inducing *Dasineura* and *Asphondylia*. These two cosmopolitan genera occur commonly on the reproductive organs of southern Australian acacias (Adair *et al*., 2000). Two groups of *Dasineura* spp. are described in this study – seven species that induce woody galls and pupate within the malformed ovary in the galls (group A) and four species that induce soft-tissued galls and pupate either in the soil or within the galls but externally to the malformed ovary (group B). The closely related biology, morphology and DNA profiles suggest a more recent speciation in group A when compared with group B. Hard-tissued galls and internal pupation may provide a better protection against biotic (e.g. parasitation) and abiotic

(e.g. regular fires) factors than more delicately built galls and external pupation. Investment in thick-walled, sturdy galls might provide group A species with better survival prospects, which possibly accounts for more intense speciation, wider distribution and higher abundances when compared with group B species.

Most Australian *Dasineura* that develop in the flowers of acacias are univoltine and well synchronized with the limited temporal availability of flowers. Multivoltinism is present in species that exploit either a single host that flowers over a large part of the year (*D. oshanesii*, *D. dielsi* and *D. glauca*) or alternate between several hosts that flower successively (*D. glomerata*).

Although oligophagy is a general pattern in herbivorous insects (Jermy, 1976, 1984), species that develop in the reproductive tissues of their host plants generally display a high degree of host specificity (Mani, 1964; Janzen, 1980; Auld, 1983; Ananthakrishnan, 1986; Dreger-Jauffret & Shorthouse, 1992). Phytophagous cecidomyiids are generally confined to a single host species or at the most a group of close relatives (Gagné, 1989, 1994). *Dasineura* from Australian acacias are no exception, with most known species restricted to one or a few closely related *Acacia* species, with only *D. glomerata* and *D. pilifera* utilizing a broader host range.

Host specificity is an important consideration in the planned use of introduced organisms for classical biological control of alien pests. Organisms with a narrow host range reduce the risk of nontarget damage and possible conflicts of interest in the country of introduction. No *Dasineura* and only one *Asphondylia* are known from African acacias (Gagné & Marohasy, 1993). In this study, all described *Dasineura* spp., except *D. oldfieldii* and *D. oshanesii*, occur on acacias that cause problems in South Africa (Adair *et al.*, 2000) and the natural host range of most of them is sufficiently narrow for consideration as biological control agents. The small size and biomass of *D. rubiformis* and *D. dielsi* galls relative to fruit, indicate that these insects are unlikely to have a resource-loading impact on the growth of their respective hosts and therefore may be compatible with the commercial exploitation of these plants, *A. mearnsii* and *A. cyclops*, in South Africa. Both cecidomyiid species can form outbreak populations and suppress fruit and seed production in Australia, especially in localities outside the natural distribution range of their host plants. *Dasineura dielsi* was recently released in South Africa as a biological control agent for *A. cyclops* and exerts significant pressure on the sexual reproduction of the plant, while spreading considerably each year. *Dasineura rubiformis* is under consideration as a seed-reducing agent for *A. mearnsii* in South Africa.

In Australia, the shrub *A. longifolia*, indigenous to the eastern coastal region (Costermans, 1981), has been introduced to Western Australia, where it is naturalized and causes ecological damage to natural ecosystems (Hussey *et al.*, 1997). *Dasineura acaciaelongifoliae* is sufficiently host specific to warrant consideration as a biological control agent of *A. longifolia* in Western Australia to limit seed production of this weed there.

A 398 bp long segment of mitochondrial cytochrome *b* gene was used to separate European *Contarinia vincetoxici* Kieffer and *C. asclepiadis* (Giraud), both from *Vincetoxicum hirundinaria* (Medicus) (Asclepiadeae), with the intraspecific nucleotide difference of forty-eight to fifty-three and interspecific difference of one to five and four to eleven, respectively (Widenfalk *et al.*, 2002). Interestingly, in the same study, the difference between four accessions of *C. asclepiadis* and an accession of *D. lysimachiae* Rübsaamen from *Lysimachia vulgaris* L. (Primulaceae) was only seventeen to twenty nucleotides. In this study, a 410 bp long segment of cytochrome *b* amplified with the same primers as in Widenfalk *et al.* (2002) helped separate eleven *Dasineura* species. Most species were well differentiated from one another at the nucleotide level, with interspecific differences between sixteen and thirty-three nucleotides. The divergence between species in group A was lower than that between species in group B, with intraspecific differences of zero to one and interspecific differences typically around thirteen but as low as two nucleotides between *D. acaciaelongifoliae* and *D. glauca*. The wide availability of sequencing techniques makes this molecular marker a useful diagnostic tool for Cecidomyiidae, enabling identification of a species based not only on the morphology of reared adult specimen series but also on individual larvae extracted from the galls. Furthermore, DNA fingerprinting can be used effectively in studies on dispersal behaviour and infestation modelling in gall midges based on the identification of adults caught in the air (Sylvén, 1970; Ko & Lee, 1975; Kolesik, 1993, 2000; Withers & Harris, 1997).

Although the molecular data largely supported morphological groups A and B, sister taxa relationships within these two groups differed. This apparent incongruence may be due to the small number of available informative morphological characters, the use of different outgroup taxa for the different datasets, or homoplasy in the molecular dataset. Due to the rapid accumulation of mutations in animal mitochondrial DNA, it is likely that some sites will have experienced multiple mutations, leading to saturation and loss of phylogenetic signal. This may be exacerbated in insect mitochondrial DNA, due to the high A + T bias of the mutations, resulting in essentially only two nucleotides available for substitution (Dowton & Austin, 1997). However, plots of the total numbers of transitions and transversions vs sequence divergence for all nucleotide positions resulted in linear plots (data not shown), indicating that the cytochrome *b* fragment sequenced is not saturated for the taxa included in this study. To address the potential artificial incongruence introduced by using different outgroup taxa for the molecular and morphological datasets, sequence data should be obtained for the same outgroups used in the morphological analysis or, alternatively, morphological characters should be scored for the molecular outgroups. For future studies on closely related *Dasineura*, increasing the length of the cytochrome *b* fragment amplified and sequenced, or additionally obtaining cytochrome oxidase I sequence data (Yukawa *et al.*, 2003) may increase the resolution in finding species

boundaries. Recently, the nuclear ITS gene has also shown phylogenetic utility in resolving lower-level insect systematic relationships (Hossain & Kambhampati, 2001; Marcilla *et al*., 2002) and might therefore also be a suitable marker for elucidating species boundaries in *Dasineura*. The same cytochrome *b* fragment as used in this paper has provided a well-resolved topology of *Asphondylia* spp. on Australian acacias (data not shown), further supporting its utility as a diagnostic marker for delineating species boundaries in Cecidomyiidae.

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