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Phyllocoptes cacolyptae (Acari: Trombidiformes: Eriophyoidea) a new species from *Eucalyptus* spp. in Italy

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Abstract

Specimens of an unknown species of *Phyllocoptes* (Eriophyidae Phyllocoptinae) were collected from the shoots of three *Eucalyptus* species (Myrtaceae) in North-Western Italy. The mite, herein described as *Phyllocoptes cacolyptae* n. sp., was associated with the death of apical buds and the development of rearranged and deformed shoots causing yield loss in the quantity and quality of green cut foliage. The damage appeared to be more severe on *Eucalyptus pulverulenta* Sims cultivar "Baby Blue", than on *Eucalyptus cinerea* F. Muell. ex Benth. and *Eucalyptus ovata* Labill. The mite was found also in the Department of Var (France). It is the first record of an eriophyoid mite on gum trees in the European and Mediterranean basin. Remarks are provided on the eriophyoid mites currently associated to *Eucalyptus* spp.

Key words: Eriophyoidea, Phyllocoptinae, gum trees, eucalypt, witches' brooms, cut foliage.

Introduction

In September 2013, plants of *Eucalyptus cinerea* F. Muell. ex Benth. and *Eucalyptus pulverulenta* Sims, cultivar Baby Blue (Family Myrtaceae), from the countryside of Imperia and Albenga (Liguria District, Italy), respectively, showed an irregular growth of shoots associated with the death of apical buds. Eriophyoid mites were found on the surface of the leaves, buds and altered shoots suggesting their involvement with the observed symptoms and damages.

More than 1,000 valid species of *Eucalyptus*, mostly native to Australia, are reported in the literature (The Plant List, 2010). Many have been introduced into other countries such as Italy where they are grown as ornamental and windbreak plants as well as for other industrial purposes (Laudonia and Sasso, 2012; Inghilesi *et al.*, 2013). Some species such as *E. cinerea*, *E. pulverulenta* cv. Baby Blue and *Eucalyptus ovata* Labill. are cultivated mainly for their cut foliage which is sold in fresh and dried bunches of branches for floral compositions.

As in the case of insects (Inghilesi *et al.*, 2013), despite the popularity of growing *Eucalyptus* spp. in Europe, the community of mites hosted by these trees is poorly investigated outside their native biogeographic regions. Until now, no eriophyoid mites had been reported on *Eucalyptus* spp. or other Myrtaceae species in Europe. For the Palaearctic region, only *Aculops jambosae* Kuang has been described from clove tree (*Syzygium aromaticum* (L.) Merrill & L.M. Perry [host originally listed as *Jambosa caryophyllus* (Thunb.) Nied.)] in Lanzhou City, Gansu (China) (Kuang, 1989).

The current study was stimulated by the researchers of the Regional Institute for Floriculture. They followed the mite infestation on the foliage of the main species of *Eucalyptus* cultivated in the Liguria District and assessed it was becoming heavier during 2014-2015, with a major economic impact on the green cut foliage yield (Martini, unpublished data). Hence, further investigations about the taxonomic identity of the mite were urgently needed. It was determined that the mite species is new to science and also the first record of a member of the superfamily Eriophyoidea from Europe and the Mediterranean basin on gum trees (Myrtaceae), requiring it to be further characterized and described.

Materials and methods

Samples of *Eucalyptus* foliage were collected in the countryside of Imperia and Albenga during 2013 and 2014 and later examined in the laboratory. Eriophyoid mites were recovered from plant material directly under a dissecting microscope or following the extraction method of Monfreda *et al.* (2007), clarified and slide mounted in Keifer's solution according to the protocol reported in Nuzzaci *et al.* (1991).

The terminology and setal notation in the morphological description of the mite follow mainly that of Lindquist (1996). The number of measured specimens (n) is given within parentheses in the description. All morphological measurements were taken with the aid of a phase contrast microscope, Olympus BX50 (Shinjuku, Tokyo, Japan), according to Amrine and Manson (1996) as modified by de Lillo *et al.* (2010), and are given in micrometres. Slight clarification should be added as follows: ventral semiannuli were counted from the first entire annulus at the back of the prodorsal shield; coxigenital semiannuli were counted medially from the

coxal region to the anterior margin of the external genitalia and were not included in the ventral semiannuli count; measurements were rounded off to the nearest integer, referring to the length of morphological traits unless otherwise specified. Range values are given in parentheses. Drawings were hand-drawn through a camera lucida according to de Lillo *et al.* (2010). Drawing abbreviations follow Amrine *et al.* (2003). The generic classification follows Amrine *et al.* (2003) and comparisons were also made with new genera described since that publication. Host plant names and their synonymies are in accordance with The Plant List (2010) on-line database. The holotype and the paratype slides of the new species are deposited in the collection of the Department of Soil, Plant and Food Sciences (Di.S.S.P.A.), section of Entomology and Zoology, University of Bari Aldo Moro, Bari, Italy; two paratypes slides of the news species are deposited in the collection of the Research Centre for Agrobiology and Pedology (CREA-ABP), Florence, Italy.

External morphology was also studied using a scanning electron microscope (SEM) TM3000 Tabletop Scanning Electron Microscope (Hitachi ltd., Tokyo, Japan). A few live specimens were treated according to the method of Nuzzaci and Vovlas (1976), they were not coated and the specimen holders were transferred on a cold trap under SEM. The mites were cryo-fixed and, then, observed at 5 kV of accelerating voltage.

Phyllocoptes cacolyptae **Valenzano, Martini, Simoni et de Lillo (figures 1-2)**

Description

F e m a l e (n = 1 0)

Body fusiform, 169 (133-192, including gnathosoma), 44 (44-50) wide. Gnathosoma 21 (20-27) projecting obliquely downwards, chelicerae 22 (16-22), setae *d* 7 (6-7), unbranched, setae *ep* 2 (no range detected). Prodorsal shield 35 (35-42) excluding frontal lobe, 42 (38-45) wide, broad sub-triangular in anterior shape with a long and distally narrow frontal lobe 14 (13-14) over gnathosomal base. Shield pattern composed of a short, weak median line on posterior half and starting further back from rear margin, almost complete admedian lines interrupted around their middle part and starting further back from rear margin, complete longitudinal submedian lines converging in middle, two pairs of arched and transverse submedian lines extending jointed from the postero-lateral corners of the prodorsal shield up to the converging point of the longitudinal submedian lines; three pairs of short lines connect the anterior transverse submedian line to the lateral margin of the prodorsal shield. Tubercles *sc* ahead the rear shield margin, subcylindrical with their bases longitudinal aligned, 20 (19-21) apart, setae *sc* 5 (5-6), directing upward and mesally. Leg I 35 (30-37), femur 12 (10-12), genu 5 (5-6), tibia 9 (9-11), tarsus 7 (6-8), ω 8 (8-10) distally thin, empodium simple, 7 (6-7), 5-rayed; setae *bv* 17 (13-17), setae *l''* 25 (16-28), setae *l'* 9 (7-10), setae *ft'* 11 (11-24), setae *ft''* 15 (15-20). Leg II 33 (27-33), femur 13 (11-13), genu 4 (4-5), tibia 8 (7-8), tarsus 7 (6-7), ω 9 (9-10) distally thin, empodium simple, 6 (6-7), 5-rayed; setae *bv* 13 (13-16), setae *l''* 15 (10-15), setae *ft'* 12 (11-15), setae *ft''* 18 (18-23). Coxae with small and numerous granules; setae *1b* 11 (8-13), tubercles *1b* 13 (11-13) apart, setae *1a* 12 (10-17), tubercles *1a* 10 (8-10) apart, setae *2a* 16 (16-27), tubercles *2a* 25 (22-25) apart. Prosternal apodeme 7 (6-8). Opisthosoma dorsally arched, with 41 (41-43) broad dorsal semiannuli, 46 (46-54) narrow ventral semiannuli (counted from first annulus after coxae II) and 6 (no range detected) semiannuli between coxae and genital coverflap. Dorsal semiannuli smooth. Ventral semiannuli with circular microtubercles on the posterior margin; last 5 (5-6) annuli with elongated and linear microtubercles. Setae *c2* 19 (19-35) on ventral semiannulus 6 (5-6), setae *d* 18 (18-25) on ventral semiannulus 17 (17-20); setae *e* 18 (16-29) on ventral semiannulus 29 (29-35); setae *f* 23 (23-33) on ventral semiannulus 41 (41-49); 5 (no range detected) annuli after setae *f*. Setae *h2* 37 (36-67) very thin at the apex, *h1* absent. Genital coverflap 10 (10-12), 19 (19-21) wide, smooth; setae *3a* 15 (13-25), 13 (12-14) apart. All long setae of coxae and opisthosoma distally very thin and often apparently broken in this last part.

M a l e (n = 8)

Body fusiform, 135-173. Prodorsal shield 36-39 plus 12-13 of the narrow anterior lobe over cheliceral base. Tubercles *sc* ahead of rear shield margin, 18-19 apart, setae *sc* 4-6 directed up and mesally. Opisthosoma with 35-42 broad and smooth dorsal semiannuli, 44-52 narrow and microtuberculate ventral semiannuli.

Type host plant

Eucalyptus cinerea F. Muell. ex Benth. (Myrtaceae), commonly known as Argyle Apple, Mealy Stringbark, Silver-leaf Stringybark or Silver Dollar Tree.

Type locality

Fossati N. farm, Taggia (Imperia) (43°50'53.39"N 7°50'52.28"E), Italy, 51 m above sea level, 5th September 2014, leg. P. Martini; sampling code 149N/1.

Type material

Holotype: a female on a microscope slide, code EC14/1, marked by a circle among 5 females. Paratypes: 25 females, 19 males and 1 nymph mounted on 10 microscope slides (code from EP14/1 to EP14/7).

Etymology

The species epithet, *cacolyptae*, comes from *κακός* = *bad*, referring to the associated negative effects on the gum trees and *καλύπτειν* = *to cover* (from the *eucalyptus* etymology, i.e., *ευ* (*eu*) = *well* and *καλύπτειν* (*kalypto*) = *to cover*).

Other host plants, localities and type material

Paratypes: 18 females and 14 males mounted on 7 microscope slides (code from ES14/1 to ES14/7), prepared from samples of *Eucalyptus ovata* Labill. (Swamp Gum or Black Gum), collected at the Brogi P. farm, Riva Ligure (Imperia), Italy (43°50'39.33"N 7°52'04.56"E), 155 m above sea level, on 5th September 2014, leg. P. Martini,

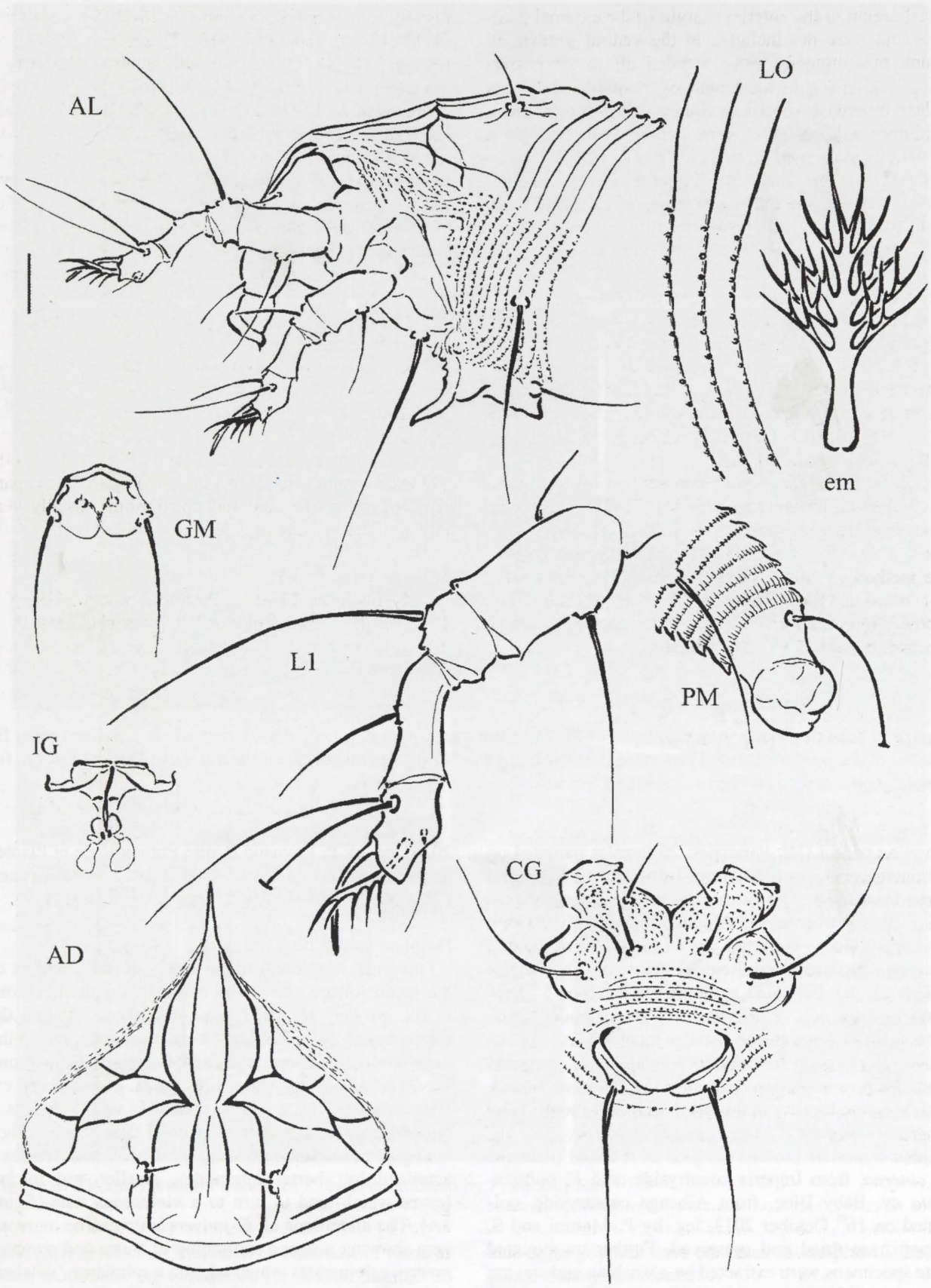

Figure 1. Schematic drawings of *Phyllocoptes cacolyptae* n. sp.: AL. Lateral view of anterior body region; AD. Prodorsal shield; CG. Female coxigenital region; em. Empodium; GM. Male genital region; IG. Internal female genitalia; LO. Lateral view of annuli; L1. Leg I; PM. Lateral view of posterior opisthosoma. Scale bar: 10 μm for AD, AL, CG, GM, IG, PM; 4 μm for LO, L1; 1.5 μm for em.

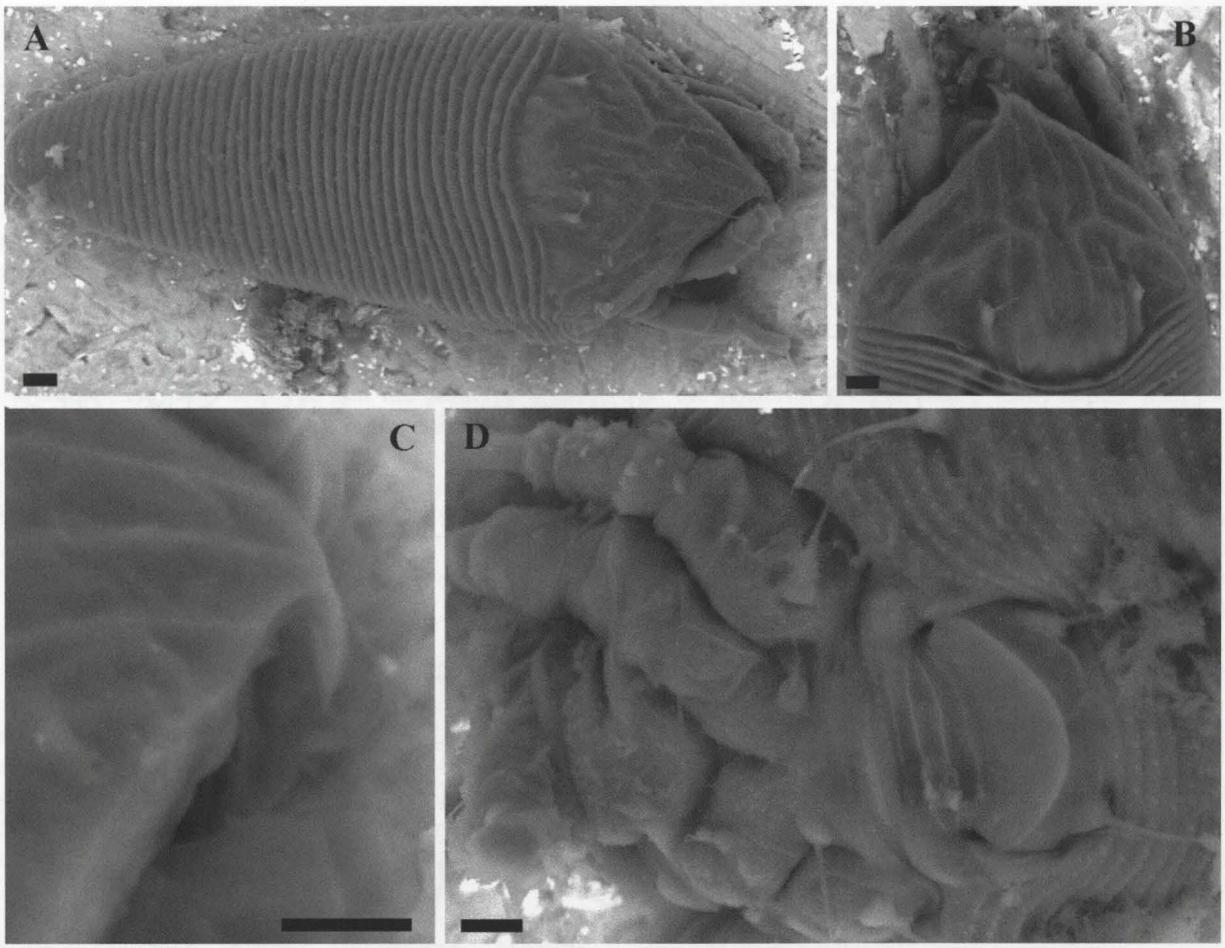

Figure 2. Scanning electron micrographs of *Phyllocoptes cacolyptae* n. sp.: A) dorsal view of an individual mite; B) detail of the prodorsal shield pattern in dorsal view; C) detail of the frontal lobe in lateral view; D) detail of the female genital and coxal region. Scale bars = 5 μm.

sampling code 122N. Paratypes: 20 females mounted on 7 microscope slides (code from EP14/1 to EP14/7) prepared from samples of *Eucalyptus pulverulenta* Sims cv. Baby Blue (Silver-leaved Mountain Gum, Silver Gum, Powdered Gum, collected at Fazio G. farm, Loano (Savona), frazione Verzi, Regione Prione (44°09'33.53"N 8°13'57.57"E), Italy, 231 m above sea level, on 5th September 2014, leg. P. Martini; sampling code 121N. Paratypes: 15 females and 9 males mounted on 9 microscope slides (code from EC13/1 to EC13/9), prepared from samples of *E. cinerea*, collected at Imperia countryside (same locality of the holotype), Italy, on 16th October 2013, leg. by P. Martini and S. Rapetti.

Other material: Further samples of infested plants of *E. cinerea*, from Imperia countryside, and *E. pulverulenta* cv. Baby Blue, from Albenga countryside, collected on 16th October 2013, leg. by P. Martini and S. Rapetti, are dried and preserved. Further un-mounted mite specimens were extracted by a washing and sieving procedure (Monfreda *et al.*, 2007) from infested plants of *E. cinerea* and *E. ovata*, collected at Taggia (Imperia), on 6th October 2014, leg. by P. Martini, preserved in Oudemans' fluid (Krantz and Walter, 2009).

Further records: This mite species was also identified in samples of *E. cinerea* collected from the city of Tanneron, Var Department, France, on 29th September 2014, leg. by P. Raynaud (Unité Entomologie et Plantes invasives, CBGP - Campus International de Baillarguet, CS 30016, 34988 Montferrier-sur-lez, France).

Relation to the host plant

This mite was found living freely on the surfaces of the green foliage and it was associated with alterations of the growing shoots. It was clearly evident that the meristems of the apical buds were damaged, causing the development of lateral buds and dwarfing of the young leaves or dieback of shoot tips. On *E. pulverulenta* cv. Baby Blue, tip death can be relatively weak whereas it can be observed a higher number of new shoots, originating from the same growing location, which are characterized by shorter internodes, smaller and thicker leaves which tend to turn to a violaceous tone (figure 3A). The alterations on *E. pulverulenta* can be more severe showing a strong shortening of stems and compact green agglomerates which take on a miniature 'witches' broom-like' appearance (figure 3B). On *E. cinerea*, the mite is associated to similar damaging effects as described for *E. pulverulenta* (figure 4A-B) which are often followed by the drying of main shoots or with the death of apical buds (figure 4C-D). The alterations were much heavier on *E. pulverulenta* cv. Baby Blue than on the other species surveyed. The damaged shoots, even

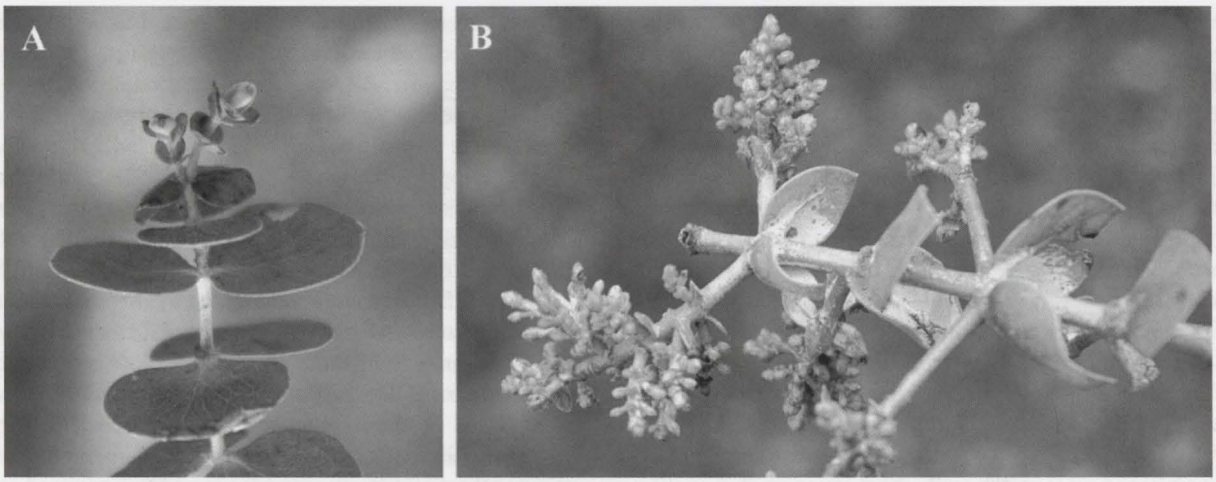

Figure 3. Alterations on the growth of shoots of *E. pulverulenta* cv. Baby Blue infested by *P. cacolyptae*. The light blue wax is a typical and own secretion of the young leaves of the cultivar.

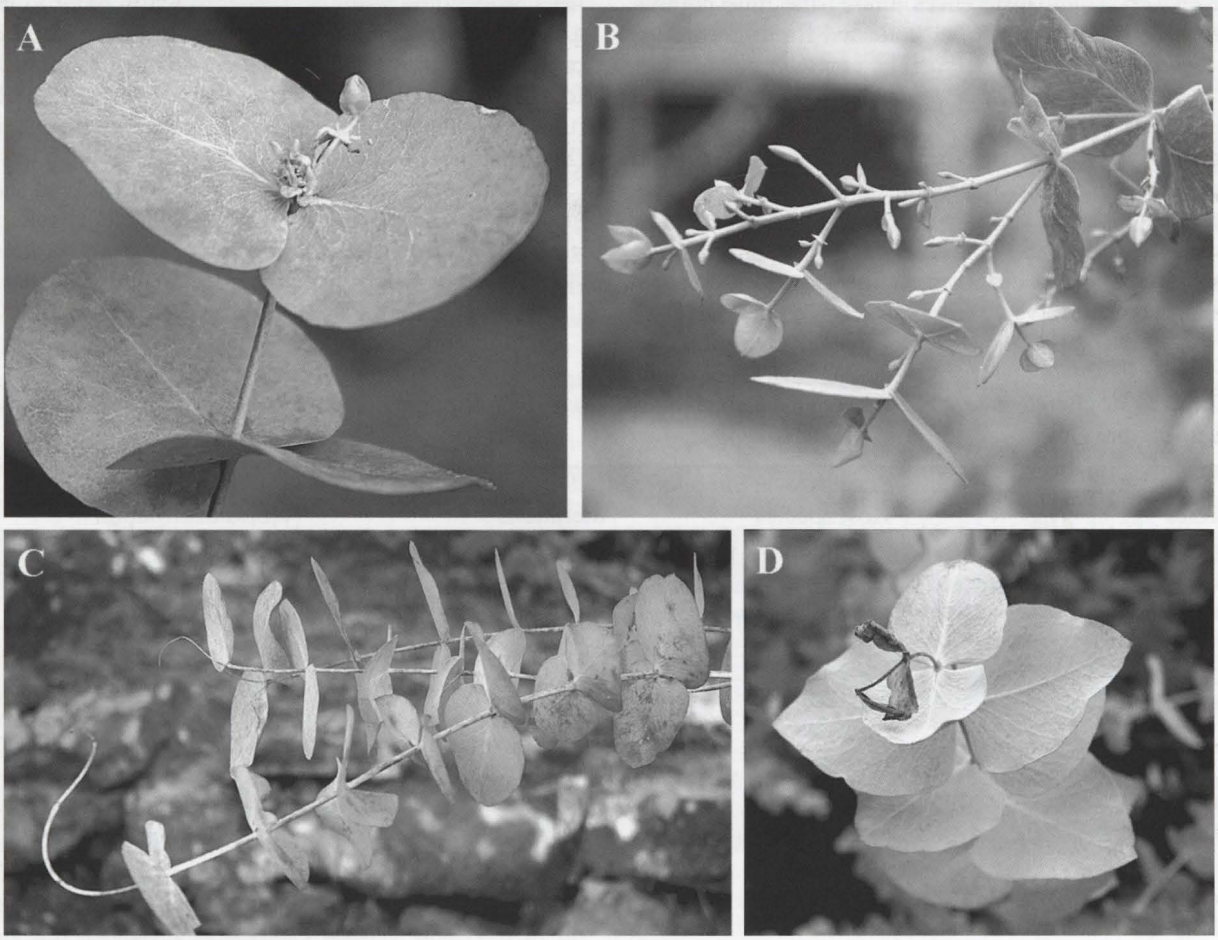

Figure 4. Alterations on the shoots of *E. cinerea* infested by *P. cacolyptae*.

upon early infestation, can have no commercial value as much more handling is required for discharging the affected green parts during the selection and preparation of commercial bunches. No regular and complete collection of data on the economic impact of the mite has been done up to know. Interviewed growers estimate a commercial loss up to 20% for *E. cinerea*, whereas in the case of heavy infestation, the whole production can be lost in *E. pulverulenta* cv. Baby Blue. In addition, also some psyllids were observed in samples of the affected *Eucalyptus* plants.

Differential diagnosis

The genus *Phyllocoptes* includes around 200 species. None of them have been collected from *Eucalyptus* trees and two of them have been recorded on other Myrtaceae, but they do not resemble the new species here described. It was compared with all currently known *Phyl-*

locoptes species and *Phyllocoptes nageli* Keifer, a needle vagrant species collected on *Pseudotsuga menziesii* (Mirb.) Franco (Pinaceae) in Oregon, USA (Keifer, 1965b), appeared to be morphologically closest to *P. cacolyptae*. Both species have a narrow frontal lobe, an articulated prodorsal shield pattern and a coxal area which is finely granulated. They differ mainly in: the prodorsal shield line arrangement (long median line and more numerous transverse lines between the admedian lines in *P. nageli* whereas the median line is short and weak with no transverse lines between the median lines in *P. cacolyptae*); the rays of the empodia (6-rayed in *P. nageli* versus 5-rayed in *P. cacolyptae*); the size of the dorsal semiannuli (wider in *P. cacolyptae* than in *P. nageli*) the coverflap ornamentation (smooth in *P. cacolyptae* versus a coverflap with short striae in *P. nageli*).

Remarks

The screening of morphological characteristics displayed very slight differences among the mite populations of *P. cacolyptae* sp. n. collected from *E. cinerea*, *E. ovata* and *E. pulverulenta*. The empodia were longer in specimens from *E. pulverulenta* (7-9 µm on legs I and 7-8 on legs II) compared to those from the other two eucalypt species (6-7 µm on legs I and 5-7 µm on legs II). Setae *1a* on coxae I was longer for the *E. pulverulenta* specimens (20-26 µm) than for those from *E. ovata* (13-24 µm) and *E. cinerea* (10-17 µm). Slight differences were also observed in the length of opisthosomal setae; however, these setae had very fine, long tips and the overall measurements could have been sensibly affected by breakage of the finest ends.

Current knowledge about the Eriophyoid mites of *Eucalyptus* spp.

Eight eriophyoid species have been described from *Eucalyptus* species to date. Seven belong to the Family Eriophyidae, Subfamily Phyllocoptinae, Tribe Phyllocoptini and one belongs to the Family Diptilomiopidae, Subfamily Diptilomiopinae (table 1). None of them have been collected from *E. cinerea*, *E. ovata* or *E. pulverulenta* and their current distribution is Antarctic (New Zealand), Australian, Indomalayan and Neotropical as based on the zoo-geographic regions according to Udvardy (1975).

Currently, three species belonging to the genus *Acadicrus* are known in the historic literature and all were found only on *Eucalyptus* spp. in Australia (Amrine and Stasny, 1994). Among those, *Acadicrus eucalypti* (Gurney) needs to be found and redescribed in order to give a confirmation of the belonging genus which was supposed to be *Acadicrus* (Amrine and Stasny, 1994). A fourth *Acadicrus* species was collected in Australia but it still requires further study and a formal description (Knihinicki *et al.*, 2010).

Six *Rhombacus* species are known and four of them are associated with *Eucalyptus* spp.: *Rhombacus asclepiadii* Keifer was collected on *Gomphocarpus fruticosus* (L.) W.T. Aiton. (originally listed as *Asclepias fruticosa* L.) (Apocynaceae) and *Rhombacus rheumella* Keifer on

Table 1. Eriophyoid mite species currently described on *Eucalyptus* spp., their host species, symptoms and current distribution.

Family Eriophyidae, Subfamily Phyllocoptinae, Tribe Phyllocoptini			
Species	Host species	Alterations	World distribution
Acadicrus bifurcatus Keifer 1965	*E. obliqua* L'Hér.	Witches' broom	Australia (Keifer, 1965a)
Acadicrus mergiferus Keifer 1965	*E. viminalis* Labill.	Witches' broom	Australia (Keifer, 1965a)
Acadicrus? *eucalypti* (Gurney 1924)	*E. stricta* Sieber et Spreng.	Witches' broom	Australia (Gurney, 1924)
Acadicrus sp. n.	*E. wandoo* Blakely	Witches' broom	Australia (Knihinicki *et al.*, 2010)
Rhombacus chatelaini Manson 1984	*E. cypellocarpa* L.A.S. Johnson, *E. regnans* F. Muell.	Vagrant, associated with brown scarifications of the leaves	New Zealand (Manson, 1984)
Rhombacus eucalypti Ghosh et Chakrabarti 1987	*E. globulus* Labill., *E. camaldulensis*, *E. grandis* W. Hill, *E. tereticornis* Sm.	Vagrant on the leaf underside	Argentina (Flechtmann, pers. comm.), Brazil (Flechtmann and Santana, 2001), India (Ghosh and Chakrabarti, 1987)
Rhombacus eucalyptifoliae Boczek 1991	*Eucalyptus* sp., *E. camaldulensis*, *E. grandis*, *E. tereticornis*	Vagrant on the leaf underside	Thailand (Chandrapatya and Boczek, 1991b)
Rhombacus morrisi Keifer 1965	*E. viminalis*, *E. tereticornis*	Witches' broom; rust on *E. tereticornis*	Australia (Keifer, 1965), India (Mohanasundaram, 1991)
Family Diptilomiopidae, subfamily Diptilomiopinae			
Species	Host species	Symptoms	World distribution
Diptilomiopus eucalypti (Boczek 1991)	*E. camaldulensis*	Vagrant on the leaf underside	Thailand (Chandrapatya and Boczek, 1991a)

Note: ? = the genus needs to be confirmed.

Rheum rhaponticum L. (originally misspelled as *rhaponicum*) (Polygonaceae). *Rhombacus eucalyptifoliae* is the replacement name for *Rhombacus eucalypti* Boczek, because the specific name was preoccupied by Ghosh and Chakrabarti (1987), see Boczek and Petanović (1995).

A further species, *Aceria rostratae* (Joshi), belonging to the Family Eriophyidae, Subfamily Eriophyinae, Tribe Aceriini, should be added to the list. It was reported as vagrant on the leaf underside of *Eucalyptus camaldulensis* Dehnh. in India, but the name and its description are not valid because they were reported on a Ph.D. thesis (Joshi, 1990), and not published in a peer reviewed scientific journal.

Another Phyllocoptinae, *Acalox ptychocarpi* Keifer, was described from the leaves of *Eucalyptus ptychocarpa* F. Muell. but it was later considered junior synonym of *Corymbia ptychocarpa* (F. Muell.) K.D. Hill & L.A.S. Johnson (Myrtaceae). More recently, this eriophyid species has been shown to cause significant damage to *Corymbia citriodora* subsp. *variegata* (F. Muell.) K.D. Hill & L.A.S. Johnson (Nahrung and Waugh, 2012).

Conclusions

As fine morphological differences are expected to exist between populations as a consequence of the host plant species, a wider morphological examination along with DNA evaluation of the new mite species should be carried out on populations associated with the different host plant species, so far *E. cinerea*, *E. pulverulenta* and *E. ovata*, as well as on other *Eucalyptus* species commonly cultivated in Italy. The studies should be aimed at proving or rejecting a morphological and genetic homogeneity of the mite populations and at explaining the slight morphological differences that were recorded and which might be probably influenced by the plant genotype (see literature in Khederi *et al.*, 2014). It would be interesting to investigate if this mite species also occurs in commercial plantations or natural stands, and to study in depth the fauna of the Eriophyoidea associated with *Eucalyptus* species in Australia, because new species have been found there on these host plant species and are still undescribed (Knihinicki, personal communication).

Currently, no effective control strategies are available against *P. cacolyptae* despite the severe damage symptoms registered; these symptoms were observed to be increasing during 2013-2015 in association with the increase of the eriophyid mite population. The damages were much more impressive and economically relevant on *E. pulverulenta* cv. Baby Blue for which up to 100% of plants were affected (Martini, unpublished data). The current data about the impact of this mite on foliage yields need to be integrated with plans of systematic collection and sampling. In addition, psyllids were detected in many cases and the role of this insects should be understood, such as potential pathogens associated to these arthropods.

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