Morphological and molecular studies of a new species of the root mealybug genus *Ripersiella* Tinsley (Hemiptera: Coccoidea: Rhizoecidae) from greenhouses in The Netherlands and a first incursion of the American root mealybug *Rhizoecus keysensis* Hambleton in Europe

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*Ripersiella emarai* is described from *Ficus cyathistipula* Warburg, *Ficus lyrata* Wargburg and *Dieffenbachia* sp. from Dutch greenhouses. The immature stages are described based on microscopic features and a key to the stages is provided for their separation. This species description is actuated by the necessity to distinguish the new species from the root mealybug *Ripersiella hibisci* (Kawai & Takagi), which is occasionally imported and shares host plants with the new species. An identification key to adults of the Dutch greenhouse species and species intercepted during import inspections based on microscopic morphological characters is given. A key to distinguish the nymphal stages is provided and keys are given to the identification of first, second and third instar nymphs of *Ripersiella multiporifera* Jansen, *R. hibisci* (Kawai & Takagi) and *R. emarai* Jansen. *Rhizoecus advenoides* Takagi & Kawai, 1971 is found to be a junior synonym of *Rh. amorphophalli* Betrem, 1940.

**Keywords:** Pseudococcidae, Rhizoecidae, *Ripersiella*, new species, The Netherlands, greenhouses

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**Introduction**

In Dutch greenhouses root mealybugs (fam. Rhizoecidae) are a common occurrence on the roots of a large number of bonsai and pot plants. Several factors influence their presence. Because of their hidden habits, it is to be expected that only a relatively small percentage of the world fauna is described yet, and as a result many new species are expected to be discovered in the future. The overview of Kozár & Konczné Benedicty (2007) of the world fauna is an important milestone in the exploration of this species-rich subterranean fauna. Rhizoecidae are continually transported all over the world on rooted plants with soil. The Netherlands is a main port in Europe for imports of plant material originating from different parts of the world. Most of these imported consignments are intensively sprayed, killing the majority of specimens, but a small number of specimens or eggs might survive, giving rise to new population growth. Most of these revived populations...
are discovered much later by accident or might by overlooked by inspectors, which may enhance the risk of unseen spreading to other parts of Europe or in the case of re-export even to other parts of the world. The new species described here, Ripersiella emarai, was only found on Ficus cyathistipula Warburg and was successfully reared on F. lyrata Warburg as well, indicating that this new root mealybug species might be able to propagate on many more host plants.

The morphology of the pre-adult stages was compared with the related Ripersiella multiporifera Jansen (2008) and R. hibisci Kawai & Takagi (1971). The last species has a quarantine status in Europe and is regulated by its presence on the Annex I/AII list of species whose introduction into Europe is prohibited. R. hibisci shares part of its hosts with that of the new species. As a result consignments with plants originating from East Asia are very often heavily treated with pesticides. In most cases only a very small number of the populations living on the roots survive this treatment resulting in the discovery of a very small number of specimens during import interceptions. Therefore pre-adult stages were studied to compare the morphology of related species with the description of those of R. hibisci (Jansen 2001). Furthermore, molecular data of cytochrome oxidase I (COI), 18S small subunit ribosomal RNA (18S rRNA) and 28S large subunit ribosomal RNA (28S rRNA) were generated from R. emarai to aid species identification at the molecular level.

**Material and methods**

Populations of the new species originating from the finding sites in Berkel en Rodenrijs and Bleiswijk were reared on under quarantine conditions for further study on infested Ficus plants in a greenhouse in Wageningen. Nymphs were collected several times to obtain enough material. For microscopic slides, specimens were cleared in 10% KOH, ethanol 70%, and acetic acid, stained with a mixture of lignin pink and acid fuchsin, cleared in clove oil and mounted in Canada balsam. Specimens of the first and second stage tend to fold in antennae and legs when mounted in Canada balsam. Therefore part of the specimens of these stages was mounted in berlese solution. About 150 specimens in total were studied and part was used for measurements. The description of each stage and the measurements are based on multiple slide-mounted specimens only. Measurements are given as ranges. The characters of the holotype fall within the range of the characters as given. The wrongly printed original figures of Ripersiella multiporifera (Jansen 2008) are given here as well. Measurements and drawings were made using an ocular micrometer on an Olympus (JAPAN) BX50 interference contrast microscope.

DNA was isolated from single specimens of R. emarai, R. hibisci, R. multiporifera and Rh. dianthi Green using the Mammalian Tissue protocol of the High Pure PCR Template Preparation kit (Roche, Basel, Switzerland) and stored at −20°C until use. Partial COI using primers PcoF1 5’-CCTTCAACTAATCATATAAATATYG-3’ (Park et al. 2010) and LepR1 5’-TAACTTTCTGGATGTCAAAAATCA-3’ (Park et al. 2011), an 18S region using primers 18S-2880 5’-CTGGTGATCTCTGAGCGAGTGCTGCAATGAGTAGTA-3’ and 18S-B 5’CGGCTGCTGGCACCAGAGTTAG-3’ (von Dohlen & Moran, 1995) and a fragment of the D2 expansion region of 28S using primers 28S-D2F AGAGATCCCAAGAAGCTGTG and 28S-D2R TTGGTGCTGTCTAGATCAG-3’ (Belshaw & Quicke, 1997) were amplified. The PCR reaction components and final concentrations were 2.5 mM MgCl2, 0.1 mM dNTPs, 0.2 μM each primer, 2.5 units Taq DNA polymerase with appropriate buffer (Qiagen, Venlo, The Netherlands), and 2 μl DNA template in a final volume of 25 μl. The PCR cycling condition for COI amplification was 3 min at 94°C; five cycles of 30 s at 94°C, 45 s at 51°C, 1 min at 72°C; forty cycles of 30 s at 94°C, 45 s at 51°C, 1 min at 72°C; 10 min at 72°C. For 18S and 28S the PCR cycling conditions were 3 min at 94°C; forty cycles of 20 s at 94°C, 1 min at 50°C, 1 min at 72°C; 10 min at 72°C. All PCR products were visualized in a 1% agarose gel stained with SYBR Safe (Lifetechnology, Carlsbad, CA, USA) and bidirectionally sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA, USA) on an Applied Biosystems 3500 Genetic Analyzer. Sequences from both strands were assembled, edited if necessary and aligned with MAFFT (Katoh et al. 2002) using Geneious ver. 7.0.6 (Biomatters, Auckland, New Zealand). All sequences were deposited in GenBank under accession numbers KM453213–KM453227.

**Ripersiella emarai** Jansen sp. n.

Figs 1–7


Etymology. The species epithet is named in honour of Atef Emara, the collector of the first specimens of the new species, in acknowledgment of his activities as an experienced phytosanitary field inspector.

Field characters

The body of the species is typical for the Rhizoecini: elongate to broadly oval depending of the age of the specimen, flattened ventrally and convex dorsally. Teneral females are more or less elongate and the hind legs reach the oostioles whereas egg-laying females possess a swollen abdomen and an oval-shaped body, the legs reaching the third or fourth abdominal segment. Normally they are very active, walking around. The body is lightly covered with a white powdery, mealy, wax secretion; legs and antenae are pale brown.

Biology. Like all members of the family Rhizoecidae, the new species was found living subterraneously on the root hairs. Most species of Rhizoecidae tend to live oligophagously or polyphagously on their hosts. The description of the new species is based on specimens found on representatives of two families and two genera (Moraceae: *Ficus cyathistipula*; *F. lyrata*; Araceae: *Dieffenbachia* sp.). During all observations, most developmental stages were present and mixed, suggesting that there is more than one generation per year, which is in accordance with observations on the life cycle of *Ripersiella hibisci* (Jansen 2001). Major factors that influence the duration of the life cycle are the temperature and the host plant. The new species is bisexual with alate males being present in much smaller numbers than females.

Description of adult female

Figs 1, 2

Slide-mounted characters. Measurements based on 12 specimens. The holotype is a teneral adult female. It is elongate-oval with almost parallel sides, length 0.72 mm (antennae and anal setae excluded), width 0.3 mm. Paratypes: adults are 640–1650 μm long and 275–960 μm wide.

Dorsum. Posterior end of body with well developed, slightly projecting anal lobes on a slightly sclerotized plate; each lobe with 7 apical setae of varying length: a group of 4 short setae 12–23 μm long near the edge of the lobe and three setae 65–85 μm long accompanied by a group of 20–25 trilocular pores, mostly only half of them visible. Anal ring 48–53 μm in diameter with 6 setae each 55–68 μm long, with an outer row of 18 cells; up to 14 well-visible spiculae in outer row of cells. Flagellate setae in bands across the segments but absent from intersegmental areas; length marginal setae around the entire body: those around the head up to 28 μm, of the thorax up to 25–30 μm and from the abdomen up to 25 μm. On each abdominal segment usually the longest setae up to 15–20 μm long, the shortest setae 8–10 μm long (including basal socket). Bitubular pores of one type, more or less oval-shaped, up to 6 μm wide and

Fig. 1. *Ripersiella emarai* Jansen, adult female, microscopic view. Photo by Maurice Jansen.

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Fig. 2. *Ripersiella emarai* Jansen, adult female. Left side is dorsal, detail drawings from top to bottom: trilocular pore, tubular duct, circulus and anal ring. Right side is ventral, detail drawings from top to bottom: bitubular pore, trilocular pore, tubular duct and internal female genitalia.
6–7 μm long (including internal part) and 3–3.75 μm high. Position and number of bitubular pores are as follows: head 2 on submargin near eyes and one in posterior median position which is sometimes lacking; prothorax 2 marginal, 2 submedian and 1 median; mesothorax 2 marginal, 1 median; metathorax 2 marginal, 2 submarginal and 1 median; abdominal segments: I: 2 submedian, II: 3 or 4 divided over the segment, III: 2 submedian or 3–4 in total, IV: 2 marginal and 1 median; V: 2 submedian, VI: 2 marginal, 1 median; VII: 2 marginal, 1 median; VIII without ducts. Ostioles well developed with edges weakly sclerotized; anterior pair with the border of both lips beset with up to 4 triloculars and 4 setae; those of posterior pair with up to 6 triloculars and 5 setae. Trilocular pores 2.5–3.5 μm wide, evenly scattered on surface, on abdominal segment I–VII: more than 70, on VIII: 18–29, several between anal lobe setae, the median part anterior of anal ring may possess an anterior row of three triloculars and a posterior row of several triloculars following the edge of the anal ring; sometimes the triloculars are scattered on the whole median surface of the segment, however. Tubular ducts 4–5 μm long, present in small numbers in most abdominal segments, a single one on one or more thoracic segments. Multilocular pores absent.

Venter. Antennae relatively slender, 6-segmented, 148–168 μm long. Length of segments: I (lateral inner side) 28–40 μm, II–IV (medially) 58–63 μm, V (medially) 18–23 μm, VI 43–49 μm. The apical segment has three long and one short sensory falcate setae, segment five has one falcate sensory seta and there is one sensory pore on the second segment. Segments I, II, IV and V have one row of setae, segment III has two rows of setae. Trilocular pores numerous, about 3 μm wide. Bitubular pores of one type present, more or less oval-shaped, each about 6–7 μm wide and 3 μm high. There is one in the frontal margin of the head between the antennae. Thoracic segments: prothorax: 0; mesothorax: 2 submarginal and metathorax: 2 submarginal. Absent on abdominal segment I, II and IV. Present on abdominal segment III: 2 on the margin, V: 2 on the margin, VI: 0–2 on the margin, VII: 2 on the margin, VIII: 2 on the margin. Flagellate setae in bands across the segments but absent from intersegmental areas and in small clear areas in the ventral thoracic region; length longest lateral setae of head 27 μm, of thorax 30 μm, of abdominal segment I–V 20 μm and from last segments 25 μm. Length of setae of the head variable, up to 30 μm, those next to cephalic plate about 25 μm, several setae in interantennal space up to 33 μm; thoracic setae less variable, up to 15 μm long, abdominal setae up to 23 μm long. Multilocular disc pores absent. Width of mesothoracic sternal apophysis 15 μm. Spiracles 30–33 μm long, peritreme 13–15 μm wide. Cephalic plate variably developed, variable in shape; with on each side one seta of about 20 μm. Legs well developed: hind trochanter + femur 125–140 μm long; hind tibia + tarsus 165–185 μm long, hind claw slender, 25–29 μm long with a few short claw digitules of about 18 μm long, in most cases about half this length as a result of damage. Anterior width coxa 55–65 μm; trochanter (measured outside) 40–47 μm long; tibia 83–95 μm long, tarsus 75–88 μm long. Ratio of lengths of hind tibia + tarsus to hind trochanter + femur 1.25–1.32. Lip-like triangular structure posterior to vulva absent. Labium (three segments combined) 67–83 μm long, width of clypeolabral shield 58–78 μm. Circulus with cell-like reticulations at its truncate distal plate, shape of distal half variable from conical to almost parallel, distal and proximal half of same length, width of circulus 30–37 μm at base, height 15–16 μm. Eyes present. Tubular ducts 4–5 μm long, present in small numbers in most abdominal segments, a single one on one or more thoracic segments. Internal genital organ chitinized, length about 38 μm, width 40 μm.

Diagnosis. Ripersiella emarai can be distinguished from female adults of the other members of the genus by the following combination of characters: bitubular pores present, ventral and dorsal claw digitules setose, shorter than claw, multiloculars absent, oral collar tubular ducts present, dorsal multiloculars absent, eyes present, antennae 6-segmented. Adult females can be identified using the key of Kozár & Konczné Benedicty (2007) up to couplet 22 and key out against R. loksae Konczné Benedicty and Kozár. Ripersiella emarai is distinguished from R. loksae by the shorter bitubular ducts which are 9 μm in length in R. loksae and up to 6 μm in length in R. emarai, the shape of the internal genital organ, the cephalic plate which is not visible in R. loksae but present in R. emarai, width of circulus which in R. loksae is 14 μm wide and in R. emarai 30–37 μm wide at base, anal ring of R. loksae with 26 cells in outer row whereas there are 18 cells in the outer row of R. emarai and the antennae of R. loksae which are about 130–140 μm long whereas those of R. emarai are 148–168 μm long.

Adult females of R. emarai can be distinguished from the preceding nymphal stage by the presence of a vulva, tubular ducts, ventral bitubulars and a larger number of bitubulars on the dorsum. Additional characters are the smaller number of triloculars and setae on dorsal abdominal segments and the absence of bitubulars on dorsal abdominal segment VI.

Description of first instar

Figs 3–5

Microscopic characters; 240–445 μm long (antennae and anal setae excluded) and 143–242.5 μm wide. Measurements based on 16 specimens.
Dorsum. Posterior end of body with well-developed membranous, slightly projecting anal lobes; each lobe with 3 apical setae of varying length: 40–44 μm, 35–38 μm and 15–18 μm. One pair of tritubular pores on the margin of abdominal segment I, 5–6 μm wide; bitubular pores absent. Anal ring 23–36 μm in diameter with 6 setae each 31–35 μm long, outer row of cells with spiculae. Each abdominal segment with one row of flagellate setae, which are absent from intersegmental areas. Length of abdominal setae on segment I–VI up to 10 μm, on segment VII–VIII 10–13 μm, anal lobe setae up to 31–38 μm. Trilocular pores 3–3.5 μm wide, numbering on head about 10, the numbers on the abdominal segments are as follows: I: 2 submarginal; II: 4, 2 submarginal and 2 marginal; III: 2–4, 2 submarginal and 0–2 marginal; IV: 2–4 at the edge of submargin and submedian; V: 4 evenly distributed; VI: 4 evenly distributed; VII: 2 submarginal; VIII: 2 submedian. Ostioles membranous, anterior pair often barely perceptible, without related triloculars and setae; anterior lip of posterior pair related with 1 seta and 1 trilocular pore. Tubular ducts and multilocular pores absent.

Venter. Antennae 6-segmented, 75–88 μm long, border between segment II and III not well indicated. Length of the segments: I: (measured laterally at the inner side) 18–25 μm, II–IV: (measured medially) 27–35 μm, V: (measured medially) 8–10 μm, VI: 35–45. Segment I–V have one row of setae. The apical segment has three sensory falcate setae at the distal half of the segment with lengths: 29 μm, 23 μm and 20 μm. Segment five has one falcate sensory seta about 10 μm long and there is one sensory pore (campaniform sensilla) on the second segment. Trilocular pores about 3 μm wide. Bitubular pores absent. Flagellate setae in a single row across the abdominal segments but absent from intersegmental areas and in small clear areas in ventral thoracic regions. Cephalic plate present but very weakly developed and hardly visible. Length of setae on the head, thoracic and abdominal segments up to 12 μm. Multilocular disc pores absent. Width of mesothoracic sternal apophysis 12–17 μm. Posterior spiracles 15–19 long, peritrema 3.8–5 μm wide. Legs well developed: hind trochanter + femur 52.5–67.5 μm long; hind tibia + tarsus 77.5–90 μm long, hind claw slender, 15–18 μm long with thin claw digitules as long as claw. Length trochanter (measured outside) 22–26; anterior width coxa 33–37.5. Ratio of lengths of hind tibia + tarsus to hind trochanter + femur 1.29–1.47. Hind tibia 31–35 μm long, hind tarsus 42–45 μm long. Labium (three segments combined) 40–55 μm long, width of clypeolabral shield 35–45 μm. Circulus at base 13–15 μm wide and 7–10 μm long; height 3–6 μm; 4 cell-like reticulations present at its truncate distal end, shape of distal half variable from conical to almost parallel, distal and proximal half of same length. No variation in the number of circuli was observed. Eyes present. Tubular ducts and internal genital organ absent. Disc pores (Fig. 4) flat, circular with a more or less three-sided structure in the centre, 4–5 μm in diameter, present in five pairs: on the head at the antennal base, on the posterior margin of the prothorax behind the labium, on mesothorax and metathorax anteriorly of the coxa of the leg, between two setae, on the second abdominal segment anteriorly of the circulus.

Diagnosis. The first-instar nymph possesses five pairs of disk pores of a type that seems distinctive of this species and this character has not yet been found in any other mealybug (Fig. 4). Because of their flatness the pores can only observed from above and may be easily missed when observed laterally. The first stage can easily be distinguished from subsequent stages by the presence of a marginal tritubular on the dorsal second abdominal segment and the absence of bitubulars. The number of triloculars on dorsal abdominal segments is mostly less than 10 whereas the number of triloculars in the second stage always is at least 12. The first-instar nymphs of both sexes are indistinguishable; no morphological groups could be distinguished.

Fig. 3. Ripersiella emarai Jansen, first-stage nymph, microscopic view. Photo by Maurice Jansen.
Fig. 4. *Ripersiella emarai* Jansen, first-stage nymph. Left side is dorsal. Right side is ventral.
Description of second-instar female

Fig. 6

Length 450–710 μm (antennae and anal setae excluded) and width 190–348 μm. Measurements based on 11 specimens.

Dorsum. In most abdominal segments several rows of spinules are present. Posterior end of body with well developed slightly sclerotized and projecting anal lobes; each lobe with 3 apical setae of about same length, 45–58 μm. Between anal lobe setae are 3 triloculars. Bitubular pores absent. Anal ring 30–40 μm in diameter with 6 setae each 38–58 μm long, outer row of cells with spiculae, cell structure as adult. Most abdominal segments with one to two irregular rows of flagellate setae, which are absent from intersegmental areas. Length of abdominal setae on segment I–VI up to 10–18 μm, on segment VII–VIII 10–16 μm; anal lobe setae up to 52–60 μm. Trilocular pores 3–3.5 μm wide, numbering on head about 15, evenly distributed on abdominal segments, 8–12 on abdominal segment I–VII and on VIII, 2–3 at the anal lobes and 1 in median position. Ostoioles membranous, without related triloculars and setae. Tubular ducts, disk pores and multilocular pores absent.

Venter. Antennae 6–segmented, 87–112 μm long, border between segment II and III not well indicated. Length of segments. I: (measured laterally at the inner side) 17–25 μm, II–IV: (measured medially) 40–54 μm, V: (measured medially) 10–15 μm, VI: 30–43 μm. Segments I–V have one row of setae. The apical segment has three sensory falcate setae, the longest one almost half way or just below the middle of the segment, length 9–11 μm, two others at the distal half of the segment 8–9 μm long. Segment five has one falcate sensory seta about 4–5 μm long and there is one sensory pore (campaniform sensilla) on the second segment. Trilocular pores about 3–3.5 μm wide. Bitubular pores absent. Flagellate setae in a single row across the abdominal segments but absent from intersegmental areas and in clear areas in ventral head and thoracic regions. Cephalic plate present, length 17–20 μm, width 32–45 μm with two setae at its border. Maximum length of setae on the head 12–22 μm, those of thoracic and abdominal segments up to 15 μm. Multilocular disc pores absent. Posterior spiracles 20–25 long, peritreme 7–8 μm wide. Legs well developed: hind trochanter + femur 76–85 μm long; hind tibia + tarsus 93–103 μm long, hind claw slender, 16–20 μm long with thin claw digitules as long as claw. Length trochanter (measured outside) 25–36 μm; anterior width coxa 33–40 μm. Hind tibia 35–48 μm long, hind tarsus 52–63 μm long. Ratio of lengths of hind tibia + tarsus to hind trochanter + femur 1.20–1.38. Labium (three segments combined) 50–60 μm long, width of clypeolabral shield 46–55 μm. One circulus between third and fourth abdominal segment, width at base 15–23 μm and 13–18 μm long; height 12–13 μm; 9 cell-like reticulations present at its circular truncate distal end which is about 5 μm in diameter, shape of distal half variable from conical to almost parallel, distal and proximal half of same length. Eyes present. Tubular ducts and internal genital organ absent.

Diagnosis. Two forms were found in second instars: one specimen was found with a small number of tubular ducts but no other specimens of this instar possessed these ducts. The gender of the second-instar nymph is based on the assumption that specimens without common ducts are females (see e.g. Gullan 2000, Kondo et al. 2004). One specimen with a low number of ducts on the whole body was observed. A third- and fourth-instar male nymph were observed as well suggesting that males are fewer in number than females. No observations of the pharate third–instar female were made. The male instars are not described.

Description of third-instar female

Fig. 7

Length 570–780 μm (antennae and anal setae excluded) and width 250–365 μm. Measurements based on 8 specimens.

Dorsum. In most abdominal segments several rows of spinules are present. Posterior end of body with
Fig. 6. *Ripersiella emarai* Jansen, second-instar female. Left side is dorsal. Right side is ventral.
Fig. 7. Ripersiella emarai Jansen, third-instar female. Left side is dorsal. Right side is ventral.
well-developed membranous, slightly projecting anal lobes; each lobe with 3 apical setae of about same length, 50–70 μm. Bitubular pores present on the head: 1 frontal in median position between antennae and 2 marginal, occasionally an additional median posterior one; prothorax: 2 on margin and occasionally 1 median; mesothorax: 2 on margin and occasionally 1 in median position; metathorax: 2 marginal, rarely an additional median one; on abdominal segments the numbers are as follows, I: on each side 1 in submarginal/submedian position; II: 0 or 1 marginal and/or 1 median, III: 2 marginal, occasionally an additional marginal one, IV: 0 or 1 median, V: 2 marginal and 1 median, VI: 0 or rarely 1 marginal, VII: 2 marginal and 1 median, VIII: 0. Anal ring 37–43 μm in diameter with 6 setae each 47–53 μm long, outer row of cells with spiculae. All setae flagellate, the abdominal ones evenly distributed but absent from intersegmental areas, those from segment I–VI up to 13 μm, those from segment VII to VIII up to 20 μm long. Thoracic setae up to 14 μm, those on the head up to 18 μm long. Trilocular pores 3–4 μm wide, numbering more than 20 on head, evenly distributed on abdominal segments: I: 16–22; II: 16–22; III: 16–20; IV: 15–19; V: 13–20; VI: 15–21; VII: 10–17; VIII: 8–10, 3 or 4 between each lobe and 2 in anterior position with the preceding segment. Ostoioles membranous, without related triloculars and setae, 30–35 μm. Tubular ducts, disk pores and multilocular pores absent.

Venter. Antennae 6-segmented, 122–140 μm long, border between antennal segment II and III not well indicated. Length of segments: I (measured laterally at the inner side): 25–30 μm, II: 13–17.5, III: 10–12.5, IV (measured medially): 12.5–16 μm, V (measured medially): 15–18 μm, VI: 40–45 μm. Segments I–V possess one row of setae. The apical segment has three sensory falcate setae, the longest one almost halfway or just below the middle of 20–25 μm, two others at the distal half of the segment 16–22 μm long. Interantennal width up to 50 μm. Segment V has one falcate sensory seta about 10 μm long. There is one sensory pore (campaniform sensilla) on segment II. Trilocular pores about 3–4 μm wide. Bitubular pores absent. A double row of longer setae medially of abdominal segment II–VI of 15–18 μm long and the rest of the segments setae up to 10–13 μm long. Flagellate setae across the abdominal segments but absent from intersegmental areas and in clear areas in ventral head and thoracic regions. Cephalic plate present, length 25 μm, width 75 μm with two setae at its border with a length of about 15–30 μm. Maximum length of setae on the head up to 20–25 μm, from thoracic region around coxae up to 11–13 μm. Multilocular disc pores absent. Posterior spiracles 25–27.5 μm long, peritreme 6–9 μm wide. Legs well developed, total length 243 μm

hind leg trochanter + femur 100 μm long, length trochanter (measured at the outside) 35–40 μm, diagonal length femur 50 μm; hind tibia + tarsus 122 μm long, hind tibia 60–63 μm long, hind tarsus 45–50 μm; hind claw slender, 16–19 μm long with thin claw digitules as long as claw. anterior width coxa 33–35 μm. Ratio of lengths of hind tibia + tarsus to hind trochanter + femur 1.07–1.33. Labium (three segments combined) 60–70 μm long, width of clypeolabral shield 62–72.5 μm. One circulus between third and fourth abdominal segment, at base 20–30 μm wide and 15–20 μm long, height 12.5 μm, 8–10 cell-like reticulations present at its circular truncate distal end which is 6–8 μm in diameter, shape of distal half variable from conical to almost parallel, distal and proximal half of same length. Eyes present. Tubular ducts and internal genital organ absent.

Diagnosis. The third nymphal stage is distinguished from the second stage and the adult stage by the number of triloculars on the dorsal abdominal segments. It has twice as many triloculars as the preceding stage and half the number of the adult. In contrast to the second stage bitubulars are present on dorsal abdominal segments I, II, III and V. The adult stage possesses bitubulars on dorsal abdominal segment VI and the numbers are regularly twice the number of the third-instar nymph. The third-instar nymphs of *R. bibisci* and *R. multiporifera* are easily distinguished by the presence of eyes and six-segmented antennae in *R. emarai*.

**Molecular diagnosis**

The COI sequence of *Ripersiella emarai* (Genbank Acc. No. [GBN] KM453213) is 81% identical to *R. bibisci* (GenBank KM453214 and KM453215), 82% to *R. multiporifera* (GenBank KM453216) and 85% to *Rhizoecus dianthi* (GenBank KM453217). This indicates that *R. emarai* can be distinguished from *R. bibisci*, *R. multiporifera* and *Rh. dianthi* on the basis of their COI sequences. Although COI is the DNA barcode for species identification in the animal kingdom (Hebert et al. 2003), no other COI data for the genus *Ripersiella* is present in GenBank or any other public database. Therefore additional sequence data of 18S rRNA and 28S rRNA for *R. emarai* (GenBank KM453218; KM453223), *R. bibisci* (GenBank KM453219; KM453220; KM453224, KM453225), *R. multiporifera* (GenBank KM453221; KM453226) and *Rh. dianthi* (GenBank KM453222; KM453227) were generated and compared to GenBank accessions of *Rh. amorphophalli* (GenBank JQ0855361), *Rb. cacticans* (Hambleton) (GenBank EU188591; EU188485), *Rh. floridanus* (GenBank EU188592; EU188486), *Rh. mayanus* (Hambleton) (GenBank EU188593; EU188487) and *R. gracilis* McKenzie (GenBank AY426074; AY427367). The interspecies variation for 18S rRNA
is rather small as the difference between R. emarai and the other species is only 2 to 5%. The 28S rRNA gene is less conserved and therefore better suited for species identification. For 28S RNA R. emarai has the highest similarity (91%) to Rh. floridanus. In conclusion R. emarai can at least be distinguished molecularly from eight members of Ripersiella and potentially from all other members.

Provisional key to the female nymphal stages of Ripersiella emarai

1. Tritubular ducts present on the first segment of the dorsal abdomen, no bitubular ducts present. Body length 240–445 μm, on each dorsal abdominal segment never more than 4 triloculars ...................... first instar
   – Tritubular ducts absent, bitubular ducts present; on most dorsal abdominal segments much more than 4 triloculars, generally at least 9 ...................... 2

2. Bitubular ducts present on dorsal abdominal segments on the margin of segments IV and VII and absent on the other abdominal segments. Generally 9–12 triloculars on dorsal abdominal segment I–VII and one trilocular on the median of dorsal abdominal segment VIII. Body length 450–710 μm. Ventral bitubulars absent, one frontal bitubular on the head ...................... second instar
   – Bitubulars present at least on dorsal abdominal segments I–III-V–VII. More than 12 triloculars on dorsal abdominal segment I–VII, on VIII at least 2 submedian triloculars and an additional few between anal lobe setae. Ventral bitubulars present or absent .............. 3

3. Ventral bitubulars confined to a frontal one on the head and absent on the rest of the body. Dorsal bitubulars present on abdominal segments I–III–V–VII, generally 2 on the margin and in most cases one in median position. Triloculars on dorsal abdominal segment I–VII generally numbering 14–18, on VIII 2 submedian triloculars near anterior edge with the preceding segment, an additional 2–4 triloculars between anal lobe setae. Body length 570–780 μm. Vulva or internal genital organ absent ...................... third instar
   – Ventral bitubulars present on meso- and metathorax and on most abdominal segments; dorsal bitubulars numbering 3–4 in most dorsal abdominal segments but absent on dorsum of segment VIII. Body length 640–1650 μm. Vulva or internal genital organ present ...................... adult female

Key to first instars

1. Antennae six-segmented. Five ventral pairs of disc pores present on the ventral side at the antennal base, thoracic segments and first abdominal segment. Length of marginal setae up to 15 μm ....... Ripersiella emarai sp. nov.
   – Antennae five-segmented. Disc pores absent. Length of marginal setae up to 50 μm. .... 2

2. Circulus absent. Dorsal setae long; those from thorax up to 40–58 μm long, of head 15–20 μm. Marginal setae on thorax up to (21) 40–58 μm long, on abdomen up to (22) 22–38 μm long ....... Ripersiella hibisci (Kawai & Takagi)
   – Circulus present. Dorsal setae short: those of thorax up to 5–13 μm, of head 13–15 μm long. Marginal setae on thorax up to 23 μm long, those of abdomen up to 20–25 μm (Fig. 8) ...................... Ripersiella multiporifera Jansen

Key to second instars

1. Antennae six-segmented. Circulus and eyes present ............ Ripersiella emarai sp. nov.
   – Antennae five-segmented. Eyes present....................... 2

2. Circulus absent. Dorsal abdominal segment I with 12–16 triloculars .............. Ripersiella hibisci (Kawai & Takagi)
   – Circulus present. Dorsal abdominal segment I with 19–24 triloculars (Fig. 9) .............. Ripersiella multiporifera Jansen

Key to third instars

1. Antennae six-segmented. Eyes present. Circulus present ........ Ripersiella emarai sp. nov.
   – Antennae five-segmented. Eyes absent. Circulus absent or present ...................... 2

2. Circulus absent. Antennal length 250–180 μm. Hind trochanter + femur 115–155 μm long .... Ripersiella hibisci (Kawai & Takagi)
   – Circulus present. Antennal length 130–145 μm. Hind trochanter + femur 105–112 μm long (Fig. 10). Ripersiella multiporifera Jansen

Key to the adult females of subterranean mealybugs of The Netherlands, predominantly found at import interceptions and in greenhouses

1. Bitubular or tritubular ducts absent, common tubular ducts present ...................... 2

2. Bitubular or tritubular ducts present, common tubular ducts present or absent ...................... 3
Fig. 8. *Ripersiella multiporifera* Jansen, first-instar nymph. Left side is dorsal. Right side is ventral.
Fig. 9. *Ripersiella multiporifera* Jansen, second-instar female. Left side is dorsal. Right side is ventral.
Fig. 10. Ripersiella multiporifera Jansen, third-instar female. Left side is dorsal. Right side is ventral.
2. Posterior abdominal segments terminating in a pair of strongly produced sclerotized anal lobes fused at basis. Large translucent pores on the hind tibiae absent. 

3. Bitubular pores present. 

4. Eyes absent. Antennae five-segmented.

5. Multilocular pores present on venter and dorsum; 0–1 circuli.

6. Multilocular pores absent from venter and dorsum. Oral collar tubular ducts absent or present around vulva. Labium 88 μm long.


10. Multilocular pores absent from venter.

11. Median and submedian areas of dorsum with tritubular pores. Labium 60–85 μm long.

12. Length antennae 175–210 μm. Length anal lobe setae 75–95 μm.

13. Labium 60–70 μm long. 

14. Circulus absent, tributular pores of one or three sizes.

15. Tributular pores of two sizes, on dorsum larger than on venter.


Note 1. *Ripersiella planetica* Williams (2004) was intercepted by M. van Merriënboer, phytosanitary inspector in The Netherlands, on *Livistona* plants originating from Sri Lanka on 4 September 2003. This was recorded by Kozár & Konczné Benedicty (2007) but the host plant was not given and it was suggested to be Cactaceae. On 19 December 2013 a population was discovered in a greenhouse at Ede on *Opuntia subulata*, leg. W. den Hartog. Malumphy (2012) recorded the species from Malta, not known to be established, but expected to survive for the immediate future.

Note 2. Specimens of *Rhizoecus keysensis* were found living on the roots of two plants of *Euphorbia neorborescens* Bruyns (Euphorbiaceae) and unidentified Cactaceae in the greenhouse of a grower at Kudelstaart in The Netherlands on 15 November 2007 and 19 February 2008, PPS402661, PPS4026582 and PPS4160263. This is the first discovery of *Rh. keysensis* in The Netherlands. The *Euphorbia* plants originated from the Canary Islands but it is unknown if both plants were already infested during import. A possibility is that one of the plants was infested and the population on the second plant is the spread resulting from the infestation. The population of *Rh. keysensis* was mixed with that of *Ripersiella mexicana* (Hambleton 1979). *Rhizoecus keysensis* is near to *Rh. caucicus* and measurements and study of the observed specimens of *Rh. keysensis* reveals that the labium is 65–85 μm long, length of antennae 120–150 μm long, tibia + tarsus of hind legs 95–118 μm long, the apical antennal segment is 95–118 μm long.
Fig. 11. *Ripersiella multiporifera* Jansen, adult female. Left side is dorsal: detail drawings from top to bottom: trilocular pore, big type bitubular pore, multilocular pore and anal ring. Right side is ventral: detail drawings from top to bottom: trilocular pore, small type bitubular pore; circulus and internal female genitalia.

about 1.5–1.7 times as long as wide, width of anal ring (56) 60–65 μm, anal lobe setae are thin, hair-like and their maximum length is 33–55 μm, anal ring with a double ring of cells, the outer one with (20) 25–28 cells and generally three cells between anterior pair of setae. *Rhizoecus cacticans* on the other hand has a labium of 82–100 μm long, the length of antennae is 170–210 μm, tibia + tarsus of hind legs are 140–195 μm long, the apical antennal segment 2–2.5 times as long as wide, width of anal ring 62–75 μm, anal lobe setae are flagellate and their maximum length 75–95 μm, anal ring with a double ring of cells, the outer one with 25–32 cells and generally 4–6 cells between anterior pair of setae.
Rhizoecus amorphophalli (Hambleton) is the only species occurring in the open in The Netherlands and was once recorded on the roots of Ballota nigra (Jansen 2001). The species was observed by the first author living on the roots of the grass Puccinellia maritima (Huds.) Parl at the sea dike near Koudekerke-Burghsluis on 16 April 2003. Later on high numbers were found living on the roots of the grass Elytrigia maritima by A. Ivens (RUG Groningen) on 5 July 2010, 8 July 2010, 21 July 2010 and 14 July 2011. This population was living in association with the ant Lasius flavus (Fabricius) in the transition zone of a dune towards a salt marsh on the Dutch Wadden Sea island Schiermonnikoog. It has also frequently been found in greenhouses (Jansen 2005). It was intercepted on imported Eugenia plants originating from China on 24 October 2008, leg. W. Zijlstra, PPS4308854, PPS4354991. Only one dead specimen was observed which could not be properly prepared and therefore the plant on which it was found was followed and reared. In June 2010 living specimens were observed on the roots of the plant.

Type material of Rhizoecus advenoides Takagi & Kawai was studied. Takagi & Kawai (1971) summarised the differences between Rh. advenoides and Rh. advenus Beardsley, which were synonymised by Hambleton (1979). Important discriminating characters of Rh. advenoides and Rh. amorphophalli Betrem (1940) are the labium, which is as long as wide, and the tubular ducts, which should miss a pale circular area around its orifice. Rhizoecus amorphophalli on the other hand should have a labium which is longer than wide and the tubular ducts possess a pale circular area around the orifice. These characters actually represent variability within populations of both species. Besides, the number of circuli in both species is variable and may be 1 or 2 and the circulus has a reticulated distal plate (Williams 1985). The number of tritubular pores on the dorsal abdominal segments is between 2 and 7, the shape of the claw digitules is variable and the third antennal segment is not constant within populations of both species. The conclusion is that Rh. advenoides Kawai & Takagi is a junior synonym of Rh. amorphophalli, which represents a rather variable taxon.

Note 5. Rhizoecus nemoralis (Hambleton) is recorded here for the first time for The Netherlands. Specimens were observed by B. Sukul, phytosanitary inspector, on plants of Agave filifera in a greenhouse at Honselersdijk on 19 June 2012. Danzig et al. (2008) presume that Rh. nemoralis and Rh. cyperalis are identical with Rh. dianthi. Only a part of the Rh. nemoralis specimens have tubular ducts, so it is possible that Rh. nemoralis and Rh. dianthi represent the same species.

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