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A new species, *Cinara tellenica* Binazzi F. et Strangi (Aphididae Lachninae) associated with *Cedrus atlantica* in the Tell Atlas of Algeria

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Abstract

A new species of *Cinara*, *C. tellenica* Binazzi F. et Strangi, is described from apterous viviparous females recorded in the Tell Atlas of Algeria on the endemic cedar *Cedrus atlantica* (Endl.) Manetti. Morphological and molecular data are provided to support the identification of the new species. A taxonomic key is also presented to differentiate the Mediterranean cedar-inhabiting *Cinara* species. Gene sequences were submitted to GenBank and type specimens deposited in the CREA Research Center collection.

Key words: aphids, new taxon, Atlas cedar, mountain range, North Africa, Mediterranean basin.

Introduction

Cinara Curtis (Aphididae Lachninae), is a genus widely distributed in the Northern Hemisphere and comprising more than 200 monoecious species all associated with conifers. The vast majority of them are native to North America (Voegtlin and Bridges, 1988) while in Europe more than 50 species have been reported so far. In North Africa, *Cinara cedri* Mimeur was originally described in Morocco in 1935 (Remaudière, 1954) and more recently, it was recorded in Algeria in Batna (Laamari *et al.*, 2013). New records for Morocco were also reported from the Rif and the Middle Atlas (Fabre *et al.*, 1999) while infestations were observed in several areas of Turkey on *Cedrus libani* A. Rich. and in Europe on the introduced *Cedrus atlantica* (Endl.) Manetti and *Cedrus deodara* (Roxburgh) G. Don (Covassi and Binazzi, 1974; Notario *et al.,* 1984; Oğuzoğlu and Avcı, 2019; Tuatay and Remaudière, 1964). Moreover, *Cinara* specimens collected on *Cedrus brevifolia* (Hook.f.) Elwes et A. Henry endemic to Cyprus (Binazzi *et al.*, 2015), were described as *Cinara cedri brevifoliae* Binazzi A after morphometric and molecular evaluation (Binazzi *et al.*, 2017; Binazzi and Roversi, 2018).

Although recent reports concerning biodiversity and conservation have increased knowledge about aphids in Algeria (Laamari *et al.*, 2010; 2013; Aroun, 2017; Chellali, 2018; Dahmani, 2019), North Africa still remains a poorly explored and investigated area, particularly in remote areas (Terrab *et al.*, 2008).

However, in 2018, a systematic survey of aphids was carried out in the Algerian Chréa Biosphere Reserve. This park, created in 1983 (decree number 83461 of July 23rd, DGF, 1983), is one of the most protected areas in Algeria and in 2002 it was classified as a UNESCO biosphere reserve. This site, which is crucial for the conservation of natural resources in North Africa, is located 50 km southwest of the capital Algiers at altitudes ranging from 1400 to 1600 meters and occupies an area of about 26000 hectares. The average monthly temperatures range from 5 °C in winter to 22 °C in summer with an annual rainfall of about 700 mm (Ozenda, 1991). For its geographical position and topographical features the Chréa Reserve is characterized by a typical vegetation gradient with three bioclimatic types: the Thermo-Mediterranean stage (200-800 m) with mixed formations of *Pinus halepensis* and dense Mediterranean maquis, the Meso-Mediterranean stage (800-1300 m) characterized by holm oak (*Quercus ilex*) coppices and the Supra-Mediterranean stage (1300-1600 m) formed by pure *C. atlantica* forest stands. The aphid survey, which was performed in the latter bioclimatic type, allowed the description of a new *Cinara* species co-existing with *Cinara cedri cedri* Mimeur on *C. atlantica*. This new taxon was identified by both morphological and molecular evaluation.

Materials and methods

In the autumn of 2017, some aphid colonies were occasionally observed on *C. atlantica* stands in two high-altitude sites, Haoud (36°26'07.19"N 02°53'48.64"E; 1450 m asl) and Ghellaï (36°26'87.30"N 02°54'20.64"E; 1473 m asl) located in the Chréa National Park, in the Tell Atlas of Algeria. Regrettably, only few immature specimens were found and no conclusive morphological identification could be thus performed. However, molecular identification revealed the presence of two clearly distinct aphid species: *C. cedri cedri* and another unidentified *Cinara* species. Therefore, in 2018, a more extensive and systematic survey on *C. atlantica* was carried out in the same above-mentioned sites, in order to collect further material of the unknown taxon. The collection of specimens was performed using a systematic sampling technique (Butter, 1998; Laffly and Mercier, 1999) that consisted of choosing the first tree randomly and then selecting all the others at regular intervals. In our case we examined approximately one tree in every two. A total of

16 trees were thus investigated, with 8 trees for each of the abovementioned sites. In total, six colonies (i.e. two twigs bearing single colonies from the Haoud site and four twigs with single colonies from the Ghellaï site) were collected out the 16 examined trees. Twigs with colonies were then stored separately in plastic bags, which were temporarily located in a cooler to keep them fresh. Specimens from each aphid colony were then divided in the laboratory: immature specimens were stored in coded tubes containing 96% ethanol for molecular identification, while mature individuals were set into different coded tubes with 70% ethanol, for morphological identification.

The new *Cinara* species is thus morphologically described on the basis of the following 18 apterous viviparous females deriving from the six previously collected colonies: i) three mature individuals and another single mature individual collected respectively from two different colonies in Haoud on July 11, 2018 (leg. Gahdab Chakali); ii) one, two, five and six mature specimens collected respectively from four distinct colonies in Ghellaï on July 17, 2018 (leg. Gahdab Chakali).

Moreover, the following specimens of *C. cedri cedri* (slides in CREA coll., Firenze, Italy) were used for morphological comparison: 3 from *C. atlantica*, Bagno a Ripoli (Florence), Italy, 15.x.2015 (sl. C10/931); 2 from *C. atlantica*, Florence, 28.v.1974 (sl. C2/102); 2 from *C. deodara*, Aci S. Antonio (Catania, Sicily), 30.x.1974 (sl. C2/110-109); 1 from *C. atlantica*, Piano Zucchi (Palermo, Sicily), 24.vi.1983 (sl. C2/122); 2 from *Cedrus* sp., Madrid (Spain), 28.vii.1981 and 02.x.1981 (sl. C2/117 and sl. C2/119); 3 from *C. atlantica*, Valenzano (Bari), 25.x.2018 (sl.C10/932).

Specimens of the new *Cinara* species were also compared with individuals of *C. cedri brevifoliae*: 9 apterae, *C. brevifolia*, Troodos Mountains, Cyprus, 05.ix.2015 (slide C10/928 C10/929-930) (slides in CREA coll.).

Specimens were mounted on slides for morphological analysis and identified using taxonomic keys from Binazzi (1984), Binazzi *et al.* (2017), Blackman and Eastop (2020) and Binazzi and Mier Durante (2002).

As regards molecular analysis, six samplings, each represented by five immature insects, were obtained in the laboratory from the same abovementioned colonies (two from the Haoud and four from the Ghellaï sites). Genomic DNA was thus extracted from the whole body of 30 single individuals. The DNeasy Blood and Tissue Kit (QIAGEN) were used according to the manufacturer's instructions and the final elution step was performed in 50.0 µl of AE Buffer. The quality of DNA preparations was evaluated with a spectrophotometer (QIAxpert, QIAGEN).

The barcode gene region belonging to the mitochondrial Cytochrome Oxidase Complex I gene (COI) was amplified according to Folmer *et al.* (1994). Cytocrome b (CytB) and Elongation Factor 1 alpha (EF1α) DNA fragments comprised between primers CP1 - CP2 (amplicon length: 798 bp) and Ef3 - Ef6 (amplicon length: 1071 bp) respectively, were amplified according to Jousselin *et al.* (2013).

PCR products were sequenced at the Centro di Servizi per le Biotecnologie di Interesse Agrario Chimico ed Industriale (CIBIACI), University of Florence, Italy, and the resulting sequences were submitted to GenBank.

Among all the *Cinara* species, only those closely related to *C. cedri* and belonging to "Clade B" according to Jousselin *et al.* (2013), were considered for phylogenetical reconstruction. Moreover, only specimens whose COI, CytB and *EF1α* sequences were available in GenBank or in the specific barcode / taxonomy database for European aphids (Cœur d'Acier *et al.*, 2014) were included in the analysis.

Gaps, repetitive sequences and ambiguous nucleotides were removed in the alignment with Gblock v. 0.91b. The final dataset had 1802 positions and 72 sequences.

The choice of an appropriate substitution model was evaluated using jModelTest2 under AICc, BIC and DT criteria. The chosen matrixes were GTR plus gamma and invariant sites.

Multilocus phylogeny was inferred considering the three loci (*COI*, *CytB* and *EF1α*) using Maximum Likelihood (ML) and Neighbour Joining (NJ) methods performing 1000 bootstrap replicates.

Results

Species identification

All specimens collected in our systematic survey in 2018 were identified both morphologically and molecularly as a new *Cinara* species. However, on the basis of previous findings there is evidence that this new taxon coexists with the already known *C. cedri cedri* in the Chrea National Park.

Cinara tellenica Binazzi F. et Strangi sp.n.

Morphological description

Apterous viviparous female (from 18 specimens) (figure 1, 2, 3)
Medium-sized body, 2.67-3.42 mm long, blackish in color, with no pattern of wax markings when alive and with four rows of roundish intersegmental sclerites on dorsal abdomen. Siphuncular cones brown to dark brown and medium sized with marked apical flange. Head, prothorax and mesothorax sclerotized. Metathorax and 1st to 6th abdominal tergites without sclerotizations. Medium-

Figure 1. A young colony of *C. tellenica* attended by *Camponotus cruentatus* (Latreille).

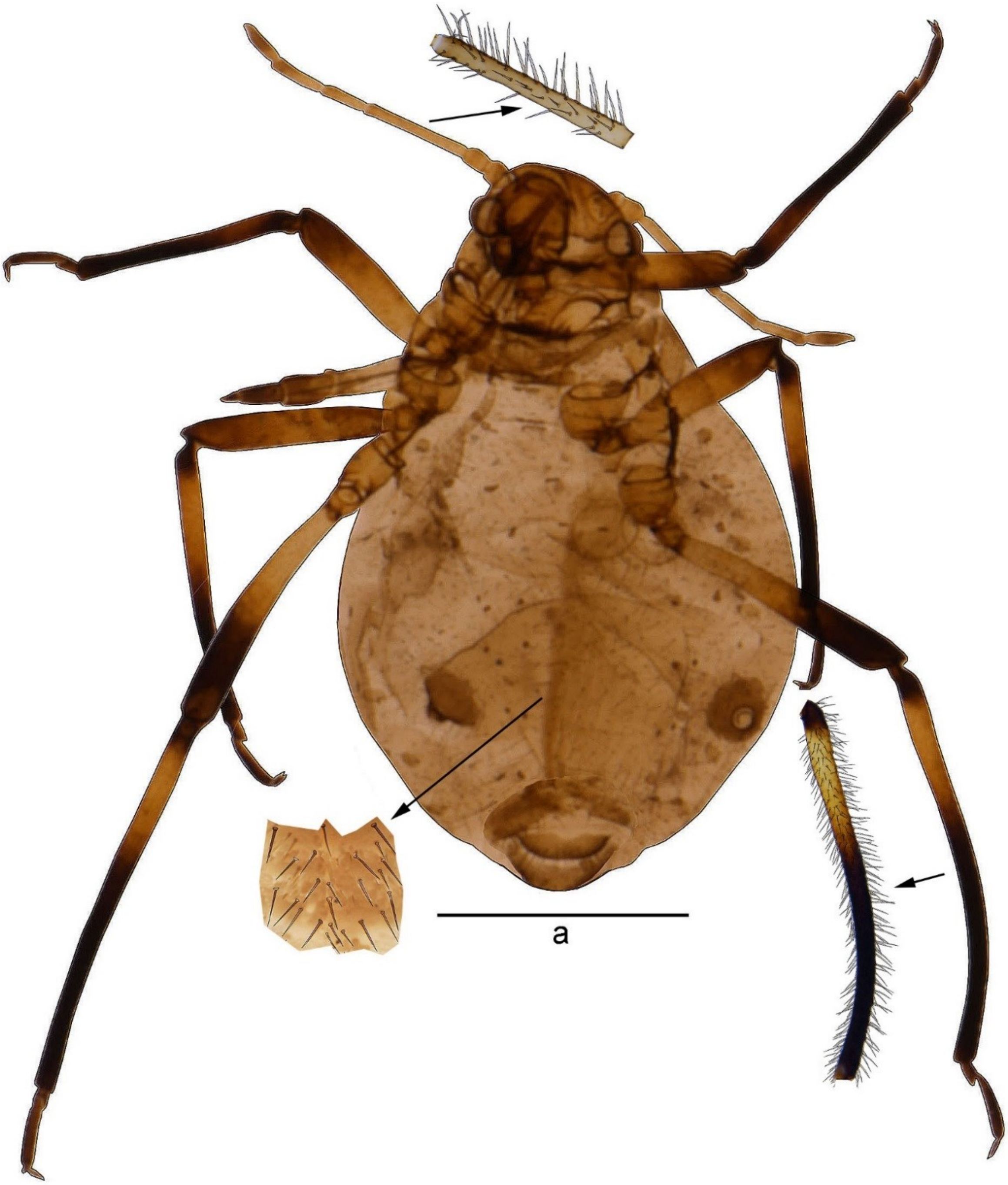

Figure 2. *C. tellenica*: the aptera virginopara (holotype). Scale: a = 1 mm.

sized mesosternal tubercle present. Abdominal tergite 7 with two sclerotic areas. Abdominal tergite 8 bears a well-sclerified solid bar interrupted in the middle. Cauda dark brown. Genital plate small and light-colored. Antennae pale brown with darker brown segments I, II, the distal part of III, the distal half of IV and V and the whole of VI. Rostrum reaches back to the fifth abdominal segment with dark brown apical segments. Legs with brown basal halves of both femora and tibiae, except for the black "knees", and with black distal halves of all the tibiae. Tarsi dark brown, paler than the distal end of tibiae.

Antennal setae light brown; abdominal tergites 1 to 7 with numerous brown and strong setae; fourth rostral segment with 6-8 accessory setae; hind tibiae with numerous strong, rather short, brown setae, the longest, equal or a little longer than the tibial diameter at the insertion point. Morphometric data are provided in table 1.

Holotype

Aptera vivipara, *Cedrus atlantica*, Chrea, Blidean Atlas, Algeria, 11.vii.2018, (slide C10/932).

Paratypes

17 apterae: 3 specimens (slides C10/932-933) collected in Haoud on 11.vii.2018 and the remaining 14 specimens collected in Ghellaï on 17.vii.2018 (slides C10/934-937). Preservation of material at the CREA Centre, Florence, Italy.

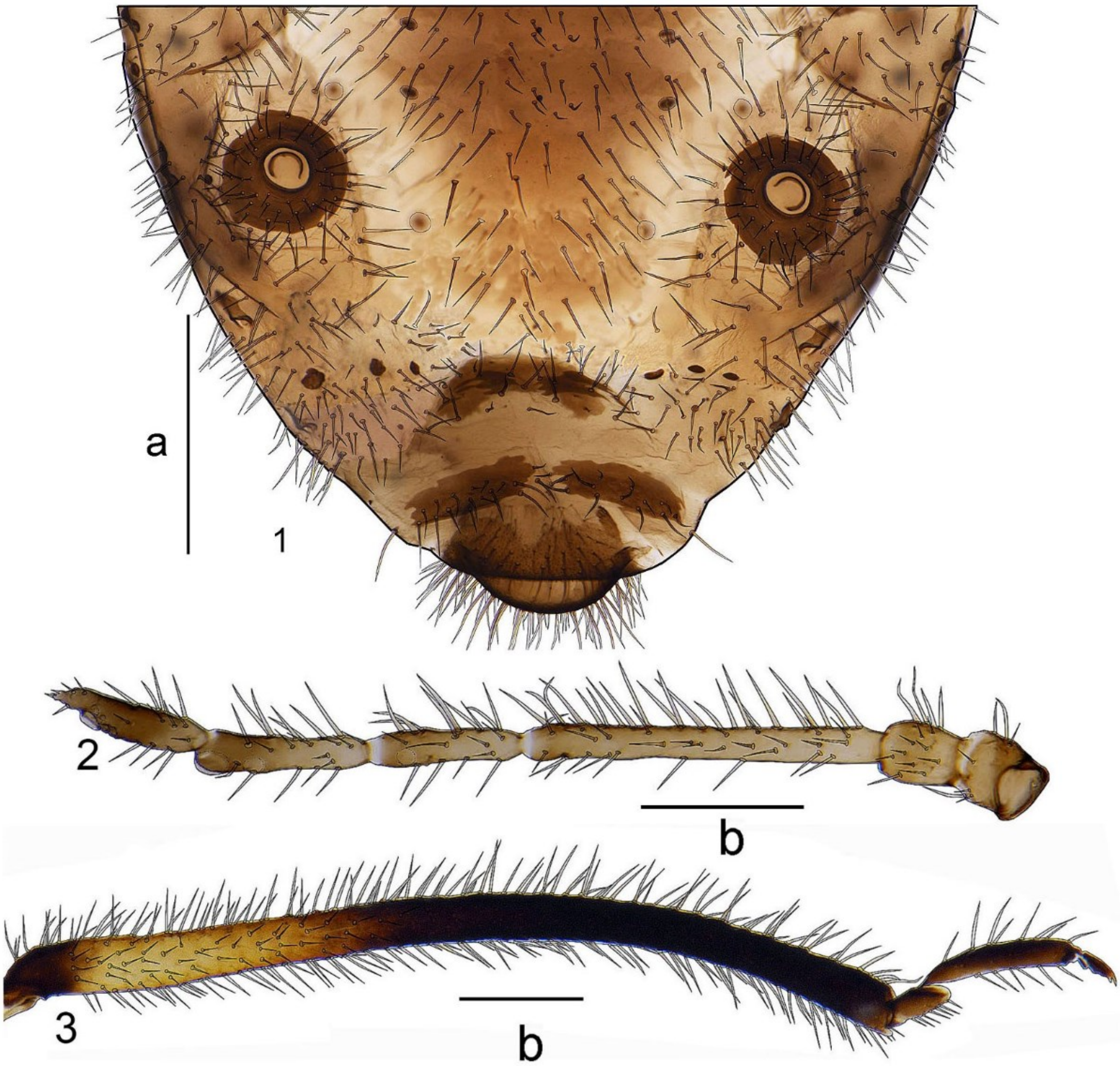

Figure 3. Morphological details of *C. tellenica*; (**1**) Dorsal aspect of posterior abdomen, (**2**) Antenna, (**3**) Hind tibia and tarsus. Scales: a = 0.2 mm; b = 0.5 mm.

Derivatio nominis

From the Algerian Tell Atlas.

Molecular analysis

Molecular results evidenced that the aphid samples collected in this study all belong to the same species. Identical COI and EF1 alpha sequences were obtained from the analysed specimens (COI GenBank Accession Numbers: MN577067, MN577068, MN577069) (EF1 alpha GenBank Accession Numbers: MN577070, MN577071, MN577072) while two different haplotypes where found in CytB locus (GenBank Accession Numbers: MN577074, MN577075, MN577076). Moreover, a third haplotype in CytB locus was found in a single specimen not included in the subsequent analyses (GenBank Accession Number: MN577073).

Phylogenetic trees obtained with both Maximum Likelihood (ML) and Neighbour Joining (NJ) methods share the same topology. Moreover, the same phylogenetic relationships among species described in Jousselin *et al.* (2013) were respected. The analysed specimens belong to a well-resolved taxon (100 bootstrap value in both NJ and ML trees) and are located in a sister clade to the *C.cedri* and *C. cedri brevifoliae* (figure 4).

Because of the phylogenetic distance between *C. tellenica* and *C. cedri* and the number of differences found in the three different analysed loci (two mitochondrial and one nuclear), we regard this as confirmation that *C. tellenica* is a new species.

Moreover, the phylogenetic tree suggests that the adaptation to the genus *Cedrus* could be a monophyletic character, even though the distance and the low support value for the most closely related species, *Cinara puerca* Hottes, do not allow a clear identification of the phylogenetic origin of this event (figure 2).

Key to apterous viviparous females of *Cinara* species on *Cedrus* (after Binazzi *et al*, 2017, modified).

1 - 5-segmented antennae.
[Small, roundish body, 1.6-2.0 mm long. Abdominal tergites 2° to 5° with sclerotized plates each bearing a rising protuberance backwards. Dorsal setae short, pale and knobbed with apical spikes. Abdominal tergite 8 bearing eight marginal setae distally ramified. On *Cedrus* shoots and twigs] . . . . ***Cinara*** **(*Cedrobium*)** ***laportei***

\- 6-segmented antennae. **2**

2 - First segment of hind tarsus short, the dorsal side 0.045-0.060 mm long with ratio dorsal to basal length 1.0-1.12. Body setae rather short, the dorsal, antennal and tibial ones less than 0.15, 0.115 and 0.13 mm long respectively. On *C. atlantica* (Algeria) . . . . ***Cinara tellenica*** **sp.n.**

\- First segment of hind tarsus dorsal side 0.060-0.090 mm long with ratio of dorsal to basal length 1.2-1.7. Body setae long, the dorsal, antennal and tibial ones 0.160-0.240, 0.120-0.170 and 0.155-0.240 mm long respectively. **3**

3 - Hind tibiae 1.5-2.1 mm long. Ratio of apical rostral segments IV to V, 2.0-2.5. Ratio of antennal segments V to VI, 1.2-1.4. Long and strong setae, those on antennal segment III and abdominal tergite 7 0.120-0.170 and 0.160-0.240 mm long respectively. On *Cedrus* . . . . ***Cinara cedri cedri***

\- Hind tibiae 1.1-1.6 mm long. Ratio of apical rostral segments IV to V, 1.8-2.1. Ratio of antennal segments V to VI, 1.0-1.2. Shorter and thinner setae, those on antennal segment III and on abdominal tergite 7 0.115-0.130 and 0.160-0.190 mm long, respectively. On Cyprus cedar, *Cedrus brevifolia*. . . . . ***Cinara cedri brevifoliae***

Table 1. Features (mm) of *C. tellenica*, *C. cedri cedri* and *C. cedri brevifoliae*. Specimens are apterous viviparous females. Collection data are in the text. (* = Main discriminant features).

Features	*C. tellenica*	*C. cedri cedri*	*C. cedri brevifoliae*
N° of specimens	18	16	9
Body length	2.67-3.42	2.86-3.55	2.21-2.57
Hind tibia length	1.66-2.09	1.54-2.11	1.16-1.68
Diameter of siphuncular cones	.210-.312	.312-.450 *	.258-.378
Length of antennal segment III	.420-.552	.390-.576	.372-.480
Number of secondary rhinaria	0-6	0-1 *	0-1
Length of antennal segment IV	.174-.264	.168-.270	.150-.186
Length of antennal segment V	.210-.240	.204-.252	.186-.222
Length of antennal segment VI	.174-.198	.162-.186	.162-.192
Length of antennal segment VI processus terminalis	.030-.048	.048-.060 *	.042-.051
Ratio antennal segment V to VI	1.1-1.3	1.2-1.4	1.1-1.2
Length of rostral segment IV	.200-.222	180-220	.174-.204
Number of accessory setae	6-8	6-8	6-8
Length of rostral segment V	.080-.090	.084-.096	.090-.102
Ratio rostral segment IV to V	2.2-2.5	2.2-2.5	1.8-2.1
1st segment hind tibia basal length	.045-.060	.042-.054	.036-.042
1st segment hind tibia dorsal length	.045-.060	.060-.090 *	.060-.072
1st segment hind tibia ventral length	.120-.132	.132-.156 *	.108-.144
dorsal length / basal length	1.0-1.12	1.2-1.7 *	1.4-1.7
Length of the 2° segment of hind tarsus	.264-.300	.282-.348 *	.258-.312
Length of the longest hair on:			
abdominal tergite 5°	.090-.150	.150-.230 *	.150-.200
abdominal tergite 7°	.090-.144	.162-.240 *	.162-.192
antennal segment III	.090-.114	.120-.168 *	.114-.132
hind tibia	.096-.132	.156-.240 *	.156-.204
N° of setae on abdominal tergite 8°	28-36	17-23 *	18-26
N° of setae on the genital plate	3-9	9-11 *	6-9

Taxonomic discussion

C. tellenica differs from the closely related *C. cedri cedri* (table 1) in having smaller siphuncular cones, a higher number of secondary rhinaria on antennal segment III and shorter 1st segment of hind tarsus. Furthermore all the body setae are shorter. Other differences are the higher number of setae on abdominal tergite 8 and the lower number of setae on the genital plate. Moreover, the hind tibiae of *C. tellenica* are darker brown except for a slightly paler section on basal part.

In the Mediterranean basin and Europe four *Cinara* species are now reported to inhabit Cedar trees, i.e., *Cinara* (*Cedrobium*) *laportei* Remaudiere, *C. cedri cedri*, *C. cedri brevifoliae* and the new species *C. tellenica*. The only known *Cedrobium* species seems to be restricted to North Africa and southern Europe while *C. cedri* s.l. has been reported as associated with *Cedrus* all over the

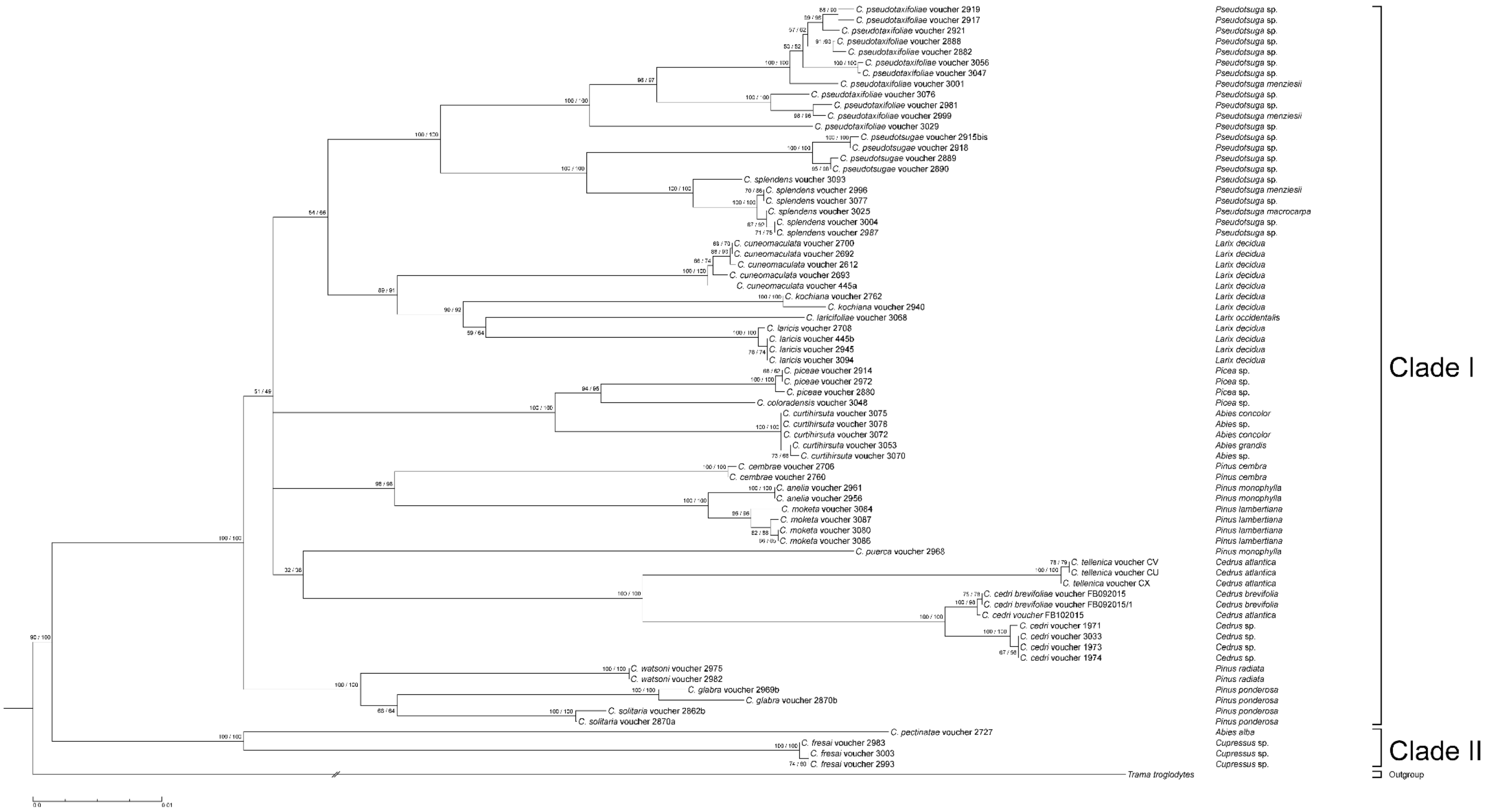

Figure 4. Phylogenetic reconstruction of *C. tellenica* based on *COI*, *CytB* and *EF1alpha* concatenated loci. Branches were annotated with bootstrap values obtained using 1000 pseudoreplicates in Neighbor Joining and Maximum Likelihood trees respectively. Species list was chosen on the basis of clades defined in Jousselin *et al.* (2013). *Trama troglodytes* von Heyden was used as an outgroup.

world (Blackman and Eastop, 2020; Millar, 1990). The other two congeneric species cited on *Cedrus* from Europe, i.e., *Cinara confinis* (Koch) and *Cinara curvipes* (Patch), are normally associated with *Abies* and only rarely found on cedar trees (Binazzi *et al.*, 2017).

The taxonomy of the genus *Cedrus* is complex and might reflect its long phylogenetic history, as it is suggested by North-Eastern Asian and North-Western-North American samples of petrified wood dating back to the Cretaceous (Blokhina and Afonin, 2007). The Himalayan *C. deodara* and the other three Mediterranean species, *C. libani*, *C. brevifolia* and *C. atlantica*, might represent thus relicts whose ancestors were much more widely distributed in Europe, Asia and North Africa before the Quaternary (Gaussen, 1964; Ferguson, 1967; Pons, 1998; Magri and Parra, 2002; Postigo-Mijarra *et al.*, 2010; Manzi *et al.*, 2011; Jasińska *et al.*, 2013). The progressive reduction of the *Cedrus* range and the formation of discontinuities in its distribution probably occurred during the late Tertiary as a consequence of climate cooling (Thompson, 2005; Utescher *et al.*, 2007; Ivanov *et al.*, 2011). During Pleistocene, the ongoing process of fragmentation could have been exacerbated by prolonged climate oscillations leading to further reduction of its range (Elenga *et al.*, 2000; Svenning, 2003; Fady *et al.*, 2008; Terrab *et al.*, 2008; Cheddadi *et al.*, 2009; Postigo-Mijarra *et al.*, 2010; Jasińska *et al.*, 2013). Therefore, the current worldwide distribution of *Cedrus*, characterized by the presence of four distinct species, could be the ultimate result of the increasing isolation occurred between the central-Asiatic populations and the Mediterranean ones (Gaussen, 1964; Pons, 1998; Qiao *et al.*, 2007; Jasińska *et al.*, 2013). Moreover, according to Terrab *et al.* (2008), the peculiarities of the *C. atlantica* genetic population structure recorded in North Africa should be interpreted in the light of its Quaternary range dynamics strongly influenced by complex local topography and climate conditions. These patterns involved the persistence of cedar formations in the Last Glacial Maximum in at least three glacial refugia situated along the south-western Mediterranean coast. Subsequently, in present-day Morocco, these areas served probably as a starting point for a new cedar expansion toward the Middle Atlas region, which has since become the major core of *C. atlantica* range (Terrab *et al.*, 2008).

However, it is worth noting that the current distribution of *C. atlantica* in North Africa certainly reflects also the severe impact of human activities in historical times. In fact, overexploitation of forest stands caused by prolonged grazing and use of cedar wood for several purposes such as shipbuilding, construction and temple decoration, is probably one of the major causes of cedar decline and further fragmentation of its original range (Terrab *et al.*, 2008; Jasińska *et al.*, 2013).

As it was evidenced by our field survey in the Chréa Biosphere Reserve, the newly described *Cinara* seems to coexist with the already known *C. cedri* previously reported for North Africa and Europe. It is not currently clear, though, how this species evolved and only some hypotheses can be formulated at this stage. Although the two species presently share the same host and geographical location, speciation could have occurred in the context of past climate changes and complex ecological processes involving aphid-host interactions. In particular, it is plausible that the increasing isolation of aphid populations due to the long-lasting and progressive fragmentation of the range of cedar in North Africa has had a fundamental role in the origin of this taxon. In a second phase, a marked further reduction of *C. atlantica* range due to dramatic thermal stresses such as the Last Glacial Maximum could have forced some populations of cedar-associated organisms such as aphids to coexist in the same Mediterranean glacial refugia.

Another possible explanation for the coexistence of the two *Cinara* species could be related to the intense human exploitation of cedar stands in recent history. In fact, the extensive trade of plant material from various locations of North Africa may have favoured the spread and establishment of aphid populations in new territories.

Preliminary observations suggest that *C. tellenica* could be quite similar to the closely related *C. cedri* in terms of either biology or ecology. Moreover, as already reported for other *Cinara* species (Binazzi *et al.*, 2016), the peculiar geographical features of the collection sites, characterized by high altitudes and cold/long winters, could be conducive to the onset of the holocycle with presence of both sexual morphs and overwintering eggs. However, this new taxon is still under investigation and additional research is needed to fully clarify its biological traits.

Furthermore, we believe that extensive aphid surveys and investigations of cedar stands, even in remote areas, are fundamental to clarify the distribution and evolutionary patterns of *Cinara* species in Northern Algeria. More generally, an increased knowledge on aphid biology and ecology in North Africa could be useful for environmental conservation, since this area is not only rich in biological diversity but it is also one of the regions of the world most endangered by anthropogenic pressure and possible future climate change (Medail and Quezel, 1997; Houghton *et al.*, 2001;Terrab *et al.*, 2008).

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