



Diversity and temporal variations of the Hemiptera Auchenorrhyncha fauna in the Ajaccio region (France, Corsica)

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Summary. During a one-year survey of the Auchenorrhyncha fauna in a maquis habitat of the Ajaccio region (Corsica), 37 species were listed including three alien species recorded in Europe in the last decades. The standard 658 bp mitochondrial COI barcode was obtained for 32 species, including 14 species never previously barcoded. Neighbor-joining analyses confirmed the delineation for all the species. However, comparisons with available sequences from mainland Europe showed that four species presented a significant intraspecific divergence (>3%), whereas a reduced interspecific divergence was found for another set of species. Complementary studies may therefore be necessary to better assess their taxonomic levels. Three species – *Euscelis lineolata* Brullé, 1832 (Cicadellidae), *Philaenus spumarius* (Linnaeus, 1758) (Aphrophoridae) and *Latilica maculipes* (Melichar, 1906) (Issidae) – accounted for up to 74.3% of the total insects collected. Although taxonomic interpretations using restricted sampling and COI sequences alone are not robust, the proportion of new sequences highlight the global lack of knowledge about the Auchenorrhyncha as a whole and the potentially presence of cryptic taxa in different clades.

Résumé. Diversité et variations temporelles de la faune des Hemiptera Auchenorrhyncha dans la région Ajaccienne (Corse). Un suivi de la faune des Auchenorrhynques dans un maquis de la région d’Ajaccio (Corse) effectué durant une année a permis l’enregistrement de 37 espèces, incluant trois espèces introduites en Europe ces dernières décennies. Le fragment standard de 658 pb du gène mitochondrial COI utilisé pour le barcoding a été obtenu pour 32 espèces, parmi lesquelles 14 ont été nouvellement séquencées. La délimitation de chacune des espèces a été confirmée par les analyses en neighbor-joining. Dans le cas de quatre espèces, les séquences obtenues pour les spécimens de Corse présentent une divergence intraspécifique significative (>3%) par rapport aux séquences de spécimens d’Europe continentale ; au contraire, une faible divergence interspécifique a été mise en évidence pour un autre groupe d’espèces. Des études complémentaires sont cependant nécessaires afin de clarifier le statut taxonomique de chacune de ces entités. Trois espèces – *Euscelis lineolata* Brullé, 1832 (Cicadellidae), *Philaenus spumarius* (Linnaeus, 1758) (Aphrophoridae) et *Latilica maculipes* (Melichar, 1906) (Issidae) – représentent à elles-seules 74,3% des insectes collectés. Bien que les interprétations taxonomiques basées sur un échantillonnage limité et sur un unique gène mitochondrial ne sont pas robustes, la proportion de séquences nouvelles et la présence d’entités génétiques divergentes mettent en évidence le manque global de connaissances concernant les Auchenorrhynques.

Keywords: abundance; COI barcode; maquis; *Philaenus spumarius*; taxon divergence

Composed of over 43,000 described species worldwide, the Auchenorrhyncha (Hemiptera) suborder is represented by the Cercopoidea (spittlebugs), Cicadoidea (cicadas), Membracoidea (leafhoppers and treehoppers), and Fulgoroidea (planthoppers) superfamilies (Bartlett et al. 2018). All members of this ubiquitous group are sap-feeders, each species usually exploiting a specific source of sap. Xylem feeders are mainly represented by spittlebug, cicada and Cicadellinae (Membracoidea, Cicadellidae) species, while treehopper, planthopper and most of the leafhopper species are usually considered as phloem feeders. The specialized Typhlocibinae subfamily (Membracoidea, Cicadellidae) is composed of mesophyll sap feeder species. Several Auchenorrhyncha species can

injure the plant and/or inoculate pathogens (bacteria, fungus or viruses) while they are feeding, and are thus considered as pests (Nielson 1968; Dietrich 2009, 2013).

The European diversity of Auchenorrhyncha is relatively incomplete, with local or national species lists produced only for France (Ribaut 1952; Della Giustina 1989; Puissant 2006), Germany (Nickel 2003) and Italy (Guglielmino et al. 2000, 2005, 2015; Mazzoni 2005; D’Urso & Mifsud 2012). In Corsica, only two published studies concern the Cicadellidae (Della Giustina & Bonfils 1978) and the cicadas (Puissant & Sueur 2001). More recently, a one-week field mission recording the Auchenorrhyncha diversity was also performed in Corsica (Chauvel et al. 2015). These regional lists can

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also be completed by online electronic resources covering the worldwide Auchenorrhyncha fauna (Dimitriev 2003) or more specific groups, such as the Cercopoidea superfamily (Soulie-Perkins 2019) or the Fulgoromorpha infraorder (Bourgoin 2019). Despite the high diversity of the Auchenorrhyncha suborder, this group is as a whole poorly known. In the scope of studying the eco-ethology and the abundance of the potential insect vectors of the plant pathogen *Xylella fastidiosa* Wells et al., 1987 in Corsica, we performed a one-year-long survey of the Auchenorrhyncha diversity in a maquis habitat of the Ajaccio region. This study will also contribute to the International Barcode of Life (iBOL) project by sequencing a fragment of the cytochrome c oxidase subunit I (COI) of all the collected species.

In this paper, we first discuss the species identification proposed by the barcoding approach of the COI sequences and highlight the taxonomy issues that could potentially need more specific taxonomic and phylogenetic studies. Then, we will present the diversity and abundance of the Auchenorrhyncha fauna in South West of Corsica (Ajaccio), but also the annual temporal variations of the most abundant species.

Materials and methods

Sample collection

From March 2017 to March 2018, a survey of the Auchenorrhyncha fauna was performed in the Ajaccio region (Corsica). Insects from the herbaceous and shrub strata were collected once a week in two close sites (GPS coordinates: 41°54'49.9"N 8°39'18.4"E and 41°55'18.9"N 8°38'46.3"E, respectively) using a sweep-net and stored in 75% ethanol. The Mediterranean vegetation at the studied sites was dominated by *Pistacia lentiscus* L., *Cytisus laniger* (Desf.) DC., *Cistus monspeliensis* L., *Olea europaea* L. var. *sylvestris* (Mill.) Lehr, *Arbutus unedo* L., *Phillyrea angustifolia* L. and *Myrtus communis* L. Cicadas were not considered in this survey, since this group, living in the canopy of the trees, requires a dedicated study.

Morphological identifications

Most of the adult specimens were identified to species level by the examination of the male appendages, based on the identification keys of Ribaut (1952) and Della Giustina (1989). Identifications were also confirmed by three international specialists, Dmitry Dimitriev (Cicadellidae), Thierry Bourgoin (Issidae and Tettigometridae) and Adeline Soulier (Aphrophoridae). The identification of the nymphs was usually more difficult using traditional morphological methods. Consequently, some specimens of the different nymph morphotypes were collected alive and reared to the adult stage. Specimens collected in the herbaceous or shrub strata were reared on *Poa annua* L. (Poaceae) or *A. unedo* (Ericaceae), respectively. While *P. annua* plants were transplanted in pots, we used cuttings of *A. unedo* placed in water-filled jars. In all cases, plants were bagged to avoid insects escaping. Voucher specimens of each species were deposited to the Office de l'Environnement de la Corse (OCIC, Corte). Similarly, Issidae, Tettigometridae and Aphrophoridae specimens are available at Thierry Bourgoin's laboratory (MNHN, Paris, France) and

Cicadellidae at Dmitry Dimitriev's laboratory (Illinois Natural History Survey, Champaign, IL, USA).

Barcoding

We also adopted a molecular approach, by sequencing a partial cytochrome c oxidase subunit I (COI) fragment (see Hebert et al. 2003) of the species collected in the studied sites or neighboring areas. Genomic DNA was extracted from the abdomen of the insects using the EZNA Insect DNA Kit (Omega Bio-tek Inc., Norcross, GA, USA). A fragment of the COI gene (658 bp) was amplified by PCR using the M13-tailed primers LCO1490puc_t1 (TGTA AACGACGGCCAGTTTTC AAC WAATCATAAAGATATTGG) and HCO2198puc_t1 (CAGGAAACAGCTATGACTAAACTTCWGGRTGWCCAA-ARAATCA) (Cruaud et al. 2010; Atiama et al. 2017). Amplifications were performed using the PCR Master Mix (2×) (Thermo Fisher Scientific, Waltham, MA, USA) in a 50 µl reaction containing 25 µl of PCR Master Mix, 0.25 µM of each primer and 250 ng of DNA template. Thermocycling conditions were: an initial denaturation at 94°C for 3 min, followed by five cycles of 30 s denaturation at 94°C, 30 s annealing at 45°C, 1 min elongation at 72°C and 35 cycles of 1 min denaturation at 94°C, 1 min annealing at 51°C, 1 min elongation at 72°C, and a terminal elongation at 72°C for 10 min. PCR products were sequenced by BIOFIDAL (Lyon, France) using the primers M13F (TGTA AACGACGGCCAGT) and M13R (CAGGAAACAGCTATGAC) in separate reactions.

Sequences were deposited in the online databases GenBank and BOLD. Most of the barcode sequences obtained were new to science, and thus relevant molecular identifications to species level were often not possible. Consequently, we selected COI sequences of related taxa available on BOLD or GenBank for the specimens for which we generated sequences for comparative purposes and clade affiliations. Based on the recent phylogenomic studies, three additional species were also used as outgroups. Thus, two species of Coleorrhyncha (Peloriidae: *Hemiodoecus leai* China, 1924 and *Xenophyes cascus* Bergroth, 1924), considered as the sister group of the Auchenorrhyncha suborder (Johnson et al. 2018) or of the Fulgoromorpha infraorder (Li et al. 2017), and one species of Aphididae (*Panaphis juglandis* Goeze, 1778), belonging to the sister group of all the taxa, were included in this study. GenBank accession numbers and BOLD sequence pages are available in the Supplementary material Table S1. Alignments and distance analyses were performed using MEGA X (Kumar et al. 2018) and Taxon DNA (Meier et al. 2006), respectively. Neighbor-joining (NJ) analyses were performed using the Kimura's 2 Parameter (K2P) distance model in MEGA X (Kumar et al. 2018). We adopted the threshold of sequence divergence of 3% usually admitted and used for barcoding based on the COI gene sequences in insects (Meyer & Paulay, 2005).

Results and discussion

Diversity – barcoding

Between March 2017 and March 2018, a total of 2562 specimens were collected in the studied area (Table 1), including 1364 adults and 1198 nymphs, corresponding to 38 and 11 morphotypes, respectively. Rearing experiments of the nymphs allowed us to identify each of the distinct morphotypes after completion of development. They all

Table 1. List and abundance of the Auchenorrhyncha species (adults and larva) collected in the Ajaccio region. For each species, the number of specimens successfully sequenced and the BIN assigned by the BOLD systems are indicated (bold: new BINs). *Larva partially misidentified – an undetermined number of larva of *Synophropsis lauri* (Horváth, 1897) corresponds to *Placotettix taeniatifrons* (Kirschbaum, 1868).

| Family | Species | Number of adults | Number of nymphs | Number of specimens sequenced | BIN |
|-----------------|---|------------------|------------------|-------------------------------|---------------------|
| Aphrophoridae | <i>Aphrophora alni</i> (Fallén, 1805) | 1 | - | 1 | BOLD:ADP5304 |
| Aphrophoridae | <i>Neophilaenus campestris</i> (Fallén, 1805) | 7 | - | 2 | BOLD:ACP8461 |
| Aphrophoridae | <i>Philaenus spumarius</i> (Linnaeus, 1758) | 294 | - | 5 | BOLD:AAB1850 |
| Cicadellidae | <i>Anoscopus assimilis</i> (Signoret, 1879) | 2 | - | 2 | BOLD:ACD5842 |
| Cicadellidae | <i>Aphrodes makarovi</i> Zachvatkin, 1948 | 6 | 30 | 1 | BOLD:ADE0937 |
| Cicadellidae | <i>Jikradia cf. olitoria</i> (Say, 1830) | 1 | - | 0 | N/A |
| Cicadellidae | <i>Aconurella prolixa</i> (Lethierry, 1885) | 4 | - | 2 | BOLD:AAZ5698 |
| Cicadellidae | <i>Allygus modestus</i> Scott, 1876 | 6 | 15 | 2 | BOLD:ACR1103 |
| Cicadellidae | <i>Anoplotettix fuscovenosus</i> (Ferrari, 1882) | 41 | 222 | 2 | BOLD:ADP1303 |
| Cicadellidae | <i>Balclutha frontalis</i> (Ferrari, 1882) | 16 | - | 2 | BOLD:AAD4769 |
| Cicadellidae | <i>Circulifer haematoceps</i> (Mulsant & Rey, 1855) | 17 | - | 2 | BOLD:ADO2697 |
| Cicadellidae | <i>Eupelix cuspidata</i> (Fabricius, 1775) | 4 | - | 2 | BOLD:ADN9562 |
| Cicadellidae | <i>Euscelis alsia</i> (Ribaut, 1952) | 12 | - | 2 | BOLD:ADN8776 |
| Cicadellidae | <i>Euscelis lineolata</i> Brullé, 1832 | 532 | 230 | 4 | BOLD:ACY0335 |
| Cicadellidae | <i>Exitianus capicola</i> (Stål, 1855) | 1 | - | 2 | BOLD:ACP7902 |
| Cicadellidae | <i>Goniagnathus guttulinervis</i> (Kirschbaum, 1868) | 4 | - | 1 | BOLD:ACP8518 |
| Cicadellidae | <i>Hishimonus hamatus</i> Kuoh, 1976 | 1 | - | 1 | BOLD:ADT2006 |
| Cicadellidae | <i>Neoliturus fenestratus</i> (Herrich-Schäffer, 1834) | 1 | - | 2 | BOLD:ADP0308 |
| Cicadellidae | <i>Opsius stactogalus</i> Fieber, 1866 | 1 | - | 0 | N/A |
| Cicadellidae | <i>Orientus ishidae</i> (Matsumura, 1902) | 1 | - | 0 | N/A |
| Cicadellidae | <i>Phlepsius spinulosus</i> Wagner, 1963 | 3 | - | 2 | BOLD:ADO1967 |
| Cicadellidae | <i>Placotettix taeniatifrons</i> (Kirschbaum, 1868) | 27 | 4 | 2 | BOLD:ADI7221 |
| Cicadellidae | <i>Selenocephalus conspersus</i> (Herrich-Schäffer, 1834) | 29 | 29 | 2 | BOLD:ADP0043 |
| Cicadellidae | <i>Synophropsis lauri</i> (Horváth, 1897) | 67 | 79* | 2 | BOLD:ACR2360 |
| Cicadellidae | <i>Thamnotettix dilutior</i> (Kirschbaum, 1868) | 9 | 34 | 2 | BOLD:ACQ1394 |
| Cicadellidae | <i>Bugraia ocularis</i> (Mulsant & Rey, 1855) | 29 | 1 | 2 | BOLD:ADO9929 |
| Cicadellidae | Eurymelini sp. | 1 | - | 1 | BOLD:ADP0191 |
| Cicadellidae | <i>Anaceratagallia ribauti</i> (Ossiannilsson, 1938) | 2 | - | 3 | BOLD:ACP5684 |
| Cicadellidae | <i>Austroagallia sinuata</i> (Mulsant & Rey, 1855) | 5 | - | 0 | N/A |
| Cicadellidae | <i>Megophthalmus scabripennis</i> Edwards, 1915 | 2 | - | 2 | BOLD:ACS2510 |
| Issidae | <i>Agalmatium bilobum</i> (Fieber, 1877) | 1 | - | 1 | BOLD:ADQ5346 |
| Issidae | <i>Agalmatium flavescens</i> (Olivier, 1791) | 14 | - | 3 | BOLD:ADO2357 |
| Issidae | <i>Issus muscaeformis</i> (Schrank, 1781) | 18 | 163 | 1 | BOLD:ADK5090 |
| Issidae | <i>Latilica maculipes</i> (Melichar, 1906) | 180 | 391 | 2 | BOLD:ADJ2737 |
| Tettigometridae | <i>Tettigometra impressifrons</i> Mulsant & Rey, 1855 | 6 | - | 5 | BOLD:ADP2274 |
| Tettigometridae | <i>Tettigometra laeta</i> Herrich-Schäffer, 1835 | 2 | - | 0 | N/A |
| Tettigometridae | <i>Tettigometra virescens</i> (Panzer, 1799) | 17 | - | 1 | N/A |
| | Total | 1364 | 1198 | 66 | 31 |

corresponded to adults already collected in the study area. However, adults belonging to two distinct Cicadellidae species, namely *Synophropsis lauri* (Horváth, 1897) and *Placotettix taeniatifrons* (Kirschbaum, 1868), were obtained for one single nymph morphotype.

The COI sequences (658 bp) were obtained for 65 adult specimens, representing 33 out of the 38 morphotypes (Table 1). Sequencing failed for five morphotypes, including four Cicadellidae – *Jikradia cf. olitoria* (Say, 1830) *Orientus ishidae* (Matsumura, 1902), *Opsius stactogalus* Fieber, 1866 and *Austroagallia sinuata* (Mulsant & Rey, 1855) – and the Tettigometridae *Tettigometra laeta* Herrich-Schäffer,

1835. In summary, 42% of the obtained BOLD identification numbers (BIN) were new, 23% led to new species identifications (i.e. BINs with no species name), 19% matched identified sequences and 16% were incongruent (see below). NJ reconstructions of the different families (Figures 1–3) revealed the presence of 32 distinct species, thus confirming most of the initial morphotypes designations. The sequences of 14 of them are new to science and correspond to species for which no COI barcode was available in the online databases. BOLD systems thus created new BINs for 13 of these genetic entities (Table 1). No BIN was assigned to the last, namely *Tettigometra virescens* (Panzer, 1799) (Tettigometridae),

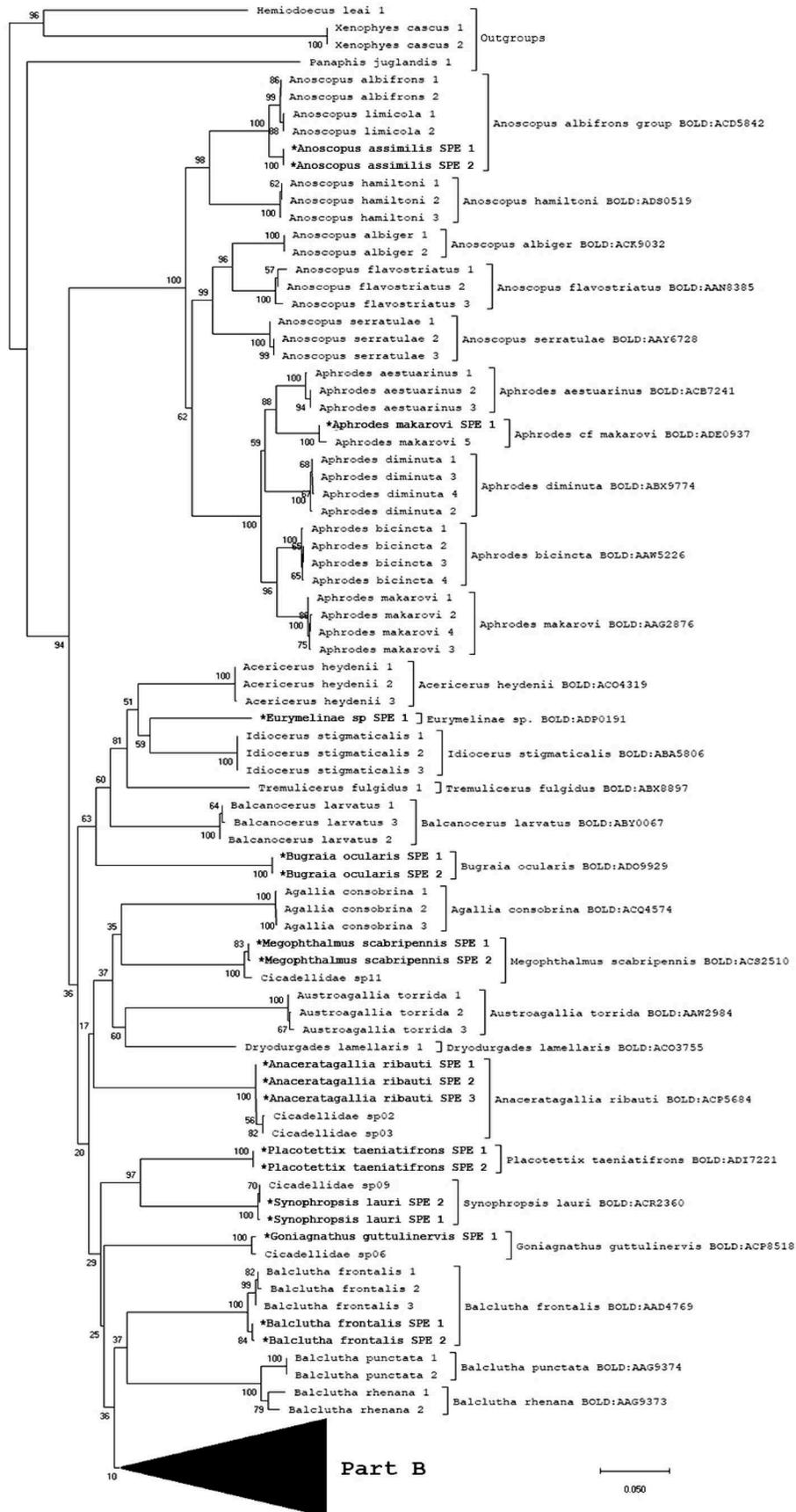


Figure 1. Neighbor-joining tree using Kimura's 2-parameter model (10,000 bootstrap replicates) illustrating distance clustering among Cicadellidae species based on 658 bp COI sequences. *Corsican specimens. For each of the distinct clusters, the species name (as recorded in the Genbank or BOLD databases) and the BIN attributed by the BOLD systems are indicated.



Figure 1. (Part B)

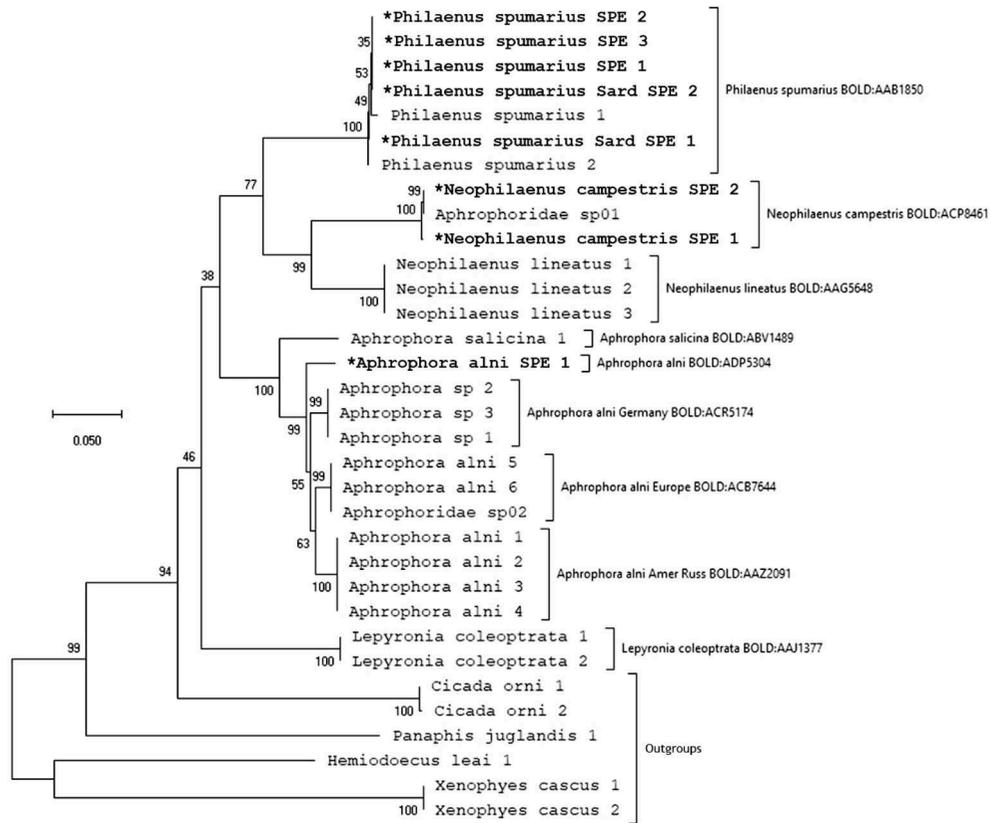


Figure 2. Neighbor-joining tree using Kimura’s 2-parameter model (10,000 bootstrap replicates) illustrating distance clustering among Aphrophoridae species based on 658 bp COI sequences. *Corsican specimens. For each of the distinct clusters, the species name (as recorded in the Genbank or BOLD databases) and the BIN attributed by the BOLD systems are indicated.

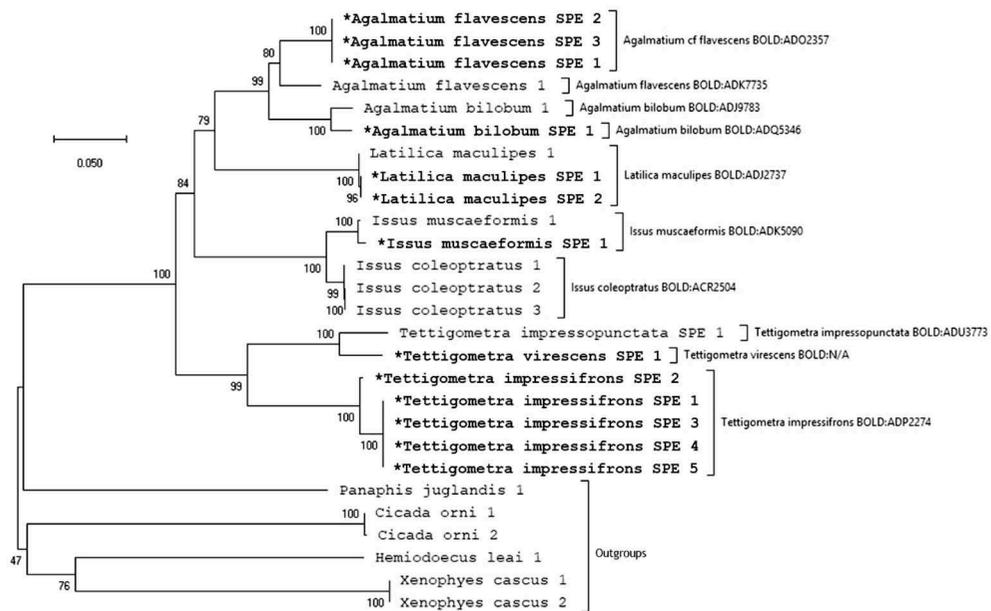


Figure 3. Neighbor-joining tree using Kimura’s 2-parameter model (10,000 bootstrap replicates) illustrating distance clustering among Issidae and Tettigometridae species based on 658 bp COI sequences. *Corsican specimens. For each of the distinct clusters, the species name (as recorded in the Genbank or BOLD databases) and the BIN attributed by the BOLD systems are indicated.

because its sequence did not fulfill the quality requirements imposed by the BOLD systems. On the other hand, 13 genetic entities corresponded to sequences already available on the online databases. While six of them were identified to the species level and corresponded to our initial identifications, the other sequences corresponded to non-identified species. However, the low genetic divergence within their respective clade (0–1.2%) leads us to think that these unnamed sequences could likely be conspecific with our named specimens.

Distinct morphotypes assigned to a same BIN

The NJ reconstruction of the Tettigometridae family (Figure 3) revealed that two distinct morphotypes belonging to the genus *Tettigometra* Latreille, 1804 (dark and brachypterous form vs. orange and winged form) were conspecific and corresponded to distinct forms of *T. impressifrons* Mulsant & Rey, 1855, thus confirming the morphological identifications by T. Bourgoïn.

The two female specimens tentatively identified as *Anoscopus assimilis* (Signoret, 1879) (Cicadellidae, Aphrodinae) appeared to belong to a clade including two additional species, *A. albifrons* (Linnaeus, 1758) and *A. limicola* (Edwards, 1908) (0.3–2.1% divergence). However, morphological identification is not possible for the females, and very challenging for the males. Consequently, identification errors cannot be excluded,

concerning the Corsican specimens or the specimens deposited in the online databases.

Same morphotype assigned to different BINs

The sequences of five taxa presented some barcoding incongruences compared to sequences present in the databases (Figure 4; Supplementary material Table S2).

The sequences of the specimens identified as *Agalmatium flavescens* (Olivier, 1791) (Issidae) presented significant divergences (5.9%) compared to the unique sequence of the conspecific taxon from Italy. To a lesser extent, the Corsican specimen of *Agalmatium bilobum* (Fieber, 1877) (Issidae) presents some genetic divergence (2.9%) compared to the unique sequenced specimen from Lebanon. However, with only a single specimen available in both cases, further consideration will be avoided.

The sequence of the unique Corsican specimen identified as *Aphrodes makarovi* Zachvatkin, 1948 (Cicadellidae, Aphrodinae) is identical to a specimen collected in British Columbia (Canada) and thus shares the same specific BIN (Supplementary material Table S2). However, they present an important genetic divergence compared to conspecific specimens from North America and Northern Europe (5.9–6.8%) and to any other species of *Aphrodes* Curtis, 1833 (5.7–7.6%). The identification of *Aphrodes* specimens is often not possible using morphological characters alone. Nevertheless, acoustic and molecular (COI barcoding)

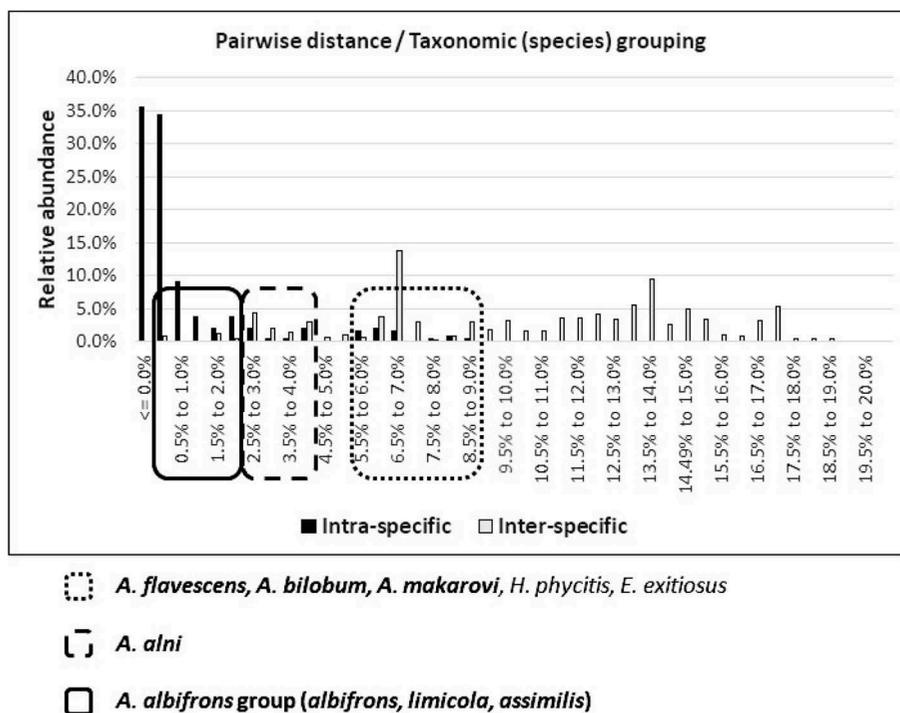


Figure 4. Intra- and inter-specific pairwise differences between the Auchenorrhyncha taxa used in this study. Groupings were generated from species identifications. Plain line box: inter-specific issue; dotted line box: intra-specific issue; dashed line box: intra-specific potential issue.

identifications of the currently recognized European species resulted in congruent results (Bluemel et al. 2014). According to the authors, these taxa presented between 4.2% and 7.0% of genetic divergence, a range similar to that observed for the Corsican and Canadian specimens (Supplementary material Table S2). It thus should be interesting to include these to clarify their position within the *Aphrodes* clade.

The sequences of *Aphrophora alni* (Fallén, 1805) (Aphrophoridae) presented between 2.4% and 4.1% of divergence (Supplementary material Table S2), which is in the range of the 3% arbitrary threshold usually admitted to distinguish conspecific from non-conspecific animal taxa based on COI barcodes (Meyer & Paulay 2005). However, four distinct BINs, corresponding to four distinct genetic lineages on the NJ tree (Figure 2), have been assigned by BOLD systems to the different existing sequences attributed to *A. alni*. The unique Corsican specimen successfully sequenced, for whom a new BIN has been created, appeared to be the most divergent taxa (3.7–4.2%). While specimens from North America and Russia correspond to a specific clade, the situation appears to be more complex for the Western European ones, which are divided into three distinct groups. Complementary studies are thus necessary to clarify the systematics and genetic structure of this taxon.

Auchenorrhyncha fauna in Corsica

The barcoding approach allowed confirming the presence of 32 different Auchenorrhyncha species in our sampled community. When considering the five well distinct morphotypes for which no sequence was obtained, it can be assumed that a total of 37 species have been recorded during the one-year survey. With up to 60.5% of specimens collected, the Cicadellidae was the most abundant family, followed by the Aphrophoridae (22.2%), the Issidae (15.6%) and the Tettigometridae (1.8%). Similarly, the Cicadellidae is the most diversified family, with up to 27 recorded species; the other families are on the contrary poorly diversified with only three (Aphrophoridae and Tettigometridae) and four (Issidae) species. To our knowledge, seven species are new records for Corsica. Four of them – *Latilica maculipes* (Melichar, 1906), *Agalmatium flavescens*, *A. bilobum* and *Issus muscaeformis* (Schrank, 1781) – belong to the Issidae family and are native European species. In return, none of the Issidae species supposed to be present in Corsica (Della Giustina & Bonfils 1978; Gnezdilov et al. 2014) was found during the survey. *Latilica maculipes*, one of the most abundant Auchenorrhyncha species in the Ajaccio region, has never been recorded in Corsica before this study. The three others species are alien Cicadellidae coming from the Americas (*Jikradia* cf. *olitoria*) or the Oriental region (*Hishimonus hamatus* Kuoh, 1976 and

Orientus ishidae); however, one single specimen of each of these leafhopper species has been observed.

Alien vector species

The alien species recorded here for the first time in Corsica have relatively recently been detected in Europe. The first European occurrence of a *Jikradia* Nielson, 1979 species (*J. olitoria*) was recorded in Italy in 2013 (Nielson et al. 2014); while this species was suspected in the SW of France from 2016 (Insecte.org 2016), it is to-date the only observation validated in Europe. The two Oriental leafhopper species *O. ishidae* and *H. hamatus* have already been detected in Europe, in Italy since 1998 (Guglielmino 2005) and in Slovenia since 2012 (Seljak 2013), respectively. Since their first observation, both species expanded their distribution range and can now be found in most of the West-European countries (Trivellone et al. 2015; Klejdysz et al. 2017). These three alien species or related ones are known to be vectors of plant pathogenic diseases, particularly phytoplasmas. These organisms are close to bacteria but with no wall and no nucleus, develop in the phloem of the plants and need phloem feeder insects (mainly Auchenorrhyncha) for dispersal (Weintraub & Beanland 2006). Phytoplasmas are known to be responsible for several hundreds of plant diseases worldwide (Firrao et al. 2005). The leafhopper *J. olitoria* is a potential vector of a phytoplasma associated with the North American Grapevine Yellows, a lethal disease of grapevines in the USA (Lenzi et al. 2019). Similarly, it has been demonstrated in Italy that the Oriental species *O. ishidae* is a vector of the phytoplasma associated with the Flavescence Dorée, which is one of the most serious diseases affecting European vineyards (Lessio et al. 2016). Species related to *H. hamatus*, such as *H. phycitis* (Distant 1908) and *H. sellatus* (Uhler 1896), are vectors of phytoplasmas affecting among others *Citrus aurantiifolia* (Christm.) Swingle (witches' broom) in China, *Solanum melongena* L. (little leaf of eggplant) in India and *Ziziphus jujuba* Mill. (Jujube witches' broom) in eastern Asia (Kusunoki et al. 2002; Hao et al. 2015). Moreover, it has been demonstrated that *H. sellatus* can transmit different phytoplasmas and thus can be the vector of different diseases (Kusunoki et al. 2002). *H. phycitis* is considered as a potential threat and is regarded as an EU quarantine pest by the European Food Safety Authority (EFSA 2017).

Eco-ethology information on the main species

Three out of the 37 recorded species represented up to 74.3% of the total number of the collected adult insects (Figure 5): *Euscelis lineolata* Brullé 1832 (Cicadellidae; 39.3%), *Philaenus spumarius* (L. 1758) (Aphrophoridae; 21.7%) and *Latilica maculipes* (Issidae; 13.3%). Among the nymphs, the more represented species were

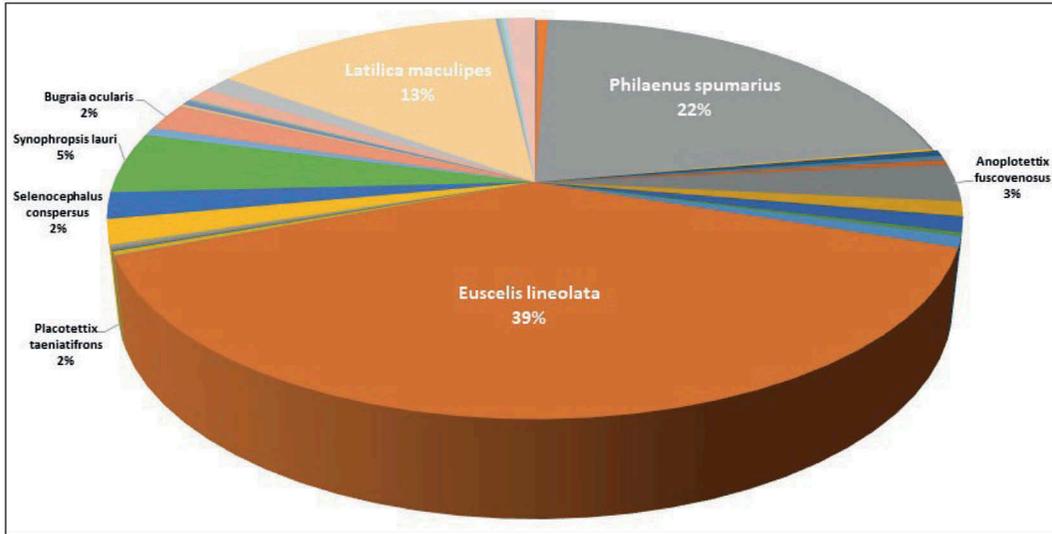


Figure 5. Auchenorrhyncha species (adults) collected during a one-year survey in the Ajaccio region. *Euscelis lineolata*, *Philaenus spumarius* and *Latilica maculipes* account for up to 74.3% of all the insects collected.

L. maculipes (32.6%), *E. lineolata* (Cicadellidae; 19.2%) and *Anoplotettix fuscovenosus* (Ferrari 1882) (Cicadellidae; 18.5%). Even if abundantly present in the studied sites, the nymphs of *P. spumarius*, developing within characteristic foams, have voluntarily not been collected since their identification was not problematic.

***Latilica maculipes* Schrank, 1781.** The planthopper *L. maculipes* is one of the most common species in the studied area. But interestingly, it also constitutes a species new for Corsica (Della Giustina & Bonfils 1978; Gnezdilov et al. 2014). Adults and nymphs, both highly mobile, can be found almost exclusively on shrubs and trees, mainly from

May to September (Figure 6). Planthoppers are considered as phloem feeders (Wang et al. 2016); however, nothing is known about the ecology of *L. maculipes* and about the ecology of the Issidae family as a whole.

***Philaenus spumarius* (Linnaeus, 1758).** The meadow spittlebug *P. spumarius* is a common species that can be found in most of the Holarctic region, in Europe, Asia and in the Americas. With up to 50 distinct morphs, formerly considered as distinct species, this highly polymorphic species has been the subject of many studies from taxonomists and geneticists (Drosopoulos et al. 2010). In

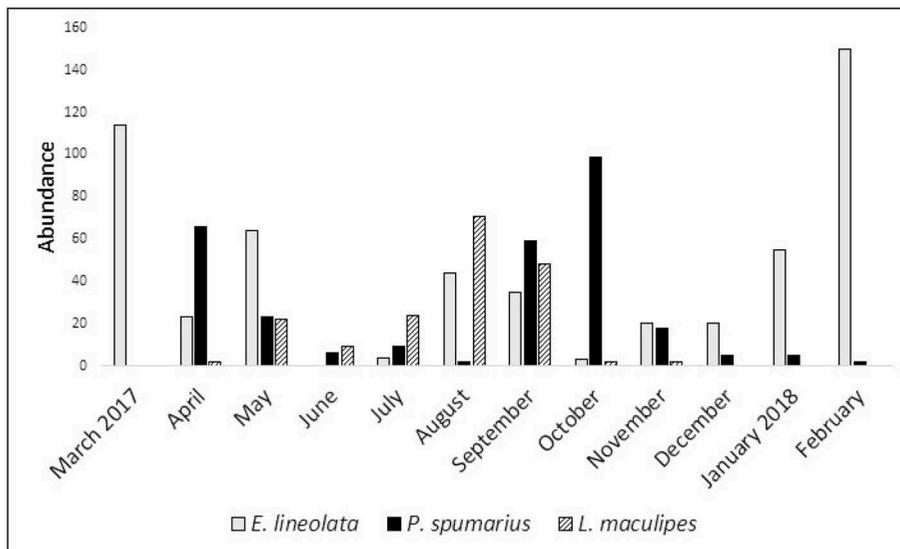


Figure 6. Monthly abundance of the main Auchenorrhyncha species collected in the Ajaccio region.

the studied area, adult specimens were first observed in spring and later in autumn/winter (Figure 6). The first appearance period corresponded to the emergence of the adults, while the second one corresponds to the mating and egg laying period. Extremely few adults were collected between these two periods, which corresponds to the warmest and driest weeks of summer. Actually, adults survive this critical period by estivating in shrubs and trees (Drosopoulos et al. 2010). However, when environmental conditions are more suitable, such as at higher altitudes and/or latitudes, estivation is not necessary and adults can be observed even in summer (Drosopoulos 2003). The nymphs of *P. spumarius*, not very mobile compared to the adults, develop mainly on grasses and shrubs, feeding on the xylem of their host plants (Yurtsever 2000). In Europe, *P. spumarius* is considered as the main vector of *Xylella fastidiosa* (Xanthomonadaceae), one of the most dangerous plant bacteria in the world (Saponari et al. 2014; Cornara et al. 2017), with up to 300 recorded host plants (Redak et al. 2004). *X. fastidiosa* is responsible for Pierce's disease, one of the most serious diseases affecting American vineyards. It is also responsible for economic damage in diverse agricultural or ornamental crops, including *Citrus* L., *Prunus* L., coffee, oleander (Redak et al. 2004) and, more recently, olive trees (EPPO 2013).

***Euscelis lineolata* Brullé, 1832.** In the Ajaccio region, *E. lineolata* was represented by two distinct morphotypes, with differences in the general coloration of the adults and in the sexual appendages of the males (Ribaut 1952). Their conspecificity was confirmed by COI sequences (Figure 1). The first morphotype, *E. lineolata* f. *stictopterus* Flor, 1861, corresponded to the winter generation, while the second one, *E. lineolata* f. *lineolata*, was found in May and later in September. The later morphotype disappeared from the survey during the driest weeks of summer, from June to mid-August (Figure 6). Nymphs were collected from May to June (f. *lineolata*) and from November to February (f. *stictopterus*). Both adults and larvae develop mainly on grasses, feeding on the phloem of the plants. This leafhopper species is predominantly recorded from Western Europe (Müller 1979). Depending on the environmental conditions, this species can present several generations each year with up to seven distinct transitional morphs, formerly considered as different species. Interestingly, this variation also concerns the sexual appendages, with the aedeagus of the males forming eunomic rows of morphs (Müller 1954, 1979). In most animals and particularly in insects, such structures are usually highly conserved for successful mating with conspecific females, and thus constitute powerful taxonomic characters. The sexual appendages are more problematic to interpret in the case of *E. lineolata* and in the related species *E. incisa*

(Kirschbaum, 1858) and *E. alsia* (Ribaut 1952) (Müller 1979). *E. lineolata* is a vector of phytoplasmas responsible for the green-petal disease and clover phyllody, affecting mainly Asteraceae and *Trifolium* L. species, respectively (Weintraub & Beanland 2006).

Conclusions

A one-year survey of the Auchenorrhyncha fauna in the Ajaccio region allowed the presence of 37 species to be recorded, among which seven appear to be new for Corsica. Three of them are alien species, recorded in Europe in the last decades. The local fauna was dominated by three species – *Euscelis lineolata* (Cicadellidae), *Philaenus spumarius* (Aphrophoridae) and *Latilica maculipes* (Issidae) – accounting for up to 74.3% of the total insects collected. Barcoding of the COI region has been successfully performed for 32 species. For 14 of them, the produced sequences are new to science and identifications are provided for seven unnamed taxa already present in the Genbank or BOLD online databases. Moreover, NJ analyses revealed incongruences for five taxa, suggesting that complementary studies may be necessary to clarify their taxonomic level. Although taxonomic interpretations using restricted sampling and COI sequences alone are not robust, the proportion of new and/or suspicious sequences highlight the global lack of knowledge about the Auchenorrhyncha as a whole and the potential presence of cryptic taxa in different clades.

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Supplementary material

Supplemental data for this article can be accessed [here](#).

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