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A new species of *Dysmicoccus* damaging lavender in French Provence (Hemiptera, Sternorrhyncha, Pseudococcidae)

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Résumé

Une nouvelle espèce de *Dysmicoccus* nuisible à la lavande en Provence (France) (Hemiptera, Sternorrhyncha, Pseudococcidae). *Dysmicoccus lavandulae* Germain, Matile-Ferrero & Williams n. sp. est décrite et illustrée. Ses séquences ADN sont présentées. L'espèce vit sur *Lavandula x intermedia* cultivée pour la production d'essence de lavande en Provence. La liste des espèces de pseudococcines vivant sur les lavandes spontanées en France est dressée. Le statut des 2 genres voisins *Trionymus* Berg et *Dysmicoccus* Ferris est discuté.

Abstract

Dysmicoccus lavandulae Germain, Matile-Ferrero & Williams n. sp. is described and illustrated, and its DNA sequences given. The species lives on *Lavandula x intermedia*, the cultivated form of lavender grown in the French region of Provence. In addition the mealybug species recorded in France on indigenous lavender are listed. The status of two related genera, *Trionymus* Berg and *Dysmicoccus* Ferris, is discussed.

Key words: *Dysmicoccus lavandulae*, mealybugs, *Lavandula* species, lavandin fields, south-eastern France

Introduction

The lavender fields in Provence, France, have been handed down through many generations of farmers. Many tourists associate these fields with their fragrance, mainly from the lavender hybrid known as lavandin. Production from these crops generates a turnover of 30 million euros, resulting in 10,000 direct and 20,000 indirect jobs, and ensures the maintenance of nearly 2,000 farms, sometimes in areas of low agricultural potential, such as mountains and dry plateau. Tourism in these regions produces a further \$1.7 billion per year.

Production is mainly in the Drôme Department, in the northern part of Provence. Lavender fields cover approximately 20,000 hectares, with 16,000 hectares planted with lavandin and the other 4,000 hectares with true lavender. About 1,600 hectares of lavandin, and 1,200 hectares of lavender are confined to organic farming.

The genus *Lavandula* (Lamiaceae) contains two main species: true lavender, *Lavandula angustifolia* Mill., and Aspic lavender (*Lavandula latifolia* Medik) which has a much stronger perfume than true lavender. Lavandin (*Lavandula x intermedia*) is a hybrid between *Lavandula angustifolia* and *Lavandula latifolia*, and has been cultivated since the 1930s. Its yield is much higher than that of true lavender. In addition, there are a few other *Lavandula* species not used in lavender production, such as *Lavandula stoechas* L., which is endemic to the

Mediterranean maquis shrubland, and on which several mealybugs (Goux, 1933, 1990a, 1990b) (see list below) and other scale insects belonging to other families have been reported.

Among important enemies of lavender is lavender decline stolbur phytoplasma which causes dieback of both lavender and lavandin, and is caused by bacteria transmitted by *Hyalesthes obsoletus* Signoret, 1865 (Hemiptera, Fulgoroidea, Cixiidae), against which only prophylactic control measures are currently used. Another important pest is a midge, *Resseliella lavandulae* (Barnes, 1953) (Diptera, Cecidomyiidae), the larvae of which develop under the bark and cause dieback.

In the early 1980s, lavandin producers faced a new problem. A mealybug was observed in the Alpes-de-Haute-Provence Department but has spread throughout the south-east, causing 45% loss of lavender essence production [Centre Régionalisé Interprofessionnel d'Expérimentation en Plantes à Parfum Aromatiques et Médicinales, (CRIEPPAM) 1995]. Later, Panis (1999) published some biological field studies, identifying the species as *Dysmicoccus multivorus* (Kiritchenko, 1936). This species is polyphagous and widely distributed in the Palaearctic Region. However, the species described by Panis is broadly-oval, the usual shape for *D. multivorus*. On the other hand, the species under discussion develops on the spike and the flower buds, feeding at the base, and is remarkably elongate, the most elongate mealybug we have seen (except perhaps for some species that live inside the leaf sheaths of grasses). It causes leaf distortion after feeding (Fig. 1). After the lavender has been harvested, any remaining mealybugs move to the lower parts of the stems, sometimes inside the hollow stems or even to the roots. We have not detected adult males but the CRIEPPAM report (1995) states that mating takes place, inferring that males could be present.

A resurgence of the problem since 2009 has led us to study this species more thoroughly. Our morphological and DNA studies have shown that the species is new and the purpose of this paper is to describe it as *Dysmicoccus lavandulae* Germain, Matile-Ferrero & Williams.

Material and methods

The DNA voucher specimens plus other preserved specimens were slide-mounted using the method described in Malausa *et al.* (2011). The illustration (Fig. 2) represents a generalised individual based on several adult females used for the description. The drawing shows the dorsum on the left and the venter on the right with enlargements of important characters around the central drawing. The enlargements are not drawn to scale. Abbreviations of the depositories of specimens are: ANSES, Laboratoire de la Santé des Végétaux, Montpellier, France; BMNH, The Natural History Museum, London, U.K.; MBK Collection, University of Cukurova, Adana, Turkey; MNHN, Muséum national d'Histoire naturelle, Paris, France.

The DNA characterization was performed on 5 specimens of *D. lavandulae*. DNA was extracted using the Qiagen DNEasy Tissue kit, following the manufacturer's recommendations. Cuticles were not destroyed by the extraction process and were kept in ethanol 96%, then slide-mounted (slides labelled as LSV1101200, 1101201, 1101202, 1101203 and 1101204). Three DNA regions were amplified and sequenced. The first one, hereafter referred to as 28S, is a ~760 bp fragment within the ribosomal region 28S-D2. The second, hereafter referred to as ITS2, is a region located between the ribosomal region 5.8S and 28S, covering the entire Internal Transcribed Spacer 2 (around 900 bp). The third one, hereafter referred to as COI, is located in the mitochondrial region of Cytochrome Oxidase Subunit I. For COI, we amplified three overlapping fragments previously used in several molecular studies on Pseudococcidae (Malausa *et al.*, 2011; Park *et al.*, 2011; Abd-Rabou *et al.*, 2012), covering in total ~1050 bp. The sequences of the primers used to amplify each region are provided in Table 1.

PCR were performed using the Qiagen Multiplex PCR kit (QIAGEN, Hilden, Germany), in a reaction volume of 25 µL. The PCR mix was composed of 12.50 µL 2X Qiagen Mastermix, 0.125 µL of each primer (initial concentration of 100 µM) and 10.25 µL of ultrapure water. PCR conditions were as follows: initial denaturation at 95°C for 15 mn; 35 cycles of denaturation at 95°C for 30 s, hybridization between 48°C and 58°C depending on the primers (see table I) for 90 s, elongation for 60 s; and final extension at 72°C for 10 mn. PCR-amplified fragments were analyzed with the QIAxcel Advanced System with QIAxcel DNA Fast Analysis cartridges (QIAGEN). PCR products were sent to Genoscreen (Lille, France) or to Beckman Genomics (Takeley, United Kingdom) for capillary electrophoresis on ABI automatic sequencers (Applied Biosystems, Foster City, CA, USA). PCR products were sequenced on both strands. Consensus sequences and alignments were created manually with Bioedit version 7.01 (Hall, 1999).

TABLE 1. Primer pairs used to amplify the three DNA regions studied and annealing temperature used in the PCR. For COI, three primer pairs were used.

DNA region	Primer sequence (5'–3')	Annealing temperature
28S	F: GAGAGTTMAASAGTACGTGAAAC	58°C
	R: TCGGARGGAACCAGCTACTA	
ITS2	F: CTCGTGACCAAAGAGTCCTG	54°C
	R: TGCTTAAGTTCAGCGGGTAG	
COI	F1: CCTTCAACTAATCATAAAAAATATYAG	48°C
	R1: TAAACTTCTGGATGTCCAAAAAATCA	
	F2: AYAATATAATRATTACWWTWCATGC	48°C
	R2: TTTWCCATTTAAWGTTATTATTC	
	F3: CAACATTTATTTTGATTTTTTGG	48°C
	R3: GCWACWACRTAATAKGTATCATG	



FIGURE 1. Leaf distortion of a young host plant (*Lavandula x intermedia*) caused by the feeding of *D. lavandulae*.

***Dysmicoccus lavandulae* Germain, Matile-Ferrero & Williams n. sp.**
(fig. 2)

Unmounted material. Body of live specimens greyish, devoid of wax with 2 short caudal wax filaments. Purplish blue in alcohol 70° (Fig. 3a), becoming immediately brown in cold KOH and then reddish brown 3 hours later.

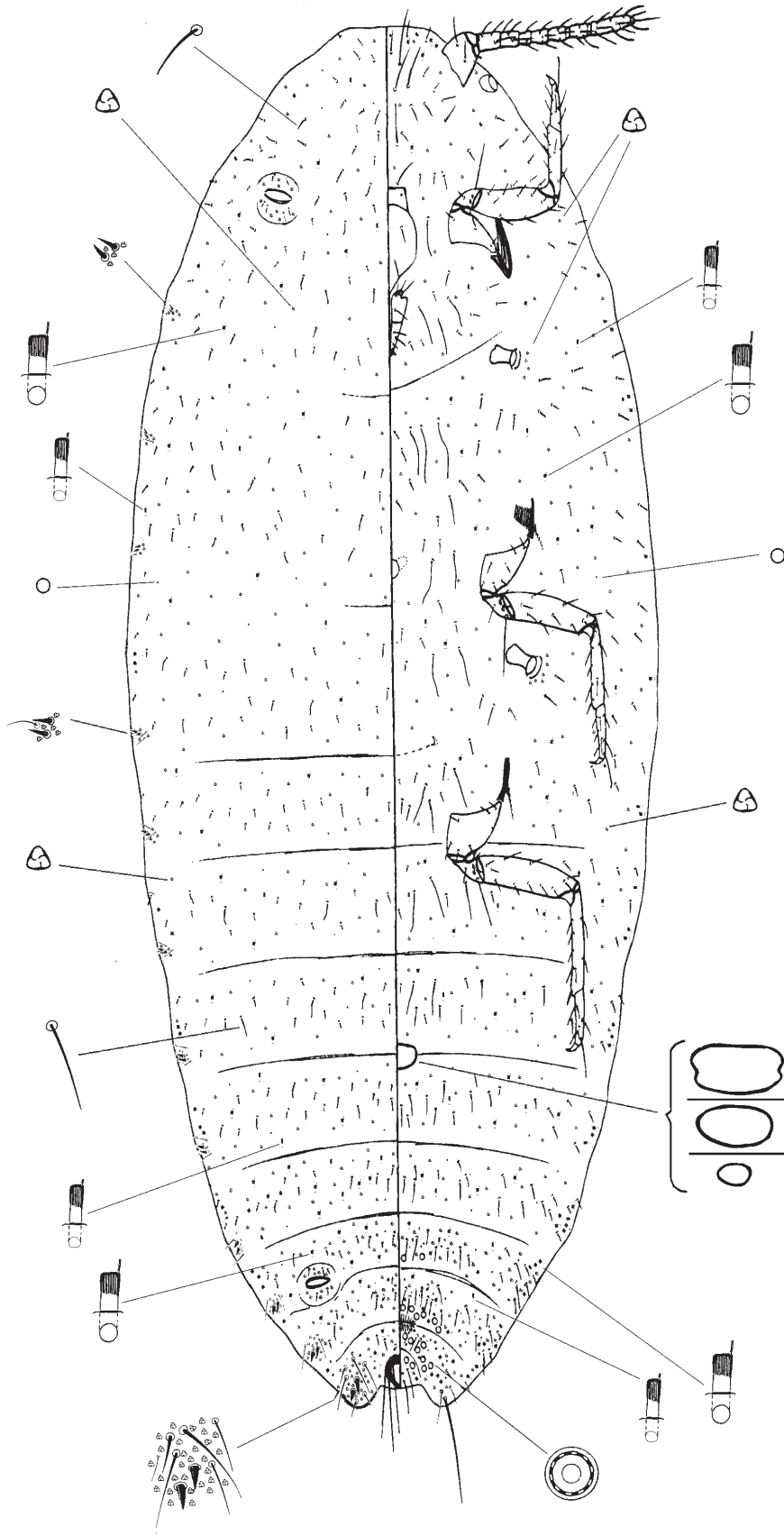
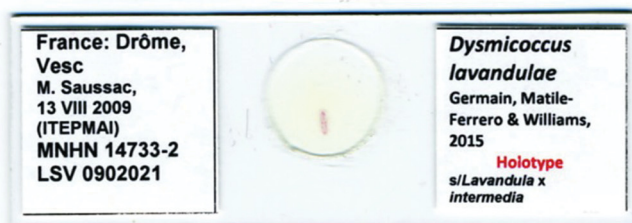


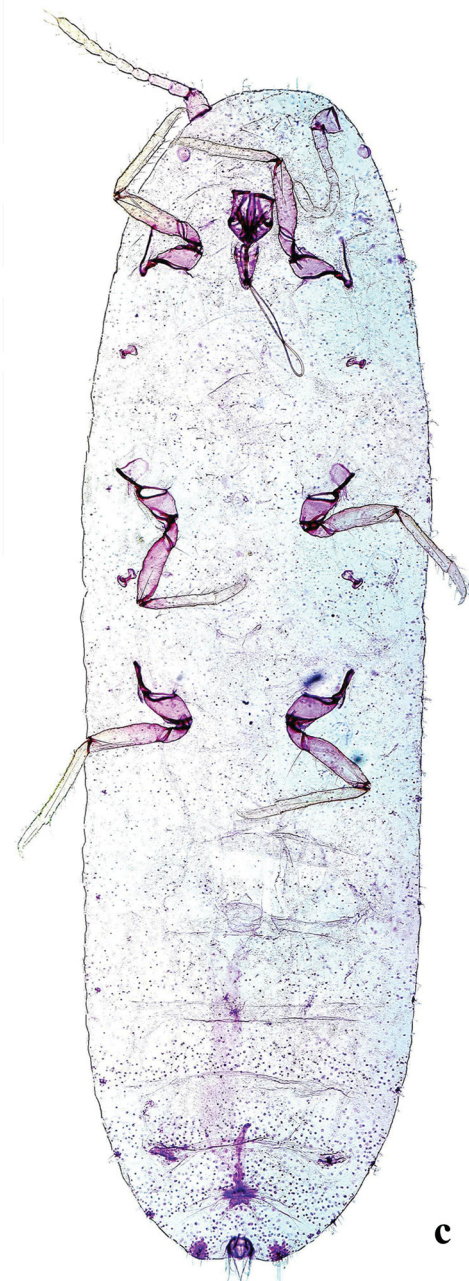
FIGURE 2. *Dysmicoccus lavandulae*, adult female



a



b



c

FIGURE 3. *Dysmicoccus lavandulae*, a, Adult female in alcohol (scale 1 mm), b, slide of the holotype, c, holotype.

Slide-mounted adult female (Fig. 3b and 3c) elongate oval, 1.44–2.96 mm long; anal lobes moderately developed, each with an apical seta 120–180 μm long. Antennae each 350–450 μm long, with 8 segments. Legs well developed; hind trochanter + femur 220–317 μm long, hind tibia + tarsus 280–410 μm long, claw about 2430 μm long. Ratio of lengths of hind tibia to tarsus 2.7. Ratio of lengths of hind tibia + tarsus to hind trochanter + femur 1.29. Translucent pores absent from hind coxa. Labium 85–125 μm long, shorter than clypeo-labral shield. Circulus present, variable in size and shape, elliptical to quadrate, 30–130 μm wide. Ostioles well developed, with inner edges of lips sclerotized, each lip with 3–5 setae and a few trilocular pores. Anal ring about 67–81 μm wide, with 6 setae, each 95–137 μm long. Cerarii numbering 12 (10–13) definite pairs. Anal lobe cerarii each with 2 conical setae, each 19–24 μm long, about 6 auxiliary setae and a group of trilocular pores. Anterior cerarii each with 2 shorter conical setae and 1 or 2 auxiliary setae.

Dorsal surface with slender setae ranging in length from 10 to 29 μm . Multilocular disc pores absent.

Trilocular pores evenly distributed, each about 4 µm wide. Discoidal pores minute, very sparse, each about 2.6 µm wide. Oral collar tubular ducts of 2 sizes, smaller ducts each 2–3.5 µm in diameter, larger ducts 3.3–5 µm in diameter; smaller ducts much more numerous and mainly forming transverse rows on last abdominal segments; larger ducts sparsely distributed among smaller ducts but most numerous on abdominal submargins.

Ventral surface with slender flagellate setae, these becoming shorter towards margins. Multilocular disc pores, each about 6.4–8.7 µm in diameter, present posterior to vulva and on segments VI and VII but never reaching margins. Trilocular pores as on dorsum, evenly distributed. Minute discoidal pores sparse. Oral collar tubular ducts, of 2 sizes, as on dorsum but less numerous.

Material examined. Holotype : adult female, FRANCE, Drôme, Vesc, on *Lavandula x intermedia*, M. Saussac, 13.VIII.2009, MNHN 14733-2, LSV 0902021 (MNHN).

Paratypes: same data as holotype, 3 adult females on 3 slides (Anses-LSV, 1 slide, BMNH 1 slide; MBK 1 slide); Drôme, Dieulefit, on *Lavandula x intermedia*, R. Bonnaure, 26.VI.2010, 12 adult females on 5 slides (Anses-LSV: 2 slides with 5 adult females; BMNH 1 slide with 2 adult females; MBK 1 slide with 2 adult females; MNHN 1 slide with 3 adult females); same data, R. Bonnaure, 13.V.2011, 2 slides with 1 adult female and 6 2nd-instar females) (Anses-LSV); same data, R. Bonnaure, 31.V.2011, 5 adult females on 5 slides (Anses-LSV); same data, R. Bonnaure, 31.V.2011, 6 adult females on 6 slides (Anses-LSV 3 slides; BMNH 1 slide; MBK 1 slide; MNHN 1 slide); same data, R. Bonnaure, 8.VI.2011, 2 adult females on 2 slides (Anses-LSV); Dieulefit, Marroux, on *L. x intermedia*, D. Matile-Ferrero & J.-F. Germain, 16.VI.2011, 1 adult female (MNHN).

Molecular characterization. After the sequence analysis, the consensus sequences obtained for 28S, ITS2 and COI were of 763 bp, 893 bp and 1045 bp, respectively. Intraspecific variation was observed in COI only: two haplotypes differing by two substitutions were observed in two and three specimens, respectively.

The consensus sequences were submitted to the NCBI database under the accession numbers KR340586 (28S), KR340587 (ITS2), KR340584 (COI haplotype 1) and KR340585 (COI haplotype 2).

The sequences obtained for COI and 28S were compared to sequences already available for other *Dysmicoccus* and *Trionymus* species (Kaydan *et al.* 2015): *Dysmicoccus multivorus* (the species most closely related to *D. lavandulae*), *Trionymus aberrans*, *Trionymus perrisi* and *Trionymus artemisiarum*. Because the PCR primers used in this work and those used by Kaydan *et al.* (2015) were different, comparisons could only be made on the 380 bp for COI and 306 bp for 28S DNA regions.

The sequence comparison, after alignment (Fig 4 and 5), reveals the extent of divergence corresponding to interspecific variations in previous studies (Malausa *et al.* 2011; Park *et al.* 2011). At COI, the divergence was >7% between *D. lavandulae* and *D. multivorus* and >9% between *D. lavandulae* and the *Trionymus* species. At 28S, the divergence was >2% between *D. lavandulae* and *D. multivorus* and >8% between *D. lavandulae* and the other *Trionymus* species. From these results, it is concluded that the new species clearly has own lineage and different from all other related species.

Comments. The new species is placed in *Dysmicoccus* Ferris and belongs to a group with a reduced number of cerarii and two sizes of oral collar tubular ducts on the dorsum and venter. This group differs from *Trionymus* Berg, a genus here regarded as restricted to species with only 1–3 pairs of cerarii. In usually possessing 12 (range 10–13) pairs of cerarii, *D. lavandulae* is similar to *D. pietroi* Marotta (Marotta, 1992), a species possessing 13 pairs of cerarii but which also has ventral multilocular disc pores on segment V (absent on this segment in *D. lavandulae*), and noticeably more tubular ducts on both the dorsum and venter. *Dysmicoccus pietroi* was described from Italy, Campania, on unidentified Poaceae and on *Cirsium* sp. (Asteraceae). *Dysmicoccus lavandulae* is also similar to some species presently included in *Trionymus* in ScaleNet, a database of the scale insects (Ben-Dov *et al.* 2015). Pending further research, we believe that *D. lavandulae* is also related to *D. multivorus* (Kiritchenko), described originally as *Pseudococcus multivorus* by Kiritchenko (1936), now a widespread species in Europe (and recorded from France by Panis (1999) and neighbouring parts of Asia. However, *D. multivorus*, as discussed by Ter-Grigorian (1973), Tereznikova (1975) and Danzig (1997), usually possesses only 4–6 pairs of cerarii.

The new species is also similar to *Trionymus angustifrons* Hall, described from Egypt on *Ambrosia maritima* (Asteraceae) by Hall (1926), and recorded from south-eastern France, by Goux (1941). Specimens are at hand prepared by Goux (MNHN), labelled Marseille, La Madrague-de-Montredon, on Compositae, 19.II.1939, coll. L. Goux. The species has also been recorded from Saudi Arabia on *Rhazya stricta* (Apocynaceae) at Rhyadh, and on *Tamarix aphylla* (Tamaricaceae) from Jizan by Matile-Ferrero (1988). In addition, it has also been recorded from Israel, on *Carthamus glauca* (Asteraceae) from Rehovot and on *Echinops viscosus* (Asteraceae) from Qazrin by

Ben-Dov (1991). *Trionymus lanatus* (Balachowsky), described from France, Port-Cros Island on *Kentrophyllum lanatum* (Asteraceae) by Balachowsky (1932), also possesses 5 or 6 pairs of cerarii and belongs to the same group. It is hoped to study the status of these species more in the near future.

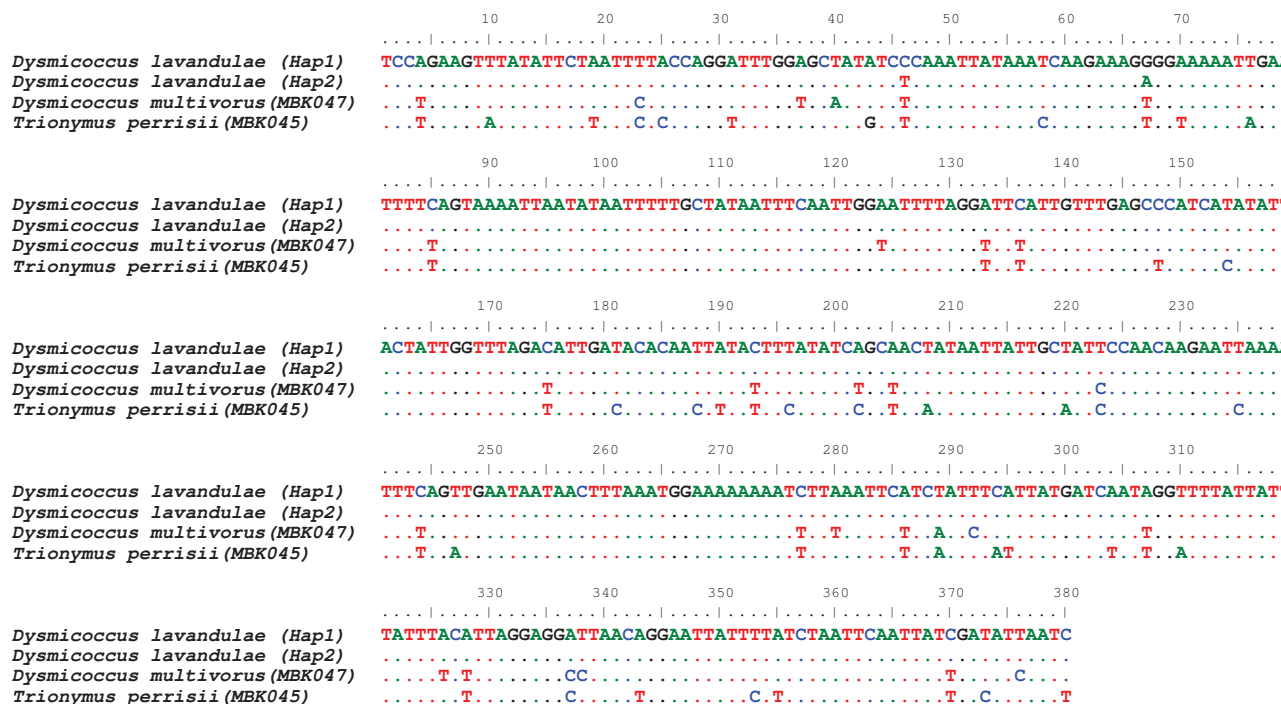


FIGURE 4. Alignment of the COI DNA sequences obtained for *D. lavandulae* and already available for other *Dysmicoccus* and *Trionymus* species. *D. lavandulae* is used as reference sequence in the alignment and only the differences to this reference are displayed in the sequences of the other species. The voucher code for the previously available sequences (Kaydan *et al.* 2015) are given in brackets.

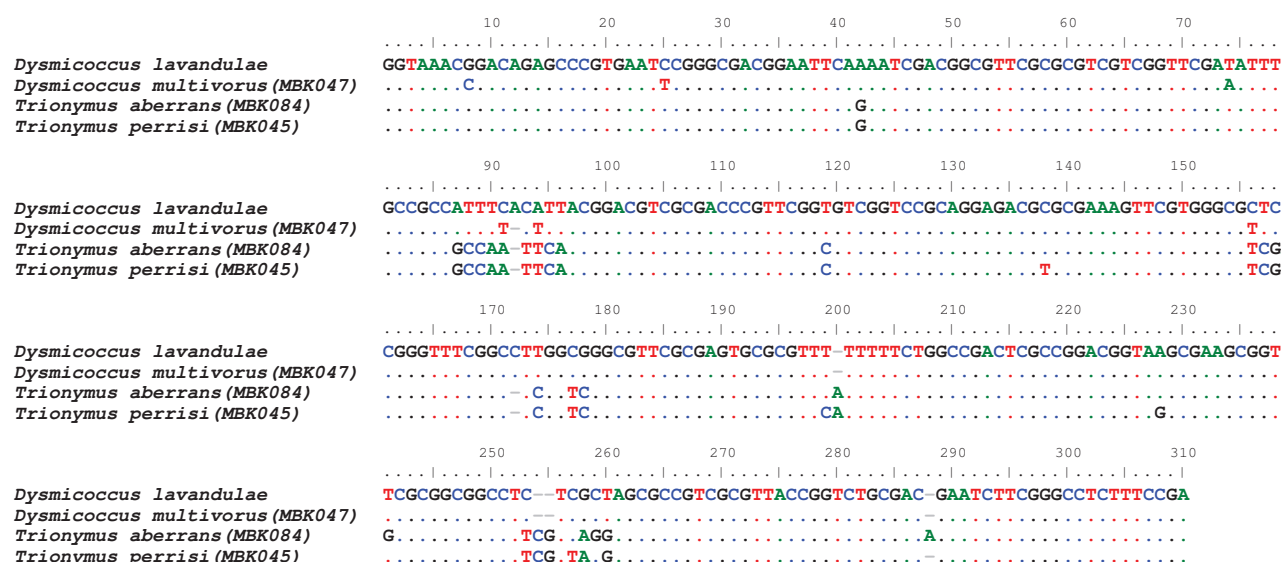


FIGURE 5. Alignment of the 28S DNA sequences obtained for *D. lavandulae* and already available for other *Dysmicoccus* and *Trionymus* species. *D. lavandulae* is used as reference sequence in the alignment and only the differences to this reference are displayed in the sequences of the other species. The voucher code for the previously available sequences (Kaydan *et al.* 2015) are given in brackets.

We have not been able to discuss the material that Panis (1999) examined but the specimens that he identified as *Dysmicoccus multivorus* collected on lavandin are normally broadly oval. The species that we have described here is considered to be different as it is usually extremely elongate, possibly because it lives mainly in the flower buds.

Mealybugs known from France (including Corsica) recorded on native lavender (*Lavandula stoechas*, *L. spica*):

Amonostherium rorismarinis (Boyer de Fonscolombe, 1834), on *Lavandula* sp. (wild), Pyrénées-Orientales, Banyuls-sur-Mer, cap du Troc, M. Canard, 18/V/1964 (MNHN), **new record**.

Atrococcus labiatarum Goux, 1941, on *Lavandula spica*, Hautes-Alpes, Colmars, L. Goux, 19.VII.1939 (MNHN), **new record**

Peliococcus lavandulae (Signoret, 1875), on *Lavandula stoechas* (roots), Alpes-Maritimes, massif de l'Estérel, route forestière des Trois Termes (near Cannes), Y. Ben-Dov & D. Matile-Ferrero, 2.V.1995 (MNHN) (Ben-Dov & Matile-Ferrero, 1995).

Peliococcus ocaanae Goux, 1990, on *Lavandula stoechas*, Corse-du-Sud, Ocana, gorges du Prunelli, L. Goux, 23.VIII.1951 (type material, MNHN) (Goux, 1990a). This name is treated as a synonym of *Seyneria porticcia* Goux by Danzig & Gavrillov-Zimin (2014) but Kaydan (2015) regarded it as a distinct species.

Seyneria gassina Goux, 1990, on *L. stoechas*, Var, Gassin, La Croix, Sylvabelle, L. Goux, 6-7.VI.1933 (type material, MNHN) (Goux, 1990b). This name is treated as a synonym of *Seyneria porticcia* Goux by Danzig & Gavrillov-Zimin (2014).

Seyneria porticcia Goux, 1990, on *L. stoechas*, Var, Le Lavandou, A.S. Balachowsky, 1.IX.1933 (MNHN), **new record**.

Seyneria seynensis Goux, 1990, on *L. stoechas*, Var, La Seyne-sur-Mer, L. Goux, 9.VIII.1930 (MNHN) (Goux, 1990b).

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