

# A review of the phylogeny of Palaearctic mealybugs (Hemiptera: Coccoomorpha: Pseudococcidae)

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## Abstract

The mealybugs form the second largest family group within the scale insects (Hemiptera: Coccoomorpha), with about 2,300 species in almost 300 genera, and is currently considered to include two families, Pseudococcidae and Rhizoecidae. D.A. Downie & P.J. Gullan undertook the first molecular phylogenetic study of the group, and recognised three major clades which more or less equated to the Pseudococcinae, Phenacoccinae and Rhizoecinae. More recently, N.B Hardy and co-workers did a similar large study with more taxa, and included a morphological data matrix based on the adult female, adult males and first-instar nymphs; their results were broadly similar to those of Downie & Gullan except they found that the Rhizoecinae were included within the Phenacoccinae. Since this latter study, the Rhizoecinae has been recognized as a separate family, Rhizoecidae, and is used as an outgroup in this study. Both previous studies used mainly Nearctic, Tropical and Australian species in their analysis but, in the present study, we use only species collected in the Palaearctic and, in addition to a molecular study, add a data matrix based on the morphology of the adult female. We (1) review the phylogeny of mealybugs, focusing on Palaearctic taxa; (2) improve our understanding of mealybug molecular phylogeny based on Palaearctic mealybug taxa; (3) add morphological characters not previously included in systematic analyses; and (4) identify morphological characters that diagnose apparently monophyletic subgroups within the Pseudococcidae. The overall congruence between gene trees provides strong support for the subfamilies Pseudococcinae and Phenacoccinae, and for the tribes Planococcini, Trabutini and Pseudococcini. Our results also strongly suggest that, as currently understood, the genera *Phenacoccus* (in Phenacoccinae) and *Trionymus* and *Pseudococcus* (in Pseudococcinae) are non-monophyletic. These results are discussed and compared with earlier studies.

## Key words

Non-monophyly, *Phenacoccus*, *Peliococcus*, *Trionymus*, morphology, tribal diagnosis.

## 1. Introduction

Along with the infraorders Aphidomorpha (aphids), Psyllomorpha (plant lice) and Aleyrodomorpha (whiteflies), the Coccoomorpha (scale insects) are members of the Sternorrhyncha (Hemiptera). The scale insects are more diverse in terms of major evolutionary lineages (families), species richness, genetic systems (such as male haploidy, hermaphroditism, facultative and obligate parthenogenesis) and morphology than any of the other

sternorrhynchan groups (HODGSON & HARDY 2013). The adult females are sap-sucking and paedomorphic (resembling nymphs), perhaps due to neoteny, and the males display complete metamorphosis (GULLAN & KOSZTARAB 1997; GULLAN & MARTIN 2009). Individual scale insects are small to minute, typically less than 5 mm long, and are cryptic in habit, often resembling parts of their host plant. Their common name derives from the appearance

of the insects themselves and also from the frequent presence of a protective covering, often of secreted wax, as found on most mealybugs. Currently, about 52 extant and extinct families are recognised within the Cocco-morpha, of which the mealybugs form the second largest family group, with 2,256 species in 291 genera in two families: Pseudococcidae (2,012 species in 273 genera) and Rhizoecidae (244 species in 18 genera) (BEN-DOV et al. 2015). Of these, about 700 species in 106 genera are known from the Palaearctic (KOZÁR 1998). Mealybugs feed on a wide variety of woody and herbaceous plants, and are often restricted to a specific part of their host. Quite a few mealybug species are confined to beneath the leaf sheaths of grasses, whilst the Rhizoecidae are mainly confined to the roots and leaf litter.

Earlier workers classified all mealybugs in a single family Pseudococcidae, which was divided into about five subfamilies, namely Pseudococcinae, Phenacoccinae, Trabutininae, Rhizoecinae and Sphaerococcinae (KOTEJA 1974 a,b; DANZIG 1980; WILLIAMS 1985; TANG 1992). The family Putoidae has been recognized as a separate family since 1969 (BEARDSLEY 1969; TANG 1992), although there is still some disagreement as to its status (GAVRILOV-ZIMIN & DANZIG 2012; DANZIG & GAVRILOV-ZIMIN 2014). Here, based on DNA evidence (COOK et al. 2002), adult male morphology (HODGSON & FOLDI 2006) and the review by WILLIAMS et al. (2011) of the status of species then included in the genus, we consider Putoidae to be a good family and, along with members of the recently recognized Rhizoecidae (HODGSON 2012) and an *Acanthococcus* species, have used it as an outgroup in our analysis.

The purpose of this paper is to: (1) review the phylogeny of mealybugs, focusing on Palaearctic taxa; (2) improve our understanding of mealybug molecular phylogeny based on Palaearctic mealybug taxa; (3) add morphological characters not previously included in systematic analyses, and (4) identify morphological characters that diagnose apparently monophyletic subgroups within the Pseudococcidae. The results are discussed and compared with earlier studies.

## 2. Materials and methods

### 2.1. Materials

Fresh specimens of 61 species (81 specimens) of Pseudococcidae plus three species of Putoidae, two species of Rhizoecidae and one species of Acanthococcidae (the latter three families used as outgroup taxa) were collected in Bulgaria, Hungary, Russia and Turkey (Table 1) and preserved in 95% ethanol.

The cuticle of each specimen used for the DNA analysis was slide mounted and used as a voucher specimen. The specimens were mounted using KOSZTARAB & KOZÁR's (1988) methodology with some modification. These specimens are deposited in the Coccoidea Collection of the University of Çukurova, Adana, Turkey (KPCT). Although these vouchers were often poor specimens, they did allow accurate species determination, especially important where mixed-species collections are suspected. Identifications were made using the plates and keys in DANZIG (1980, 1997, 1998, 2001, 2003, 2006, 2007), DANZIG & MILLER (1996), KOSZTARAB & KOZÁR (1988) and WILLIAMS (2004). Only non-parasitised adult females were used for DNA extraction.

### 2.2. Molecular studies

Prior to DNA extraction, all specimens were examined under the microscope for the presence of parasitoids. DNA was extracted from a single parasitoid-free adult female with the DNA-easy tissue kit (Qiagen, Inc, Valencia, CA).

PCR products were generated from a mitochondrial gene, cytochrome oxidase I (COI), and one nuclear gene: a fragment of the D<sub>2</sub> and D<sub>3</sub> regions of the large subunit ribosomal DNA gene (28S). Primers for both amplification and sequencing were 5' – CAA CAT TTA TTT TGA TTT TTT GG – 3' (C1-J-2183 aka Jerry) and 5' – GCW ACW ACR TAA TAK GTA TCA TG – 3' (C1-N-2568 aka BEN3R, designed by T.R. Schultz, Smithsonian Institution) for COI; 5' – TCG GAR GGA ACC AGC TAC TA – 3' (A335 REVERSE) and 5' – GAG AGT TMA ASA GTA CGT GAA AC – 3' (S3660 FORWARD) for 28S. PCR reaction components and final concentrations were 1.5–2.5 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, and 1 unit *Taq* polymerase in a proprietary buffer (PCR Master Mix, Promega Biotechnology), 0.2 μM of each primer, and 5 μl DNA template in a final volume of 25 μl. The PCR cycling protocol for COI was 95°C for 7 m, followed by 40 cycles of 95°C for 1 m, 45 °C for 1 m, and 72°C for 1 m 30 s with a final extension at 72°C for 5 m. The protocol for 28S was 94°C for 4 m followed by 35–45 cycles of 94°C for 1 m, 49–52°C for 1 m, and 72°C for 1 m, 30 s with a final extension at 72°C for 4 m.

PCR products were purified and sequenced by ION-TEK (İstanbul, Turkey). Contigs were assembled using CodonCode Aligner v. 3.7.1 (CodonCode Corp.) and multiple alignments were performed by using ClustalW in BioEdit for each gene region. Each alignment was controlled visually and cut if there were big gaps in the alignments. Gaps of this kind can occur if there were too many taxa in the study. All alignments and morphological matrix were then combined by using MacClade 4.08 (MADDISON & MADDISON 2005). Please see supplementary file for the combined data.

### 2.3. Phylogenetic analyses

Maximum likelihood (ML) and Bayesian analyses were used to estimate trees. The data were analysed for each locus separately and with data for the two loci combined. ML trees were constructed using Mega6, with four data partitions: 3 codon positions for COI, and one partition for 28S. A separate GTR, nucleotide substitution model was applied to each partition. Ten thousand non-parametric bootstrap replicates were performed using GTR, with every 100 bootstrap tree used as the starting tree for ML optimisation. Phylogenies were also reconstructed with Bayesian inference methods using MRBAYES v. 3.1.2 (RONQUIST & HUELSENBECK 2003) under the packed program Geneious 5.6 by adding morphological characters. For this analysis, morphological character data were concatenated to the DNA data using Python. The evolution of the morphological data was modelled with an mk1 model (character changes unordered), with coding set to variable. We applied a separate GTR model with gamma-distributed rates and a proportion of invariant sites (GTR) to each partition, using default priors. Four Markov chains, three hot and one cold (program default), were run simultaneously for five million generations, with trees sampled every 1000 generations. A plot of log-likelihood over time was examined, and the first 1000 trees, generated before the analysis had achieved stationary, were discarded as ‘burn-in’.

### 2.4. Morphological studies

All specimens which had been used for molecular studies were used for the morphological analyses. The characters used for compiling the data matrix were those of HARDY et al. (2008), with some modifications. The morphological matrix was concatenated to the molecular data as explained above (2.3) and analysed using Bayesian inference methods.

### 2.5. Morphological characters

All characters refer to adult females. Those listed under “Additional characters” below are additional to those in HARDY et al. (2008). The morphological character matrix is presented in Table 2.

#### *Venter*

1. Number of antennal segments: (1) 9; (2) 8; (3) 7; (4) <7.
2. Apical antennal segments: total number of fleshy setae: (0) >5; (1) 5; (2) <5.
3. Number of intersegmental setae on each antenna: (0) 3 pairs; (1) 1 pair.
4. Antennae: basiconic sensilla: (0) present; (1) absent.

5. Antennae: coeloconic sensilla: (0) absent; (1) 2 present on apical antennal segment, plus one on both of segments II and V; (2) only on apical segment.
6. Antennae with clavate setae: (0) absent; (1) present.
7. Labium, number of setae on middle segment: (0) 2 pairs; (1) 1 pair.
8. Labium, apical segment: number of setae on anterior surface (excluding fleshy subapicals) (see KOTEJA 1974b): (0) >3; (1) 3.
9. Labium, apical setae (see KOTEJA 1974b): (0) absent; (1) present.
10. Labium, number of fleshy subapical setae (see KOTEJA 1974b): (0) 2; (1) 4.
11. Ventral sclerotization on head: (0) absent; (1) present.
12. Legs, development: (1) well developed; (2) absent.
13. Legs, number of campaniform sensilla on each side of trochanter: (0) 4; (1) 2.
14. Legs, translucent pores on hind legs: (0) present; (1) absent
15. Legs, distribution of translucent pores on hind legs: (0) absent; (1) on coxa as a pore-plate only; (2) on coxa and tibia; (3) on femur only; (4) on femur and tibia; (5) on tibia only; (6) on coxa, femur and tibia.
16. Legs, denticle on claw: (0) present; (1) absent.
17. Legs, claw: (0) typical (broad basally, not extended); (1) long and slender.
18. Legs, tarsal digitules: (0) undeveloped/absent; (1) developed.
19. Multilocular disc pores with 6 or more loculi: (0) present throughout venter; (1) confined to abdomen; (2) absent; (3) present on abdomen and near spiracles; (4) near spiracles only.
20. Quinquelocular disc pores: (0) present; (1) absent.
21. Trilocular disc pores: (0) present; (1) absent.
22. Discoidal pores: (0) present; (1) absent.
23. Oral-collar ducts on dorsum: (0) present; (1) absent.
24. Circuli, structure: (0) evaginated and truncate; (1) sessile.
25. Circuli, distribution: (0) more than one; (1) only one, on abdominal sternite III; (2) only one, in intersegmental membrane between abdominal sternites III and IV; (3) absent.
26. Spiracles: (0) small, typical size for coccoids; (1) grossly developed, without shrunken trilocular pores lining the atrium; (2) grossly developed, with shrunken trilocular pores lining atrium.
27. Anal lobe bar: (0) absent; (1) present.
28. Posterior abdominal segments (V–VIII): (0) membranous; (1) sclerotised.
29. Eyes: (0) present; (1) absent.
30. Multilocular disc pores and/or tubular ducts in clusters: (0) absent; (1) present.
31. Vulva: (0) directed ventrally; (1) directed posteriorly.

#### *Dorsum*

32. Ostioles: (0) both pairs present; (1) posterior pair present, anterior pair absent; (2) both pairs absent.

Table 1. Collection details of specimens and outgroup taxa from which DNA was extracted.

Species name	Coll. Code	Code	Location	Date	N	E	Host plant	Collector
<b>Pseudococcidae</b>								
<i>Antonia evelynae</i> Gavrilov	MBK050	4673	Russia, Sochi	10.v.2003			<i>Phyllostachys</i> sp.	M.B. Kaydan
<i>Antonia graminis</i> (Maskell)	MBK076	4249	Turkey, Hakkari, Başkale	22.v.2008	37°48'480"	044°05'146"	Poaceae	M.B. Kaydan
<i>Artemioccus bispinus</i> (Borchsenius)	MBK149	4562	Turkey, Hakkari road	02.ix.2009	37°39'067"	043°52'994"	<i>Artemisia</i> sp.	M.B. Kaydan
<i>Atrococcus achilleae</i> (Kiritschenko)	MBK008	4643	Bulgaria, W. Rhodopes	24.vi.2009			Undetermined	I. Gavrilov
<i>Atrococcus achilleae</i> (Kiritschenko)	MBK227	4570	Turkey, Van, Başkale	02.ix.2008	38°07'093"	044°05'640"	<i>Salvia</i> sp.	M.B. Kaydan
<i>Atrococcus arabianae</i> (Ter-Grigorian)	MBK077	4389	Turkey, İğdir, Turluca, Gaziler	12.vi.2008	40°06'717"	043°34'183"	<i>Artemisia</i> sp.	M.B. Kaydan
<i>Atrococcus paludinus</i> (Green)	MBK020	4655	Bulgaria, Alibotosh	19.vi.2009			<i>Tritilium</i> sp.	I. Gavrilov
<i>Atrococcus saxatilis</i> (Ter-Grigorian)	MBK128	4616	Turkey, Artvin, Borçka	17.ix.2009	41°21'182"	041°41'162"	<i>Lactuca</i> sp.	M.B. Kaydan
<i>Atrococcus saxatilis</i> (Ter-Grigorian)	MBK292	4926	Turkey, Artvin, Şavşat Road	16.vi.2010	41°10'322"	041°58'542"	Undetermined	M.B. Kaydan
<i>Cerauto pilosellae</i> Šulc	MBK065	4241	Turkey, Hakkari, Başkale	22.v.2008	37°39'058"	043°52'976"	<i>Euphorbia</i> sp.	M.B. Kaydan
<i>Cheatooccus phragmitis</i> (Marchal)	MBK074	4242	Turkey, Hakkari, Esendere Road	23.v.2008	37°42'405"	044°32'040"	<i>Phragmites</i> sp.	M.B. Kaydan
<i>Chorozeucus malabadiensis</i> Kaydan	MBK088	4326 (b)	Turkey, Diyarbakir, Malabadi	26.v.2008	38°09'335"	041°12'785"	<i>Chrysopogon gryllus</i>	M.B. Kaydan
<i>Coccidihystrix samui</i> Kozár & Konzne Benedicty	MBK014	4649	Hungary, Nagykovoiicsi	05.xi.2005			D-Yac	F. Kozár
<i>Coccira circumscripta</i> (Kiritschenko)	MBK034	1402	Turkey, İğdir, Gaziler	03.v.2005	40°06'332"	043°29'423"	Undetermined	M.B. Kaydan
<i>Coccira comari</i> (Kunov)	MBK010	4645	Bulgaria, Alibotosh	19.vi.2009			<i>Rosa</i> sp.	I. Gavrilov
<i>Crisocheucus metaxvae</i> (Danzig)	MBK286	4789	Turkey, Erzurum, Tercan Road	08.vii.2010	39°35'506"	039°52'499"	<i>Quercus</i> sp.	M.B. Kaydan
<i>Eupiercia europaea</i> (Newstead)	MBK150	4627	Turkey, Kars, Ardahan Road	16.ix.2009	40°47'718	043°06'344	<i>Thymus</i> sp.	M.B. Kaydan
<i>Eupiercia tonlinii</i> (Newstead)	MBK049	4672	Russia, Voronezh, Plunogrié	13 – 16.vi.2003			Undetermined	M.B. Kaydan
<i>Foncolombia tschadaevae</i> (Danzig)	MBK107	4623	Turkey, Kars, Ardahan Road	16.ix.2009	41°04'320"	041°50'112"	Poaceae	M.B. Kaydan
<i>Heterobrevennis guillanae</i> Kaydan	MBK205	4530	Turkey, Van, Gevaş	09.vi.2009	38°16'663"	043°03'898"	Poaceae	M.B. Kaydan
<i>Heterobrevennis kozari</i> Kaydan	MBK089	4325	Turkey, Bitlis-Siirt Border	26.v.2008	38°11'772"	041°49'067"	<i>Cynodon dactylon</i>	M.B. Kaydan
<i>Heterocheucus nudus</i> (Green)	MBK043	4666	Russia, Voronezh, Hrafskaya st.	29.v.2004			Poaceae	M.B. Kaydan
<i>Longiocheucus clarus</i> (Borchsenius)	MBK075	4250	Turkey, Hakkari, Başkale	23.v.2008	37°55'816"	044°04'631"	Poaceae	M.B. Kaydan
<i>Mirococcopsis multicirculus</i> Kaydan & Gavrilov	MBK230	3798	Turkey, Van, Balçesaray	25.vi.2007	38°08'765"	042°51'506"	Poaceae	M.B. Kaydan
<i>Mirococcopsis amophila</i> Bazarov & Nurmatov	MBK101	4357	Turkey, İğdir, Turluca, Gaziler	29.v.2008	40°06'218"	043°27'952"	Undetermined	M.B. Kaydan
<i>Mirococcopsis inermis</i> Hall	MBK232	3379	Turkey, Van, Başkale, Hoşap	25.v.2007	38°15'268"	043°52'669"	Brassicaceae	M.B. Kaydan
<i>Neotrionymus monstator</i> Borchsenius	MBK118	4557	Turkey, Van, Hakkari Road	02.ix.2009	37°41'157"	043°57'433"	<i>Phragmites communis</i>	M.B. Kaydan
<i>Parahadania amena</i> Ter-Grigorian	MBK239	2017	Turkey, Van, Özalp Road	05.vii.2005	38°39'522"	043°57'287"	<i>Bromus</i> sp.	M.B. Kaydan
<i>Peliococcus arvensis</i> (Kiritschenko)	MBK235	1686	Turkey, Van, Muradiye, Şelale	08.vi.2005	39°03'388"	043°45'453"	<i>Poa bulbosa, Taraxacum</i> sp.	M.B. Kaydan
<i>Peliococcus priesneri</i> (Laing)	MBK173	4467	Turkey, Ağrı, Doğubeyazıt, İshakpaşa	03.vi.2009	39°31'905"	044°07'100"	<i>Cynodon dactylon</i>	M.B. Kaydan
<i>Peliococcus cheisonensis</i> (Kiritschenko)	MBK038	1531	Turkey, Hakkari, Çukurca	17.v.2005	37°14'836"	043°36'646"	<i>Mentha</i> sp.	M.B. Kaydan
<i>Peliococcus cheisonensis</i> (Kiritschenko)	MBK157	4516	Turkey, Muş, Bulank Road	10.vi.2009	38°57'220"	041°56'557"	Undetermined	M.B. Kaydan
<i>Peliococcus cheisonensis</i> (Kiritschenko)	MBK184	4482	Turkey, Kars, Kagıman Road	04.vi.2009	40°12'011"	043°02'827"	<i>Convolvulus arvensis</i>	M.B. Kaydan
<i>Peliococcus latitubulatus</i> Danzig	MBK058	4265	Turkey, Hakkari, Üzümcü	22.v.2008	37°29'899"	043°35'368"	<i>Papaver rhoas</i>	M.B. Kaydan
<i>Peliococcus turanicus</i> (Kiritschenko)	MBK007	4642	Bulgaria, W. Rhodopes				Apiaceae	I. Gavrilov
<i>Peliococcus turanicus</i> (Kiritschenko)	MBK068	4298	Turkey, Hatay, Harbiye	27.v.2008	36°07'859"	036°08'653"	<i>Papaver</i> sp.	M.B. Kaydan
<i>Peliococcus sp.</i>	MBK192	4549	Turkey, Elazığ, Doğukent	08.vi.2009			<i>Cornus</i> sp.	M.B. Kaydan
<i>Pelionella glanduliferus</i> (Borchsenius)	MBK080	4379	Turkey, İğdir, Doğubayazıt Road	29.v.2008	39°47'487"	044°08'630"	<i>Euphorbia seguieriana</i>	M.B. Kaydan
<i>Pelionella kansui</i> Kaydan	MBK199	4503	Turkey, Van, Hakkari Road	06.vi.2009	38°22'248"	043°35'176"	<i>Euphorbia seguieriana</i>	M.B. Kaydan
<i>Pelionella kansui</i> Kaydan	MBK277	4857	Turkey, Ani Kars Road	14.vi.2010	40°34'302"	043°30'520"	<i>Euphorbia</i> sp.	M.B. Kaydan
<i>Pelionella multipora</i> Kaydan	MBK108	4585 (a)	Turkey, Hakkari, Başkale Road	02.ix.2009	37°58'790"	044°04'775"	<i>Euphorbia sequiriana</i>	M.B. Kaydan
<i>Phenacoccus abditus</i> Borchsenius	MBK131	4556	Turkey, Hakkari, Çukurca yolu	01.ix.2009	37°29'902"	043°34'230"	<i>Cynodon dactylon</i>	M.B. Kaydan
<i>Phenacoccus aceris</i> Signoret	MBK027	4662	Hungary, Budapest	11.v.2005			<i>Fraxinus excelsior</i>	F. Kozár
<i>Phenacoccus alibotosh</i> Gavrilov	MBK017	4652	Bulgaria, Alibotosh	19.vi.2009			<i>Tritilium</i> sp.	I. Gavrilov

Table 1 continued.

<i>Phenacoccus chatakicus</i> Kaydan & Kozár	MBK241	3903	Turkey, Van, Çatak-Narlı	03.vii.2007	37°55'258"	042°59'138"	Undetermined	M.B. Kaydan
<i>Phenacoccus emansor</i> Williams & Kozárzhenskaya	MBK174	4531	Turkey, Van, Gevaş	09.vi.2009	38°16'663"	043°03'898"	<i>Bifora radians</i>	M.B. Kaydan
<i>Phenacoccus evelinae</i> (Tereznikova)	MBK155	4590	Turkey, Hatay, Erzin	08.ix.2009	37°01'224"	036.09'561"	<i>Sorghum halepense</i> + <i>Cynodon dactylon</i>	M.B. Kaydan
<i>Phenacoccus hordei</i> (Lindeman)	MBK134	4619	Turkey, Kars, Ardahan Road	16.ix.2009	41°04'320"	041°50'112"	<i>Thymus</i> sp.	M.B. Kaydan
<i>Phenacoccus incertus</i> (Kiritshenko)	MBK120	4567	Turkey, Van, Hakkari Road	02.ix.2009	37°39'067"	042°52'992"	<i>Cynodon dactylon</i>	M.B. Kaydan
<i>Phenacoccus kareliniae</i> Borchsenius	MBK210	4534	Turkey, Bitlis River	09.vi.2009	38°20'899"	042°01'898"	<i>Avena sterilis</i>	M.B. Kaydan
<i>Phenacoccus nurnamatovi</i> Bazarov	MBK055	4300	Turkey, Hatay, Harbiye	27.vi.2008	36°07'859"	036°08'653"	<i>Avena fatua</i>	M.B. Kaydan
<i>Phenacoccus phenacocoides</i> (Kiritshenko)	MBK164	4483	Turkey, Kars, Kağızman	04.vi.2009	40°12'011"	043°02'827"	Poaceae	M.B. Kaydan
<i>Phenacoccus pumilus</i> Kiritshenko	MBK098	4350	Turkey, İğdir, Türüca, Gaziler	29.v.2008	40°06'218"	043°27'952"	Undetermined	M.B. Kaydan
<i>Phenacoccus querculus</i> (Borchsenius)	MBK233	3503	Turkey, Van, Gevaş, Artos	05.vi.2007	38°16'159"	043°08'421"	<i>Quercus</i> sp.	M.B. Kaydan
<i>Phenacoccus</i> sp.	MBK060	4296	Turkey, Hatay, Harbiye	27.v.2008	36°07'859"	036°08'653"	Poaceae	M.B. Kaydan
<i>Phenacoccus</i> sp.	MBK062	4283	Turkey, Hakkari, Yüksekova, Ortaç Road	23.v.2008	37°40'881"	044°03'376"	Poaceae	M.B. Kaydan
<i>Phenacoccus</i> sp.	MBK295	4918	Turkey, Artvin	16.vii.2010	41°10'917"	041°50'764"	Apiaceae	M.B. Kaydan
<i>Phenacoccus specificus</i> Matesova	MBK200	4472	Turkey, Kars, Kağızman Road	04.vi.2009	40°16'351"	042°52'275"	<i>Thymus</i> sp.	M.B. Kaydan
<i>Phenacoccus strigosus</i> Borchsenius	MBK073	4246	Turkey, Hakkari, Yüksekova Road	23.v.2008	37°40'919"	044°03'589"	Undetermined	M.B. Kaydan
<i>Phenacoccus teigrigianae</i> Borchsenius	MBK099	4356	Turkey, İğdir, Türüca, Gaziler	29.v.2008	40°06'218"	043°27'952"	<i>Pegaronum harmala</i>	M.B. Kaydan
<i>Phenacoccus transcasicus</i> Hadzibejli	MBK160	4446	Turkey, Kars, Kağızman	19.ix.2008	40°08'160"	043°07'115"	<i>Malus communis</i>	M.B. Kaydan
<i>Planococcus citri</i> (Risso)	MBK147	4587	Turkey, Hatay, Erzin	08.ix.2009			<i>Cyperus rotundus</i>	M.B. Kaydan
<i>Planococcus ficus</i> (Signoret)	MBK167	4455	Turkey, Gaziantep, Pistacia Research Institute	04.viii.2008	37°03'395"	037°20'464"	<i>Morus</i> sp.	M.B. Kaydan
<i>Planococcus ficus</i> (Signoret)	MBK202	4582	Turkey, Hatay, Samandağ	08.ix.2009	36°04'852"	035°59'907"	<i>Vitis vinifera</i>	M.B. Kaydan
<i>Planococcus vivae</i> (Nasonov)	MBK133	4575	Turkey, Gaziantep, Nur mount, Bağçe Road	07.ix.2009	37°11'045"	036°43'007"	<i>Cupressus</i> sp.	M.B. Kaydan
<i>Pseudococcus comstocki</i> (Kuwana)	MBK148	4602	Turkey, Artvin, Şavşat Road	17.ix.2009	41°10'280"	041.58'492"	<i>Pinus granatum</i>	M.B. Kaydan
<i>Pseudococcus cryptus</i> Hempel	MBK216	4687	Turkey, Hatay, Samandağ	22.vii.09			<i>Citrus</i> sp.	M.B. Kaydan
<i>Pseudococcus viburni</i> (Signoret)	MBK238		Turkey, Artvin, Hopa				Undetermined	M.B. Kaydan
<i>Pseudococcus viburni</i> (Signoret)	MBK307	4735	Turkey, Gümüşhane 10 km	07.vii.2010	41°00'306"	039°43'995"	Undetermined	M.B. Kaydan
<i>Spilococcus vashlovnicus</i> Danzig	MBK234	3990	Turkey, Hakkari, Çukurca Road	04.vii.2007	37°28'725"	043°32'831"	Brassicaceae	M.B. Kaydan
<i>Trabutina crassipinosa</i> Borchsenius	MBK181	4476	Turkey, İğdir, Digor Road	04.vi.2009	40°07'278"	043°37'708"	<i>Tamarix</i> sp.	M.B. Kaydan
<i>Trabutina crassipinosa</i> Borchsenius	MBK231	3592	Turkey, İğdir, Digor Road	13.vi.2007	40°67'291"	043°37'233"	<i>Tamarix</i> sp.	M.B. Kaydan
<i>Trionymus aberrans</i> Goux	MBK084	4346	Turkey, Diyarbakır, Silvan	28.v.2008	38°01'290"	040°29'388"	<i>Agropyron repens</i>	M.B. Kaydan
<i>Trionymus artemisiarum</i> (Borchsenius)	MBK182	4461	Turkey, Van, Muradiye	03.vi.2009	39°03'682"	043°45'468"	<i>Artemisia</i> sp.	M.B. Kaydan
<i>Trionymus artemisiarum</i> (Borchsenius)	MBK206	4467	Turkey, Ağrı, Doğubeyazıt, İshakpaşa	03.vi.2009	39°31'905"	044°07'100"	<i>Artemisia</i> sp.	M.B. Kaydan
<i>Trionymus multivorus</i> (Kiritshenko)	MBK201	4522	Turkey, Van, Akdamar	09.06.2009			Apiaceae	M.B. Kaydan
<i>Trionymus persisii</i> (Signoret)	MBK045	4688	Russia, Voronezh, Hrafskaya st.	14.v.2003			<i>Poa</i> sp.	M.B. Kaydan
<i>Trionymus persisii</i> (Signoret)	MBK302	4934	Turkey, Kars, Kağızman Road	17.vii.2010	40°16'283"	42°57'598"	Poaceae	M.B. Kaydan
<i>Volvicoccus volvifer</i> (Goux)	MBK179	4529	Turkey, Muş, Bulank	10.vi.2009	33°45'709"	041°53'478"	Poaceae	M.B. Kaydan
<i>Volvicoccus volvifer</i> (Goux)	MBK308	4715	Turkey, Bitlis Adilcevaz, Ahlat Road	29.vi.2010	38°47'809"	42°425'93"	<i>Stipa</i> sp.	M.B. Kaydan
<b>Putoidae</b>								
<i>Puto israelensis</i> Ben-Dov	MBK019	4654	Turkey, Antalya, Elmali	13.v.2005			<i>Quercus coccifera</i>	S. Ülgentürk
<i>Puto megniensis</i> (Borchsenius)	MBK165	4554	Turkey, Van, Gevaş, Tatvan	09.vi.2009	38°23'265"	042°47'444"	Undetermined	M.B. Kaydan
<i>Puto superbus</i> (Leonardi)	MBK048	4671	Bulgaria, Rila mountains	27.vii.2008			Poaceae	M.B. Kaydan
<i>Puto superbus</i> (Leonardi)	MBK188	4533	Turkey, Bitlis river	09.vi.2009	38°20'899"	042°01'898"	<i>Aegilops</i> sp.	M.B. Kaydan
<b>Rhizoecitiae</b>								
<i>Ripisietella</i> sp.	MBK298	4946	Turkey, Artvin, Borçka	16.vii.2010	41°22'168"	041°39'155"	Poaceae	M.B. Kaydan
<i>Ripisietella parva</i> (Danzig)	MBK204	4512	Turkey, Bitlis, Adilcevaz, Ahlat road	11.vi.2009	38°47'809"	042°42'593"	<i>Sedum</i> sp.	M.B. Kaydan
<b>Acanthococcidae</b>								
<i>Acanthococcus aceris</i> Signoret	MBK029	4664	Hungary, Budapest	11.v.2005			<i>Acer campestre</i>	F. Kozár

33. Cerarii, surrounding cuticle: (0) sclerotised; (1) membranous.
34. Cerarii, number of marginal pairs: (0) 0; (1) 18; (2) 16–17; (3) 2–15; (4) 1.
35. Cerarii, number of stout setae per anal lobe cerarius: (0) 0; (1) 2; (2) >2.
36. Cerarii, additional cerarii on dorsum in mid-line: (0) absent; (1) present.
37. Cerarii, auxiliary setae: (0) present; (1) absent.
38. Cerarii, with minute discoidal pores: (0) present; (1) absent.
39. Cerarii, number of trilocular pores on each anal cerarius: (0) 5–10; (1) 11–20; (2) >30.
40. Hairlike/setose setae on derm surface apart from those on cerarii: (0) present; (1) absent.
41. Lanceolate/conical spine-like setae on derm surface apart from those on cerarii: (0) absent; (1) present.
42. Multilocular disc pores (those with more than 6 loculi): (0) present; (1) absent.
43. Quinquelocular disc pores: (0) present; (1) absent.
44. Trilocular pores: (0) present; (1) absent.
45. Tritubular/bitubular pores: (0) absent; (1) present.
46. Oral-collar tubular ducts: (0) present, with collars; (1) present, without collars; (2) as oral-rim ducts (mushroom type); (3) absent.
47. Tubular ducts with satellite discoidal pores: (0) absent; (1) present.
48. Anal ring, development: (0) exposed, ring complete; (1) exposed, with pronounced interruption along anterior margin, appearing V-shaped; (2) deeply invaginated.
49. Anal ring, cortex (outermost ring): (0) complex, several pores deep; (1) simple, single row of round pores; (2) lacking pores; (3) as a series of linear pores; (4) as spinules.
50. Anal ring, medulla (inner ring): (0) simple, single row of round pores; (1) reticulated network of large polygonal pores; (2) several cells, especially those along anterior margin, with a reticulate appearance, reticulations not extending to lightly pigmented areas inside ring; (3) more pronounced reticulation, extending onto the lightly pigmented area inside ring.
51. Multilocular disc pores and/or tubular ducts: (0) present together in groups; (1) not in groups, if both present, then pores and ducts separate.

#### *Additional characters*

52. Ventral oral rim tubular ducts: (0) present; (1) absent.
53. Ventral multilocular disc pores and oral collar tubular duct present together in a cluster anterior to anterior spiracle: (0) absent; (1) present.
54. Enlarged tubular ducts on margin of dorsum: (0) absent; (1) present.
55. Labium basal segment: (0) with two setae; (1) with three setae.
56. Macrotubular ducts: (0) absent; (1) present.
57. Anal ring spinules: (0) setose; (1) dome-shaped.

Following the analyses, the morphological characters diagnosing each clade were studied and further characters were identified which appeared to be apomorphic for particular clades (see under each section below).

## 3. Results

### 3.1. Molecular alignment

The alignment obtained from the molecular analysis comprised 1250 characters, of which 400 were from the COI dataset and 850 from 28S (Fasta files in the electronic supplement: Files 1–3).

### 3.2. Phylogenetic trees

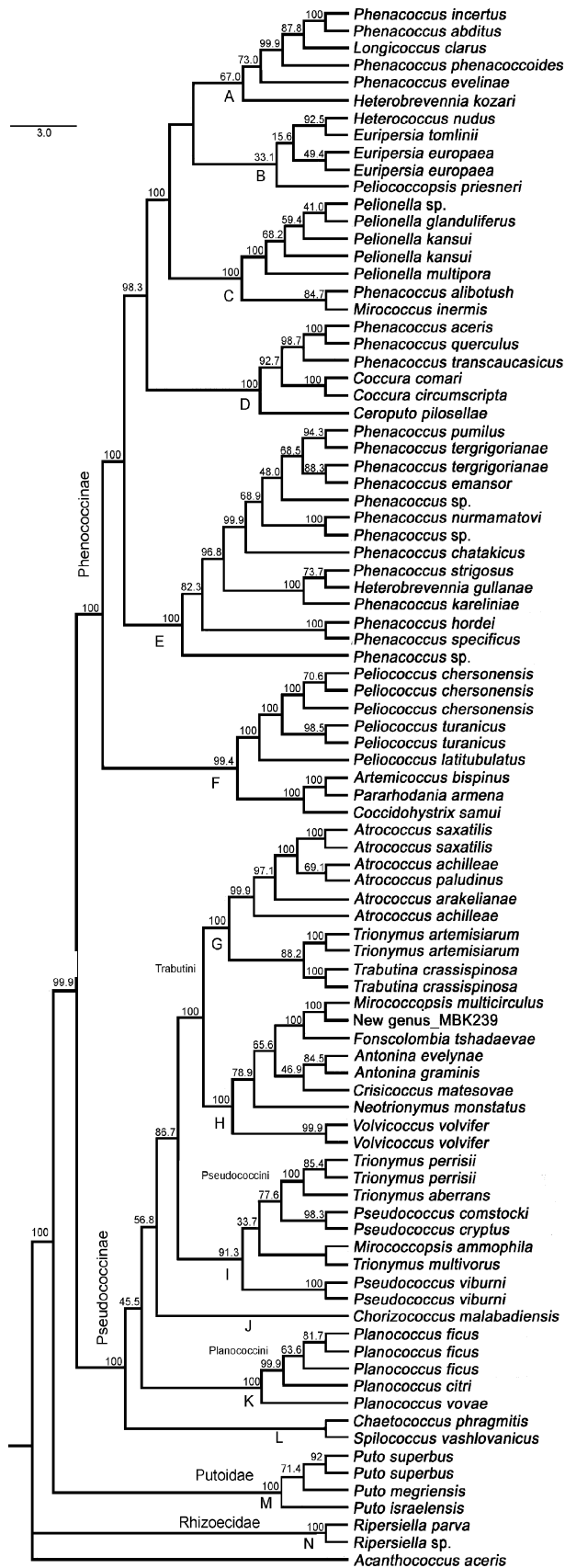
The maximum-likelihood tree (ML) recovered from the analysis of the COI and 28S data is presented in Fig. 1, with posterior probabilities (PP) values above each node. The morphological character matrix is presented in Table 2. The Bayesian analysis results support the results from the combined molecular and morphological data analysis.

We obtained two principal clades within Pseudococcidae from our Palaearctic samples, which we equate to the Pseudococcinae and the Phenacoccinae, each with strong support (99.9%). We also obtained strong support for the division of the Pseudococcinae into three tribes (Planococcini, Trabutinini and Pseudococcini).

#### 3.2.1. Phenacoccinae

The apomorphic morphological character states that diagnose the adult females of this subfamily are: (i) tarsal digitules setose “(18-0)”; (ii) claws with a denticle “(16-0)”; (iii) presence of quinquelocular pores “(20-0)”; (iv) antennae usually nine-segmented “(1-1)”; (v) anal ring with dome-shaped spinules in outer ring “(57-1)”, and (vi) spine-like setae on dorsum “(41-1)” (Fig. 2). Our results strongly suggest that the genus *Phenacoccus* is not monophyletic, as its species are currently included in three of the main clades. Within the Phenacoccinae, we recognise six clades (Clades A–F in Fig. 1), all but two with Bayesian values greater than 95%.

Clade F includes two subclades, of which one has *Artemicoccus bispinus* (Fig. 3), *Coccidohystrix samui* and *Pararhodonia amena* (Fig. 4), all of which share the absence of dorsal ostioles “(32-2)”, and which are sister to 3 *Peliococcus* species (with 6 specimens), all of which have dorsal spinose setae with an associated trilocular pore at their base (Fig. 1D). The species in this clade tend to be found on xerophilous plants and are widespread on



**Fig. 1.** Palaeartic pseudococcid phylogeny based on 28S and COI gene regions plus morphological characters, with three species of Putoidae, two species of Rhizoecidae and *Acanthococcus aceris* (Acanthococcidae) as outgroup taxa. Figures above nodes refer to posterior probabilities (PP).

steppe flora. This clade is sister to the remaining Phenacoccinae.

The remaining Phenacoccinae fall into two main clades: clade E and the group of subclades A–D. Clade E includes 12 species of *Phenacoccus* plus *Heterobrevennia gullanae*, all of which share: (i) spine-like setae on the intersegmental membranes between antennal segments VI and VII “(3-0)” (Fig. 5), and (ii) multilocular disc pores on the dorsum. Further work is needed to clarify differences between the species in this clade and those in clades A–D, including the possible transfer of *H. gullanae* to whatever taxon is formed from the species in clade E.

Clade A has four *Phenacoccus* species plus *Longicoccus clarus* and *Heterobrevennia kozari*, all of which share: (i) “dome-shaped” setae on the intersegmental membranes between antennal segments VI and VII (Fig. 6), and (ii) a reduced number of pores in the anal ring (generally with an inner ring of pores and an outer ring of spinules: Fig. 7).

Clade C contains the genus *Pelionella* Kaydan (KAYDAN 2015), with the following combination of characters: (i) multilocular disc pores each with two rings of loculi, in clusters on dorsum, differing from those on venter which have only a single ring of loculi; (ii) dorsal setae spine-like, each lacking trilobular pores near their base, and thus differing from cerarian setae; (iii) a circulus on abdominal segment III “(25-1)” (rather than in the intersegmental membrane between III and IV); and (iv) multilocular disc-pore clusters with dorsal oral collar tubular ducts of one or two sizes, each cluster with the smallest ducts in the centre and the larger ducts among the disc pores towards the outside (Fig. 8).

Clade D includes three *Phenacoccus* species (namely *P. aceris* (the type species of *Phenacoccus*), *P. querculus* and *P. transcaucasicus*), two *Coccurea* species and *Ceroputo pilosellae*, all of which have similarly-shaped, wide circuli (sometimes more than one), which perhaps provide good attachment to their hosts (Fig. 9). The three *Phenacoccus* species are found on Rosaceae, Fagaceae, Oleaceae, etc and are regarded as wood feeding species. It is clear from this study that *Phenacoccus* is not monophyletic because there are several species groups within the genus. The generic concept of *Phenacoccus*, therefore, needs further study. Although the two *Coccurea* spp. and *Ceroputo pilosellae* are placed in the Clade D, it is clear that these species do not belong to *Phenacoccus sensu stricto*. It is here considered that their presence in Clade D is due to lack of sampling and therefore that their separation will be clear when further taxa are studied.

Thus, the genera which fell within the Phenacoccinae in our study are *Artemicoccus* Balachowsky, *Ceroputo* Šulc, *Coccidohystrix* Lindinger, *Coccurea* Šulc, *Euripersia* Borchsenius, *Heterococcus* Ferris, *Heterobrevennia* Kaydan, *Longicoccus* Danzig, *Mirococcus* Borchsenius, *Pararhodania* Ter-Grigorian, *Peliococcus* Borchsenius, *Peliococcopsis* Borchsenius, *Pelionella* Kaydan and *Phenacoccus* Cockerell.



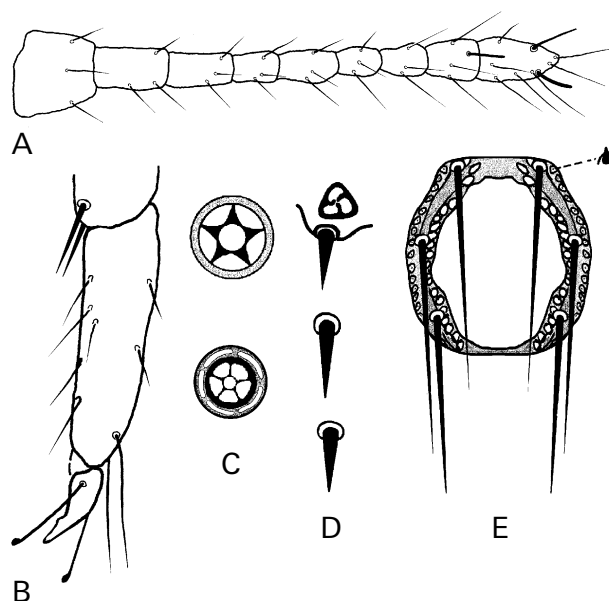


## 3.2.2. Pseudococcinae

The apomorphic morphological character states that appear to diagnose the adult females of this subfamily are: (i) all tarsal digitules apically knobbed “(18-1)”; (ii) claws without a denticle “(16-1)”; (iii) quinquelocular pores absent “(20-1)”; (iv) antennae generally with eight or fewer segments “(1-2,3,4)”; (v) anal ring with setose-like spinules “(57-1)”, and (vi) hair-like dorsal setae “(40-0)” (Fig. 10). As in the Phenacoccinae, some genera appear to be non-monophyletic, particularly *Trionymus*, species of which occur in two main clades, but also *Pseudococcus* Westwood which has species in two separate subclades. In this study, the Pseudococcinae were divided into eight clades (clades G–L, Fig. 1).

The Trabutinini are considered to be represented by the species in clades G + H, and includes the genera *Antonina* Signoret, *Atrococcus* Goux, *Crisicoccus* Ferris, *Fonscolombia* Lichtenstein, *Mirococcopsis* Borchsenius, *Neotrionymus* Borchsenius, *Trabutina* Marchal and *Volvicoccus* (Goux). This group is diagnosed by the following character states: (i) their blue-black colour both when alive and especially when in KOH, and (ii) tubular ducts of rather variable size but with a distinctly sclerotized structure (sclerotized collar or rim, or with a sclerotized inner part) “(46-0)” (Fig. 11). The five species of *Atrococcus* (*A. saxatilis* with two specimens) in clade G all have oral rim ducts “(46-2)” (Fig. 11a) and form a well-supported monophyletic clade (99.9%). Clade G also contains *Trionymus artemisiarum*, the only *Trionymus* species in this clade. Unlike most other *Trionymus* species (which are in clade I, Figs. 13, 14), *T. artemisiarum* (Fig. 12) has: (i) oral collar ducts with a sclerotized inner end to the duct; (ii) fewer setae on the hind tibia (a total of nine on *T. artemisiarum*; 12 on *T. perrisii*; Fig. 13; 20–22 on *T. multivorus*; Fig. 14) and (iii) *T. artemisiarum* also has a different placement and number of coeloconic sensilla on each antenna (i.e., two on each apical segment, and one on segment IV, compared with two on each apical segment, and one on both segments II and IV on *T. multivorus* and *T. perrisii*) (Figs. 12, 13, 14).

Clade I is considered to equate to the Pseudococcini, and here includes three species of *Trionymus*, three species of *Pseudococcus* plus *Mirococcopsis ammophila*. These species share the following character states: (i) each anal lobe cerarius with a large number of trilocular pores, hair-like setae and simple discoidal pores “(38-0), (39-2)”; (ii) dorsal setae similar to those on the venter, all hair-like/setose; and (generally) (iii) tubular ducts with associated discoidal pores. In addition to *Trionymus* and *Pseudococcus* spp., DOWNIE & GULLAN (2004) and HARDY et al. (2008) also included some *Dysmicoccus* Ferris species in the Pseudococcini (*D. brevipes* (Cockerell), *D. neobrevipes* Beardsley, *D. boninsis* (Kuwana) and *Dysmicoccus* sp.). Their tribal diagnosis was “adult females .... with 16 or 17 pairs of cerarii with auxiliary setae” (and this also applies to all species included in our clade I). No *Dysmicoccus* were available in our study although a few species do occur in the Palaearctic. The three *Pseudococ-*



**Fig. 2.** Diagnostic features of adult females of Phenacoccinae: (A) nine-segmented antennae, (B) legs (tarsal digitules setose; claw with denticle); (C) presence of quinquelocular pores; (D) dorsal setae spine-like; (E) anal ring with an outer ring of dome-shaped spinules.

*cus* species included in this study occur in two separate subclades, i.e., *P. viburni* in one subclade, diagnosed by the presence of simple pores next to the eyes, and *P. cryptus* and *P. comstocki* in another subclade that lacks simple pores next to the eye (WILLIAMS 2004).

Clade J contains only *Chorizococcus malabadiensis* Kaydan (described recently by KAYDAN et al. 2014; Fig. 15), diagnosed by the following combination of character states: (i) oral rim ducts on both dorsum and venter “(46-2)”; (ii) translucent pores present on hind femur and tibia “(14-0)”, and (iii) cerarii restricted to the last two abdominal segments “(34-3)”. In the Palaearctic region, *Chorizococcus* species share the presence of oral rim tubular ducts with *Atrococcus*, *Spilococcus* Ferris and *Pseudococcus*. However, of these four genera, only *Atrococcus* is believed to be restricted to the Palaearctic, the other three genera (*Chorizococcus*, *Pseudococcus* and *Spilococcus*) are thought to have originated from elsewhere. These four genera occur in separate clades in our analysis and further studies are needed to resolve their generic position.

Clade K equates to the tribe Planococcini, which is here monophyletic and well supported (100%). The tribe is well characterized in having: (i) an anal lobe bar “(27-1)”; (ii) 18 pairs of marginal cerarii “(34-1)”, and (iii) slightly flagellate cerarian setae (COX 1989; DANZIG & GAVRILOV-ZIMIN 2010). In our study, the Planococcini clade included only *Planococcus* species.

Thus, the genera that fall within the Pseudococcinae in our study are *Antonina* Signoret, *Atrococcus* Goux, *Chaetococcus* Maskell, *Chorizococcus* McKenzie, *Cri-*

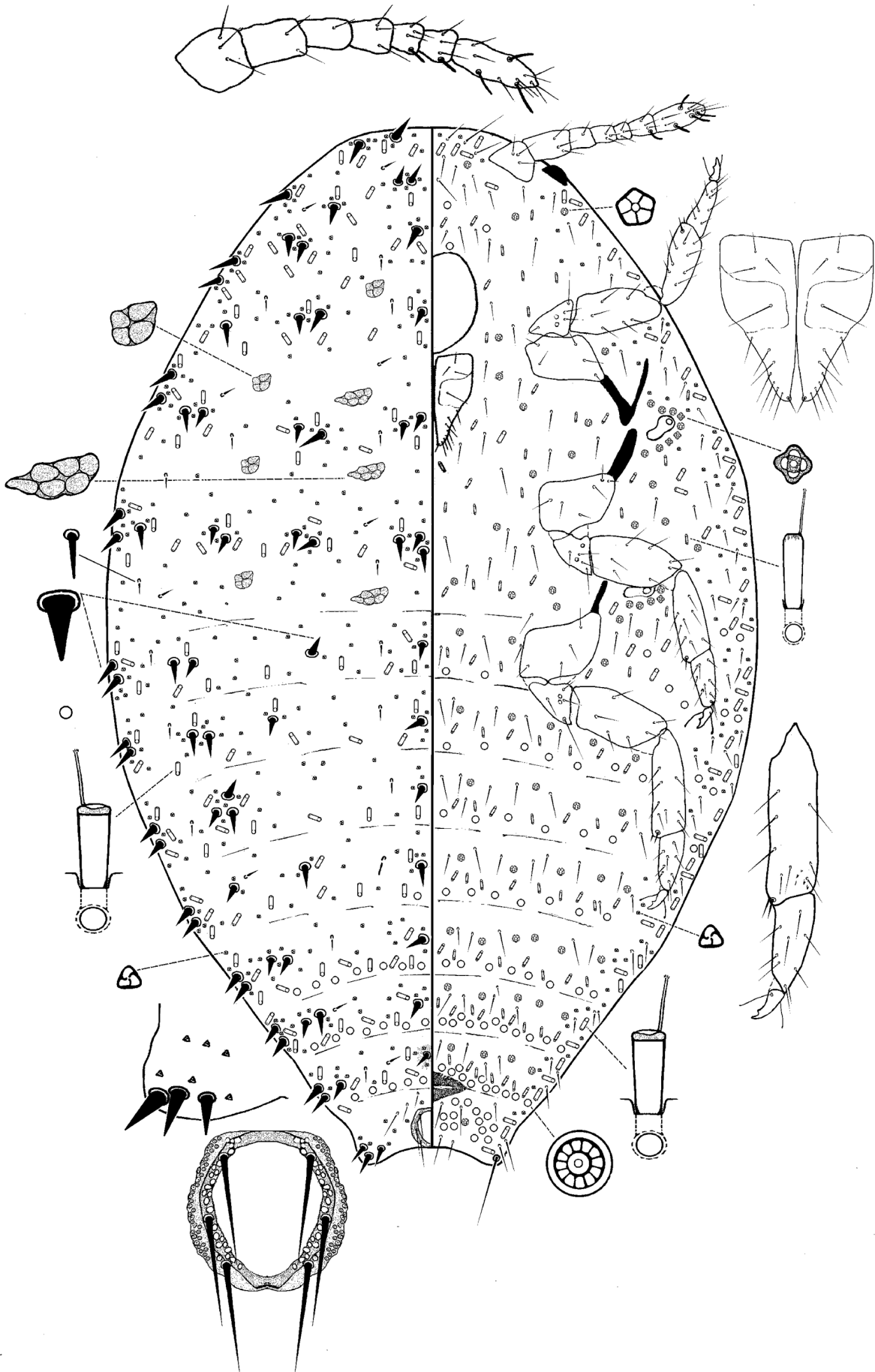


Fig. 3. *Artemicoccus bispinus* (Borchsenius), paratype, female, original (after DANZIG et al. 2012) (Note: absence of dorsal ostioles).

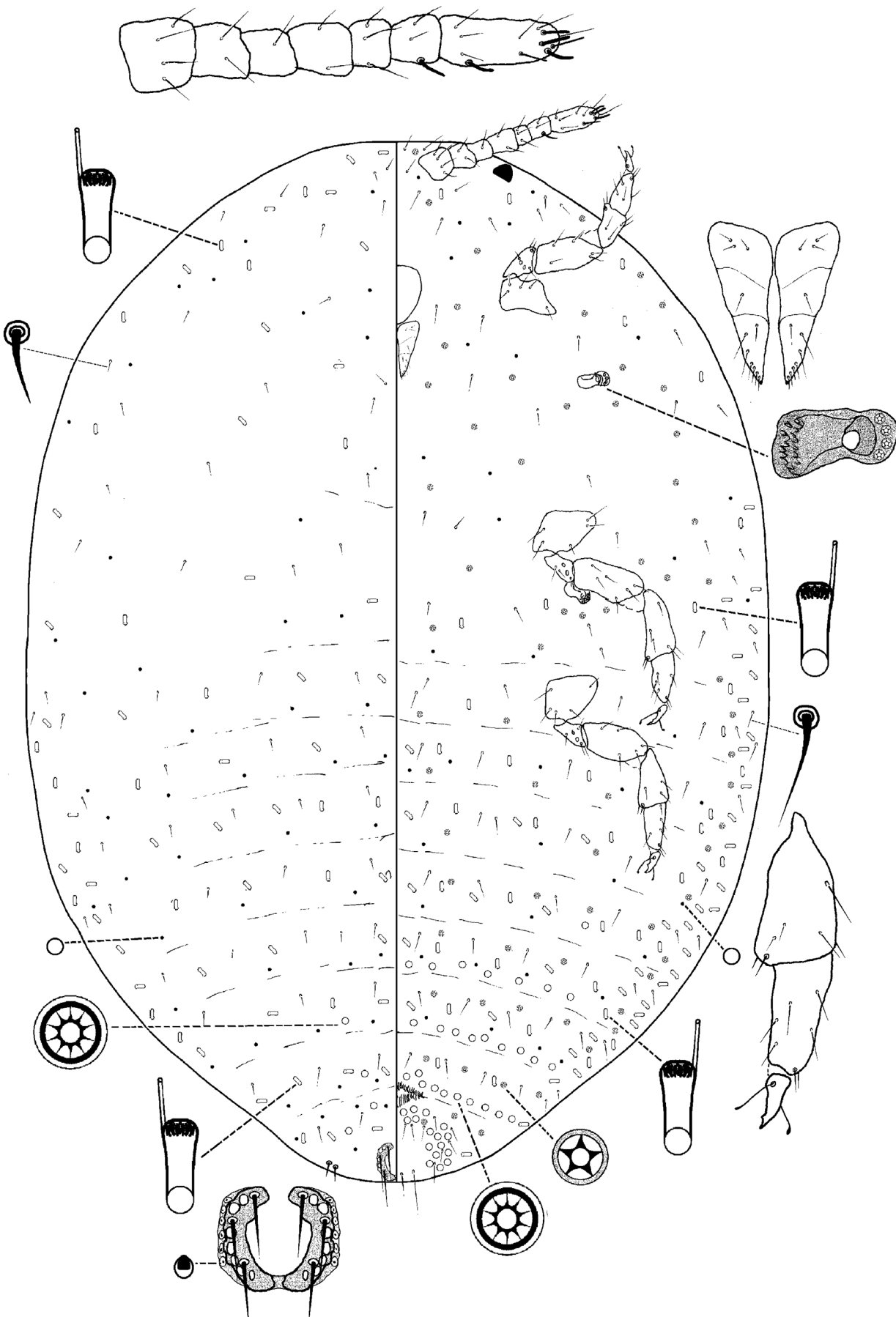
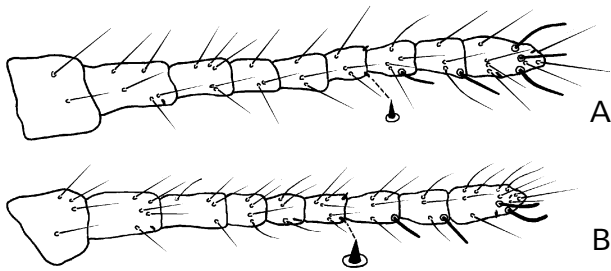
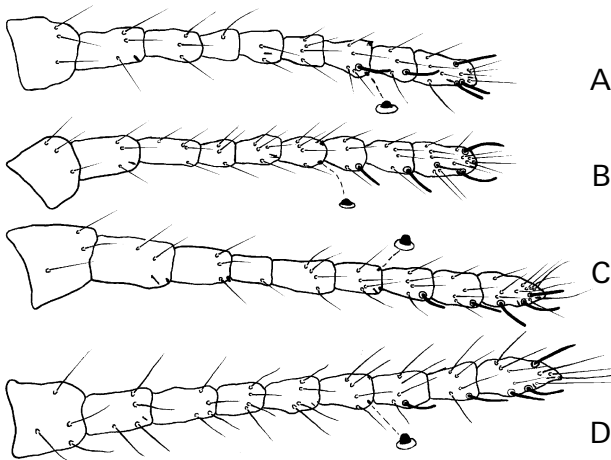


Fig. 4. *Pararhodania armena* Ter-Grigorian (after KAYDAN & KOZÁR 2010) (Note: absence of dorsal ostioles, presence of a large denticle on the claw, reduced antennae and an anal ring with dome-shaped spinules).



**Fig. 5.** Antennae of (A) *Phenacoccus nurmatovi* Bazarov and (B) *Phenacoccus specificus* Matesova showing the spine-like setae on membrane between segments VI and VII.

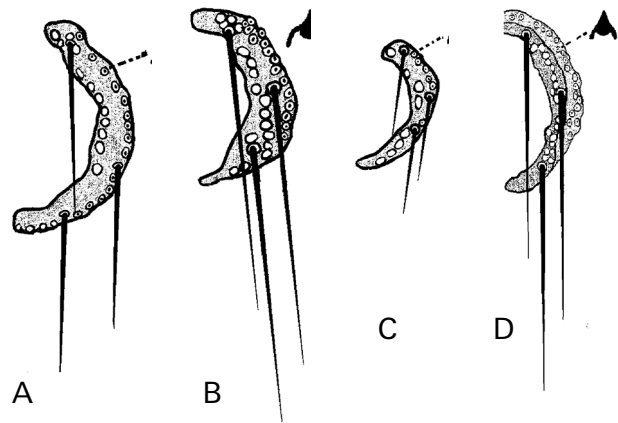


**Fig. 6.** Antennae of (A) *Phenacoccus incertus* (Kiritshenko), (B) *Phenacoccus phenacoccoides* (Kiritshenko), (C) *Longicoccus clarus* (Borchsenius) and (D) *Heterobrevennia kozari* Kaydan showing dome-shaped setae on membrane between segments VI and VII.

*siccoccus* Ferris, *Fonscolombia* Lichtenstein, *Mirococcopsis* Borchsenius, *Neotrionymus* Borchsenius, *Planococcus* Ferris, *Pseudococcus* Westwood, *Spilococcus* Ferris, *Trabutina* Marchal, *Trionymus* Berg and *Volvicoccus* (Goux).

#### 4. General discussion

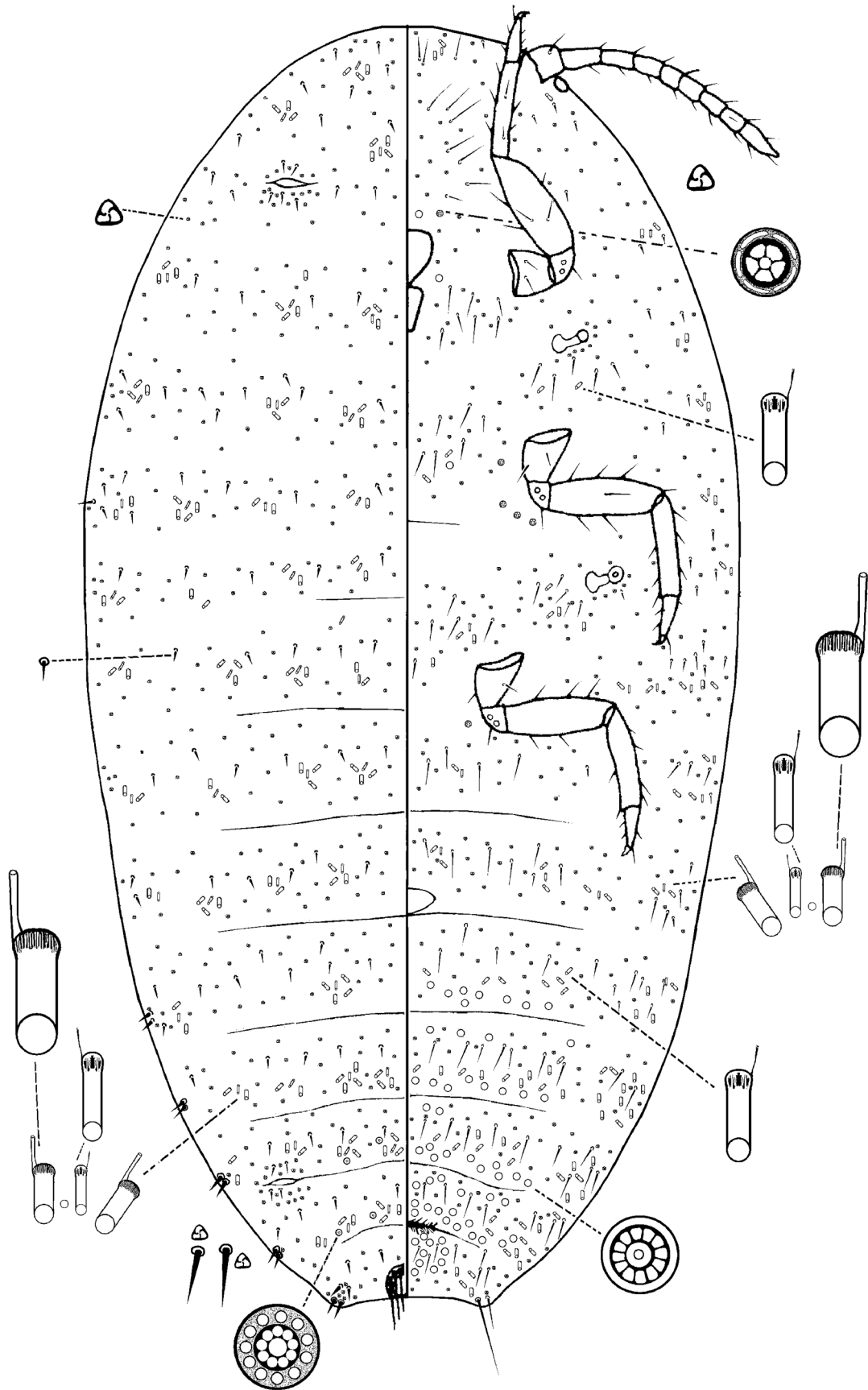
DOWNIE & GULLAN (2004) undertook the first molecular phylogenetic study of the Pseudococcidae, using 64 species from 35 genera, plus an aclerdid, a monophlebid and four putoids as outgroup taxa, and recognised three major clades which more or less equated to the Pseudococcinae, Phenacoccinae and Rhizoecinae. However, the boundaries of these clades differed from those of earlier authors, apart from KOTEJA (1974c). In addition, DOWNIE & GULLAN (2004) distinguished four Pseudococcinae subclades which they equated to the tribes Planococcini, Trabutinini, Pseudococcini and an informal *Ferrisia* group. These relationships were largely poorly resolved, apart from Rhizoecinae appearing to be sister to the other taxa. DOWNIE & GULLAN (2004) included the genera *Pseudococcus*, *Dysmicoccus*, *Trionymus* and a few smaller genera in the Pseudococcini; *Planococcus* and possibly *Planococcoides* Ezzat & McConnell in the Planococcini; *Amonostherium* Morrison & Morrison, *Antonina*, *Balanococcus* Williams, *Nipaeococcus* Šulc, the non-African species of *Paracoccus* Ezzat & McConnell, plus a few other genera in Trabutinini, and *Ferrisia* Fullaway and *Anisococcus* Ferris in the *Ferrisia* group. They considered that *Antonina* and *Chaetococcus* Maskell were not sister taxa and therefore that the Sphaerococcinae either did not exist or was paraphyletic. In addition, in DOWNIE and GULLAN's study, the Phenacoccinae contained just *Phenacoccus* and *Heliococcus* Šulc.



**Fig. 7.** Anal rings of (A) *Longicoccus clarus*, (B) *Phenacoccus evelinae* (Tereznikova), (C) *Phenacoccus phenacoccoides* (Kiritshenko) and (D) *Heterobrevennia kozari* Kaydan showing shape of spinules in outer ring (Note: pores restricted to inner ring only; outer ring composed of spinules, number of both [i.e pores and spinules] reduced compared with other clades in Phenacoccinae).

LAN (2004) distinguished four Pseudococcinae subclades which they equated to the tribes Planococcini, Trabutinini, Pseudococcini and an informal *Ferrisia* group. These relationships were largely poorly resolved, apart from Rhizoecinae appearing to be sister to the other taxa. DOWNIE & GULLAN (2004) included the genera *Pseudococcus*, *Dysmicoccus*, *Trionymus* and a few smaller genera in the Pseudococcini; *Planococcus* and possibly *Planococcoides* Ezzat & McConnell in the Planococcini; *Amonostherium* Morrison & Morrison, *Antonina*, *Balanococcus* Williams, *Nipaeococcus* Šulc, the non-African species of *Paracoccus* Ezzat & McConnell, plus a few other genera in Trabutinini, and *Ferrisia* Fullaway and *Anisococcus* Ferris in the *Ferrisia* group. They considered that *Antonina* and *Chaetococcus* Maskell were not sister taxa and therefore that the Sphaerococcinae either did not exist or was paraphyletic. In addition, in DOWNIE and GULLAN's study, the Phenacoccinae contained just *Phenacoccus* and *Heliococcus* Šulc.

HARDY et al. (2008) did a further large study (as an extension of that of DOWNIE & GULLAN 2004) that included a further 33 species from 27 genera, giving a total of 97 species in 35 genera and, in addition to analysing molecular data, they added a morphological data matrix based on adult females, adult males and first-instar nymphs. Their results basically agreed with those of DOWNIE & GULLAN (2004) except that the Rhizoecinae fell within the subfamily Phenacoccinae. However, more recently, HODGSON (2012) compared the morphology of the adult



**Fig. 8.** *Pelionella glanduliferus* (Borchsenius) (after DANZIG 2001, with modifications) (Note: (i) dorsal multilocular disc pores, each with two rings of loculi, in clusters; (ii) ventral multilocular disc-pores with only a single ring of loculi; (iii) spine-like dorsal setae, each lacking trilobular pores near their base; (iv) a circulus apparently on abdominal segment III; and (v) oral collar tubular ducts of two sizes on dorsum).

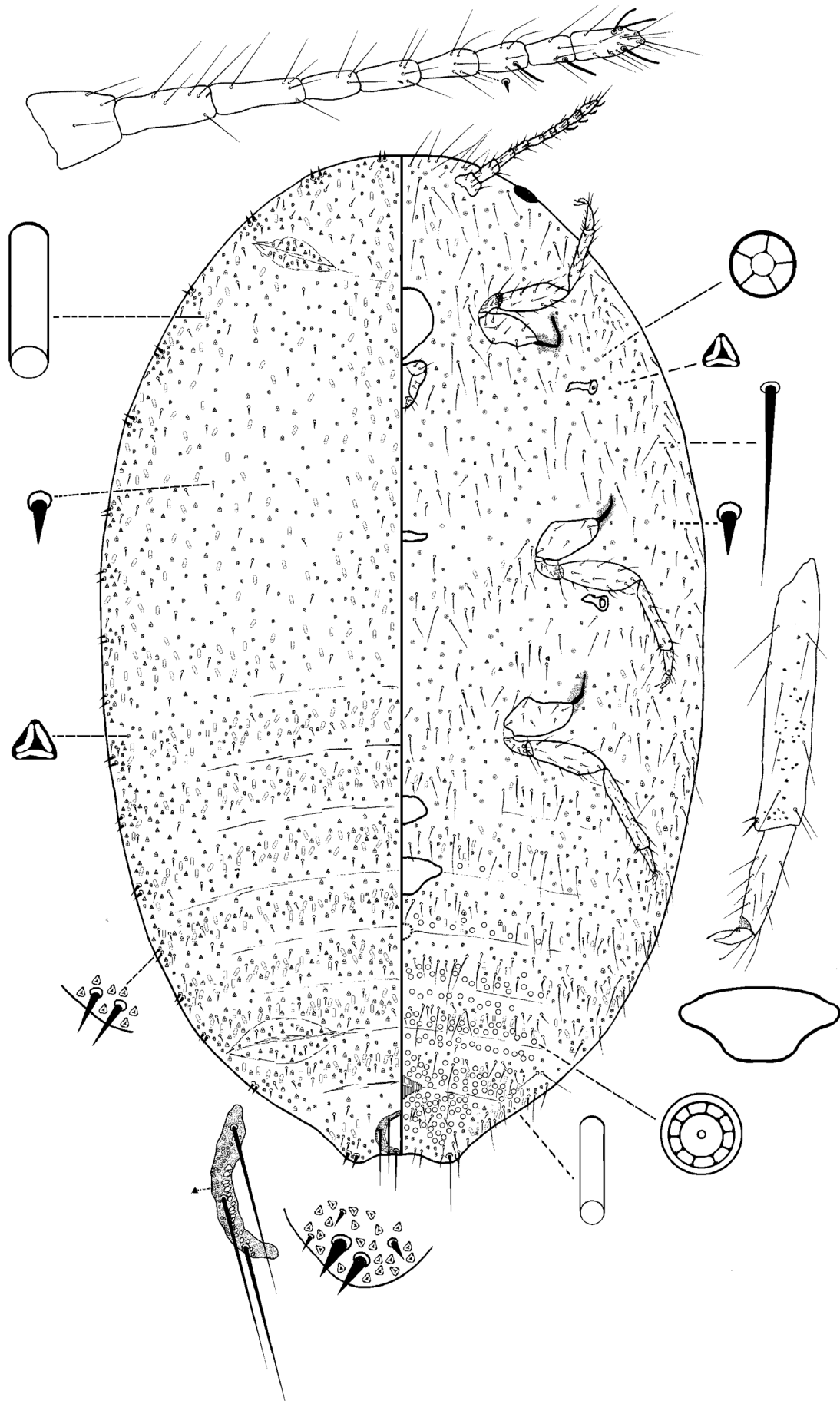
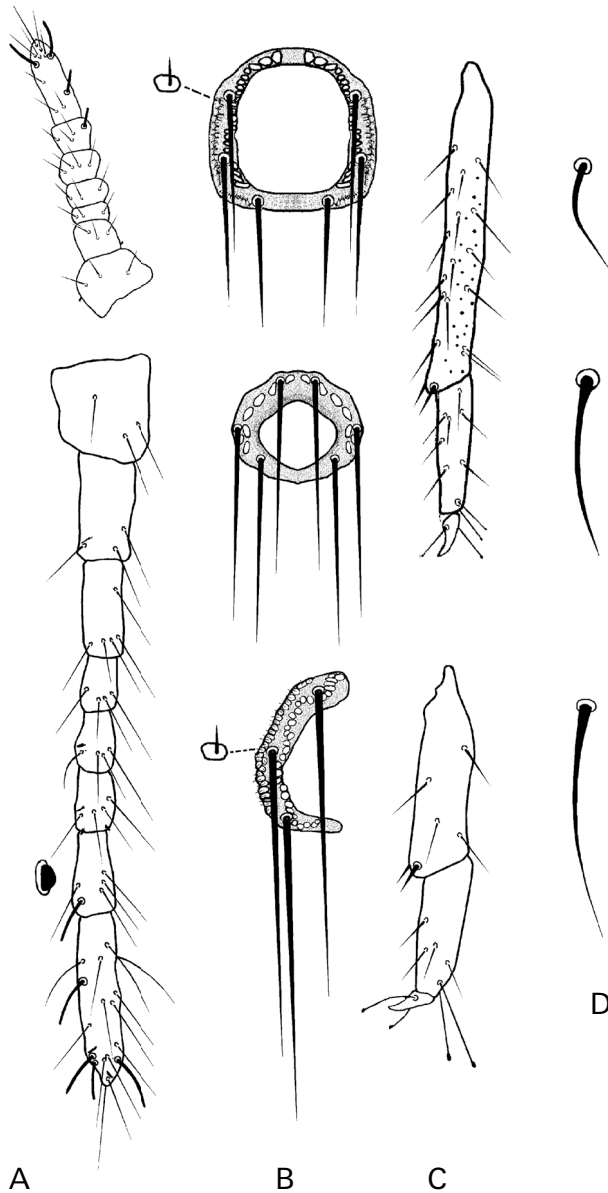
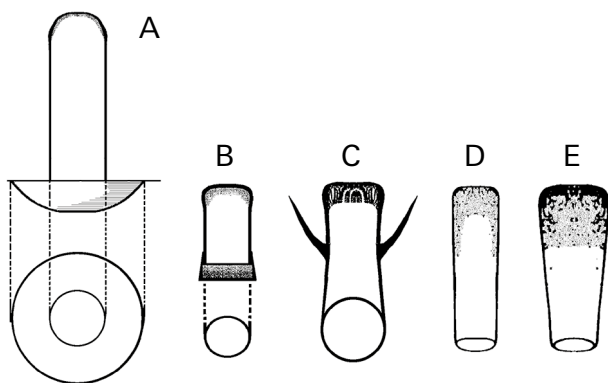


Fig. 9. *Phenacoccus aceris* (Signoret), original (Note: presence of three circuli, one unusually large).



**Fig. 10.** Diagnostic features of adult female Pseudococcinae: (A) antennae with eight or fewer segments; (B) anal ring with setose-like spinules; (C) legs with knobbed tarsal digitules and claws without a denticle; and (D) dorsal setae hair-like.



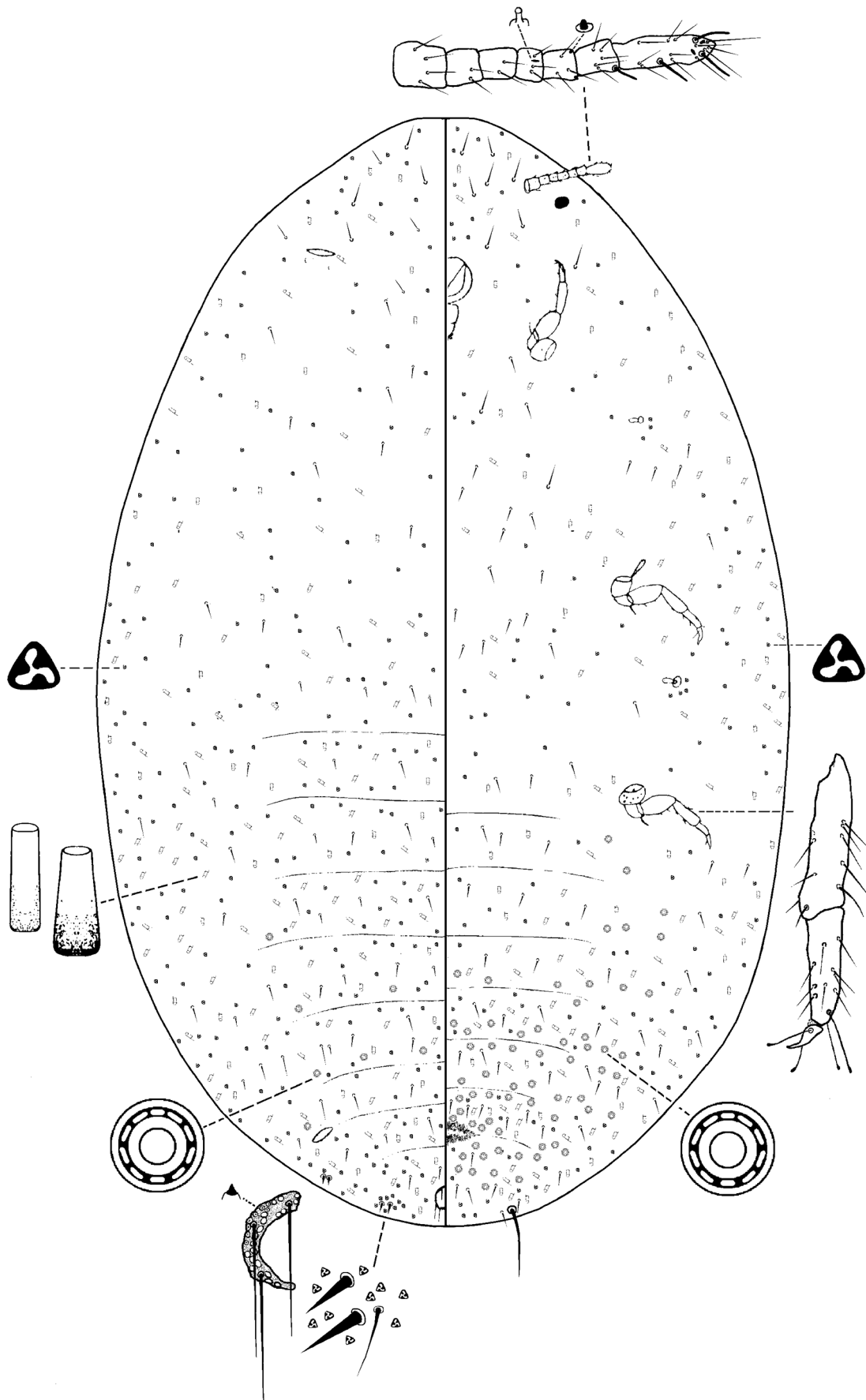
**Fig. 11.** Tubular ducts as found in three pseudococcid genera: (A,B) *Atrococcus*; (C) *Volvicoccus*, and (D,E) *Trabuttina*.

males of the rhizoecine, phenacoccine and pseudococcine mealybugs and concluded that the hypogaecic mealybugs formed a separate family, Rhizoecidae Williams, sister to the Pseudococcidae. Examples of the Rhizoecidae were therefore included as outgroup taxa in our analysis.

Earlier, COOK et al. (2002) and GULLAN & COOK (2007) had undertaken a molecular study of most families in the Coccoidea and concluded that *Puto* was not a neococcoid but might be sister to or part of a sister group to the neococcoids. Although this conclusion has not been accepted by all authors (e.g., GAVRILOV-ZIMIN & DANZIG 2012; DANZIG & GAVRILOV-ZIMIN 2014), *Puto* has been shown to have eubacterial endosymbionts in different genera from those of the Pseudococcidae (BUCHNER 1965; TREMBLAY 1989), and the Putoidae was recognised by HODGSON & FOLDI (2006) based on adult male characters. Since then, WILLIAMS et al. (2011) have reviewed the taxonomic history of *Puto* and concluded that Putoidae Tang is a valid family. Moreover, WILLIAMS et al. (2011) considered that *Ceroputo* was not a putoid but belonged to the Phenacoccinae, supporting the observations of several earlier workers (e.g., KOTEJA 1974b; TANG 1992; HARDY et al. 2008). Thus, the “mealybugs” are currently considered to include two families: Rhizoecidae Williams and Pseudococcidae Westwood.

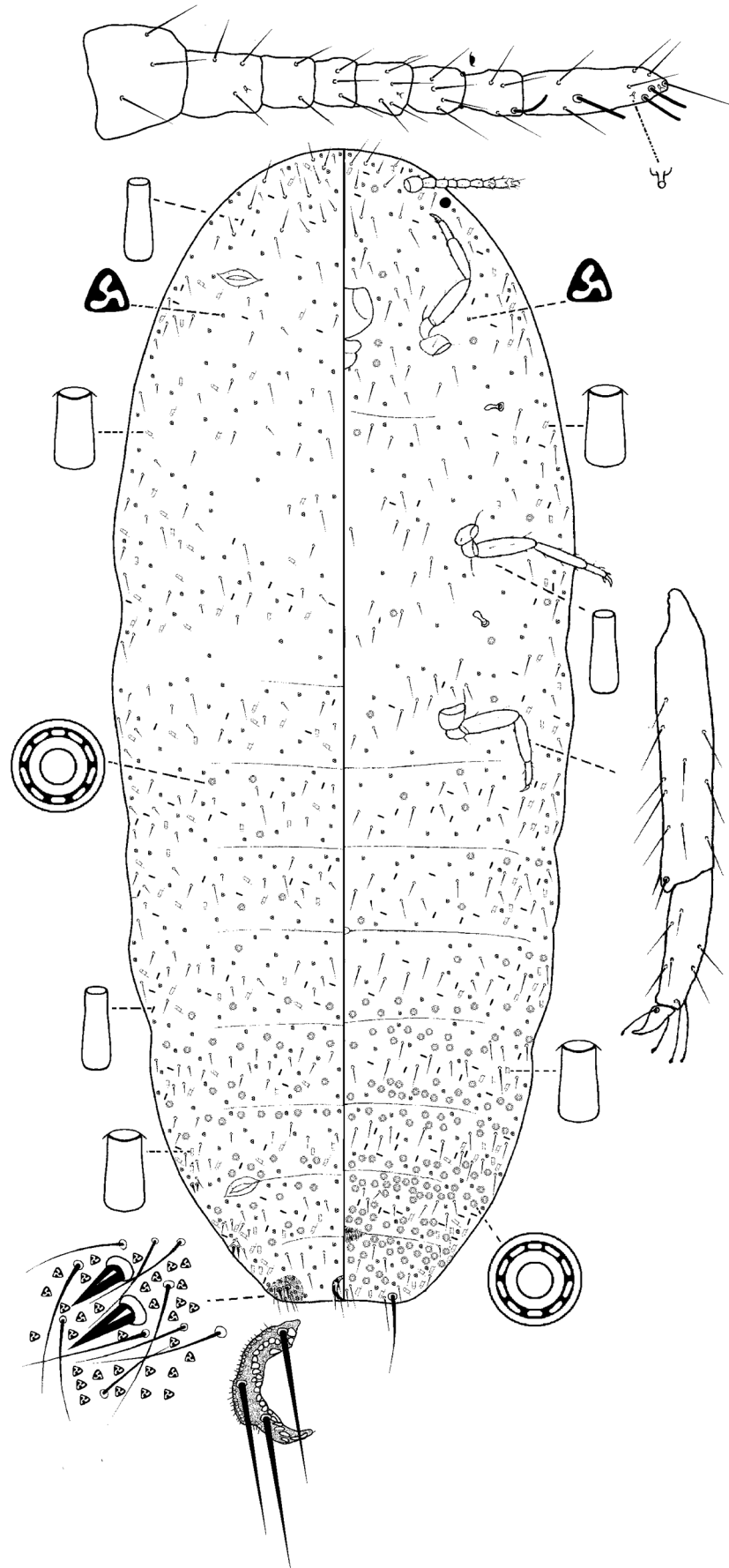
These two previous studies (DOWNIE & GULLAN 2004; HARDY et al. 2008) had included mainly species from the Holarctic and Australasia, whereas our analysis used species collected entirely within the western Palaearctic. Of the 61 pseudococcoid species (81 specimens) used in the present analysis, only seven had been included in the earlier studies. Nonetheless, our results have much in common.

The present analysis produced a well-supported tree, with about 65% of all internal nodes with Bayesian support greater than 90% (and with 44% of nodes greater than 95%). This proportion is slightly less than obtained by HARDY et al. (2008). On the other hand, both of the earlier studies had constructed two major clades, which they equated to the subfamilies Phenacoccinae and Pseudococcinae and our analysis produced 100% support for the monophyly of both subfamilies. This division was also recently supported by an analysis of adult male morphology (HODGSON & HARDY 2013). Within the Phenacoccinae, we obtained at least three well-supported clades, one (clade F) with several *Peliococcus* species, one (clade E) with mainly *Phenacoccus* species (although *Phenacoccus* appeared to be non-monophyletic) and a third clade (consisting of subclades A–E) with a wide range of genera including the recently described genus *Pelionella* Kaydan. It is clear that some of the systematic relationships suggested by these clades need further study. HARDY et al. (2008) provided a list of genera that they considered belonged to the Phenacoccinae. To this list can be added *Artemicoccus* Balachowsky, *Heterobrevennia* and possibly *Euripersia* Borchsenius. This latter genus had been included in the Pseudococcinae by HARDY et al. (2008) but the two species included in this study clearly fall within the Phenacoccinae (but they may, of

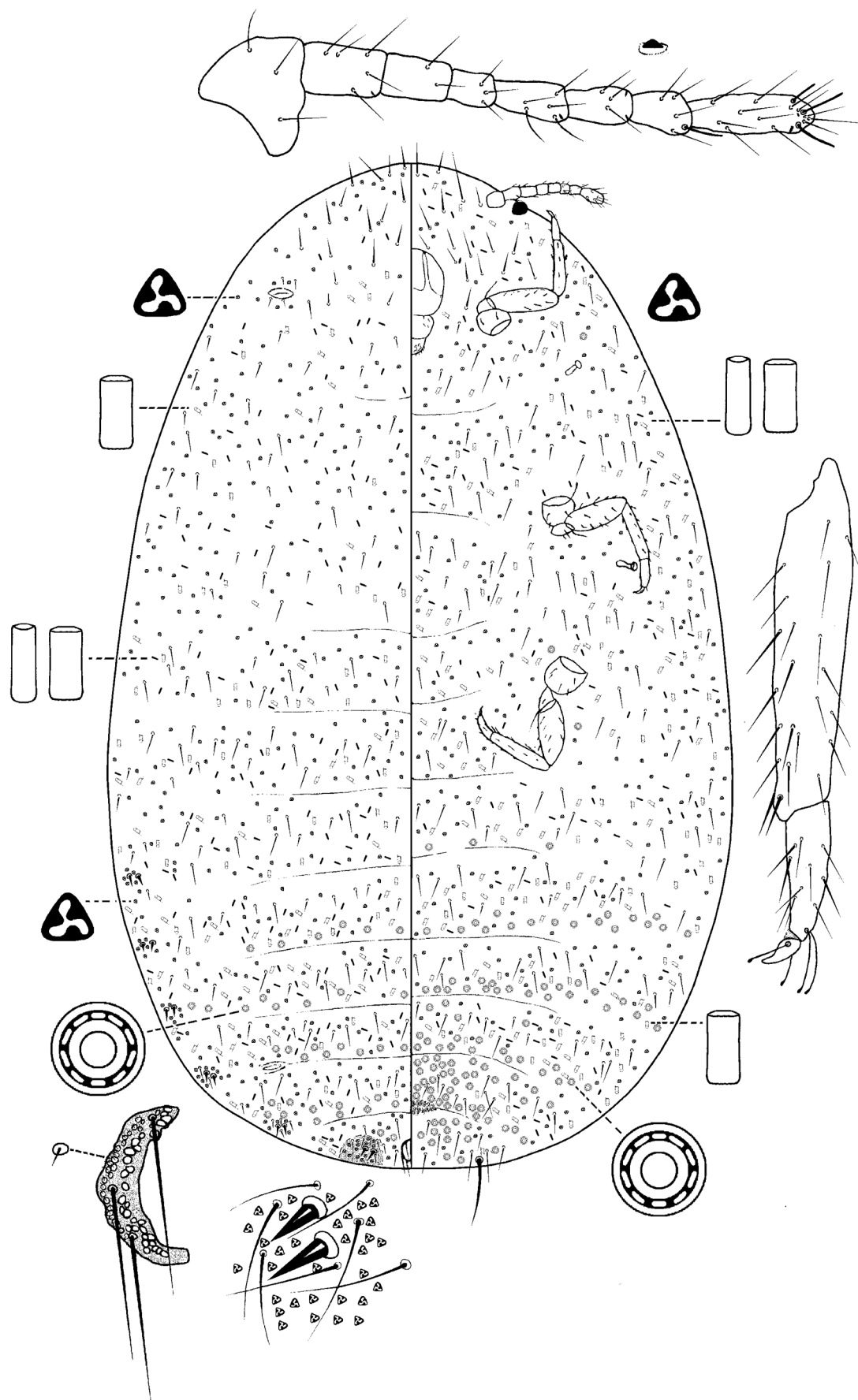


**Fig. 12.** *Trionymus artemisiarum* (Borchsenius) (after TER-GRIGORIAN 1973, with modifications) (Note: (i) oral collar ducts with a sclerotized inner end; (ii) only nine setae on hind tibia; and (iii) two coeloconic sensilla on apical antennal segment, plus one sensillum on segment IV but none on segment II).

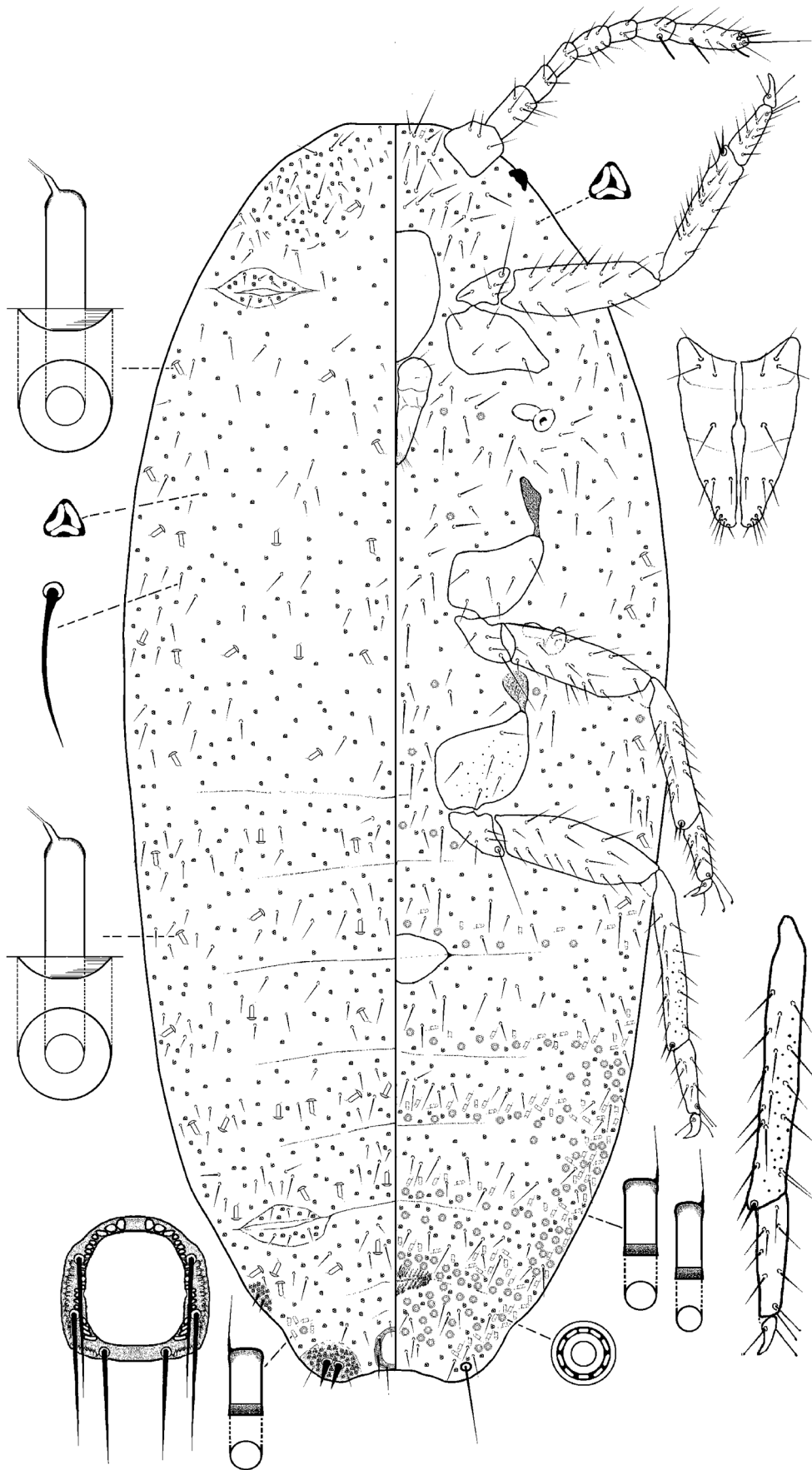




**Fig. 13.** *Trionymus perrisii* (Signoret) (after TER-GRIGORIAN 1973 with modifications) (Note: (i) oral collar ducts lacking a sclerotized inner end; (ii) a total of 12 setae on each hind tibia; and (iii) two coeloconic sensilla on each apical antenna segment, plus one on both segments II and IV).



**Fig. 14.** *Trionymus multivorus* (Kiritchenko) (after TER-GRIGORIAN 1973 with modifications) (Note: (i) oral collar ducts lacking a sclerotized inner end; (ii) a total of 20–22 setae on each hind tibia; and (iii) two coeloconic sensilla on each apical antenna segment, plus one on both segments II and IV).



**Fig. 15.** *Chorizococcus malabadiensis* Kaydan (after KAYDAN et al. 2014) (Note: (i) oral rim ducts on dorsum and venter; (ii) translucent pores present on hind coxa and tibia; and (iii) cerarii restricted to the last two abdominal segments).

course, not be congeneric with *E. amnicola*, the type species of *Euripersia*).

Within the Pseudococcinae, our analysis provides strong support for the tribes Trabutinini (100%), Planococcini (100%) and Pseudococcini (90%). The possible characters diagnosing these tribes are discussed above. Our study did not include any species related to *Ferrisia* or to *Antonina* and *Chaetococcus* and so we can offer no comment on the *Ferrisia* group of the earlier studies or on the Sphaerococcinae. As in the previous studies, there is much non-monophyly in many of the genera, particularly *Phenacoccus* in the Phenacoccinae and *Trionymus* and *Pseudococcus* in the Pseudococcinae.

Very recently, DANZIG & GAVRILOV-ZIMIN (2014) published a new concept of Pseudococcidae phylogeny based on their morphological studies. Their results are very different from those of the present work, particularly in the generic concepts of *Atrococcus*, *Phenacoccus* and *Euripersia* and the relationships between *Chaetococcus* and *Antonina*.

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## Electronic Supplement Files

at <http://www.senckenberg.de/arthropod-systematics> (“Contents”)

**File 1:** kaydan&al-coccoideaphyl-asp2015-electronicsupplement-1.fas. – Combined alignment of 28S and COI gene regions plus morphological characters, with three species of Putoidae, two species of Rhizoecidae and *Acanthococcus aceris* (Acanthococcidae) as outgroup taxa.

**File 2:** kaydan&al-coccoideaphyl-asp2015-electronicsupplement-2.txt. – Alignment (uncleaned) of 28S gene region with outgroup taxa.

**File 3:** kaydan&al-coccoideaphyl-asp2015-electronicsupplement-3.fas. – Alignment (uncleaned) of COI gene region with outgroup taxa.

