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Morphs of *Philaenus* species, candidate *Xylella fastidiosa* vectors

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Abstract

The genus *Philaenus* presents a well-known colour polymorphism, allowing discrimination of morphs and engaging non-skilled practitioners in species identification. This study considers a collection of approximately 2500 *Philaenus* spp. individuals from southern Italy and northern Tunisia. We felt a morph valid if in our collection or recorded in a minimum of two different references from different geographical locations or timepoints. Reviewing the literature for phenotypes allowed the development of a key to discriminate between the 25 available phenotypes. The study suggests that *Philaenus spumarius* has twenty-three morphs, followed by the eight morphs of *Philaenus signatus*, the seven of *Philaenus tesselatus*, the six of *Philaenus italosignus*, *Philaenus maghresignus*, and *Philaenus tarifa*, the two of *Philaenus loukasi*, and one of *Philaenus arslani*. *P. maghresignus* and *P. tesselatus* show all morphs cited in the literature plus a *gibba* morph based on single female individuals. *P. spumarius* was absent from Tunisian collections; therefore, all data on *P. spumarius* rely on Italian specimens. De-greasing revealed the true phenology of individuals, allowing the classification of ambiguous individuals. Light microscope and SEM observations in *P. spumarius* recently collected in southern Italy (Apulia region) revealed six concave hairless spots on the pronotum corresponding to the dark spots of *impressa* morph, a morph hitherto only known from North America only. *Xylella fastidiosa* was isolated and described in Nearctic. The recent finding of *impressa* morph in Italy may suggest a different route of bacterium introduction in the Old Word by adult vector importation.

Key words: insect-borne pathogen, Hemiptera Aphrophoridae, xylem-sap feeders, IPM.

Introduction

Until recently, xylem-sap feeders Hemiptera have not been considered a severe pest or threat to plant health in the Mediterranean. Today these insects play a primary role in transmitting xylem-inhabiting pathogens in the area. This attitude pushes these species to be key pests for Mediterranean olive tree protection (Picciotti *et al.*, 2021).

The meadow spittlebug *Philaenus spumarius* L. 1758 (Hemiptera Aphrophoridae) is the primary vector of *Xylella fastidiosa pauca* ST53 (Xfp53), the causal agent of the Olive Quick Decline Syndrome (OQDS) that threatens olive groves and oil production (Saponari *et al.*, 2014) impacting agroecosystems in Salento (Apulia) (Pavan *et al.*, 2021). Moreover, other Aphrophoridae species like *Neophilaenus campestris* (Fallen 1805) and *Philaenus italosignus* Drosopoulos et Remane (2000) can acquire and transmit Xfp53, but with a lesser infecting ability if compared to *P. spumarius* (Bragard *et al.*, 2019).

Xylella fastidiosa Wells *et al.* 1987 (Xf) (Wells *et al.*, 1987) appeared in the USA as the causal agent of Pierce's disease in grapevines (*Vitis vinifera* L.) (Hopkins and Purcell, 2002). Later, Xf caused leaf scorching, yield losses, and death of economically important crops in several other economic plants (Hernandez-Martinez *et al.*, 2006; Greco *et al.*, 2021). The induced disease results in losses of millions of dollars in fruits and horticultural and ornamental crops (Redak *et al.*, 2004; Schneider *et al.*, 2021).

Adults of *Philaenus* species are medium-small Cicadomorpha (5.5 to 6.0 mm long and 2 to 2.5 mm large) (Weaver and King, 1954; Drosopoulos and Asche, 1991). The immatures and adults are either polyphagous (Drosopoulos and Asche, 1991; Wood and JoneS, 2020) or specialised (Drosopoulos and Remane, 2000; Drosopoulos, 2003) xylem-sap feeders recorded on more than 1000 host and food plants.

Philaenus adults show multiple sympatric heritable colours pattern morphs within the same species (Weaver and King, 1954; Owen and Wiegert, 1962; Kapantaidaki *et al.*, 2021), being the same morphs slightly phenotypically different in different geographic areas (Farish and Scudder, 1967).

There are several hypotheses regarding the ratio for polymorphism in *Philaenus* species. Natural and genetic selection are the main drivers of morphs variation and frequency in *Philaenus* populations (Yurtsever, 2000b; Maryańska-Nadachowska *et al.*, 2012). Moreover, data suggest that polymorphism in *Philaenus* species is not a consequence of the host plants (Drosopoulos, 2003).

Differences among *Philaenus* morphs consist of the presence, extension, and intensity of marks on the dorsal side of the head, prothorax, and tegmina (Owen and Wiegert, 1962; Halkka, 1964; Halkka *et al.*, 1967; Yurtsever, 2018). Usually, the morphs cluster into melanic and non-melanic (Honěk, 1984; Stewart and Lees, 1996; Yurtsever, 2018).

In the past, different morphs supported the claim for different *Philaenus* species until Nast (1972) synonymised with *P. spumarius* with more than 50 names, previously considered valid species.

Currently, ten species belong to the genus *Philaenus*, namely: *Philaenus arslani* Abdul-Nour et Lahoud 1996; *Philaenus elbursianus* Tishechkin 2013; *Philaenus italosignus* Drosopoulos et Remane 2000; *Philaenus iranicus* Tishechkin 2013; *Philaenus loukasi* Drosopoulos et Asche 1991; *Philaenus maghresignus* Drosopoulos et Remane 2000; *Philaenus signatus* Melichar 1896; *Philaenus spumarius* (L. 1758); *Philaenus tarifa* Remane et Drosopoulos 2001; and *Philaenus tesselatus* Melichar 1899. Each *Philaenus* species shows several morphs, except *P. arslani*, which exists in a single morph. We do not consider the *P. elbursianus* and *P. iranicus* because they do not inhabit the Mediterranean environment.

The numerous studies detailing *Philaenus* and its morphs suggest that a review of the literature is due to the actual invasion of Xf in the Mediterranean and the challenge of candidate or vectors Philaenus identification. Moreover, updating Mediterranean *Philaenus* species with their associated morphs knowledge will help their census in an area of recent invasion. Furthermore, the actual Xf introduction from America to the Mediterranean and the consequent invasion suggests reviewing the gross morphology of vectors and candidate vectors of the pathogen for possible use in Integrated Pest Management (IPM) tuning (Fierro *et al.*, 2019; Liccardo *et al.*, 2020). Moreover, the genetic and molecular approach to frequent and rare morph studies will benefit from single research identifying all morphs. Finally, the present study focuses on *Philaenus* morphs collected in Italy and Tunisia, and the valid *Philaenus* morphs phenotypes allow their quick identification.

Materials and methods

Area of collection

The studied *Philaenus* species originate from a six-year (October 2015-October 2021) survey program of Xf candidate vectors in POnTE, XF-actor and Cure-XF H2020 projects. Investigations and insect collecting were carried out from February to October in olive groves, urban and natural areas in southern Italy (Apulia region) and northern Tunisia, including Bizerte, Cap Bon, and Jendouba territories.

P. spumarius and *P. italosignus* morphs exist in new collections from southern Italy. *P. maghresignus* and *P. tesselatus* morphs were selected in the Tunisian collection (Bouhachem *et al.*, 2019; Lahbib *et al.*, 2019) as expected with the distribution suggested by (Drosopoulos and Remane, 2000; Drosopoulos, 2003; Drosopoulos *et al.*, 2010). The bulk of studied *Philaenus* consists of about 2500 specimens. Data and descriptions of not collected morphs are from literature.

Morph validity

We consider a morph valid based on our collections and literature metadata reporting a morph at least in two references, far in collections place and year of publication. We consider good only the morphs recognised by several authors, including uncommon and rare morphs. We did not assume intermediate morphs.

Several authors studied the genetic inheritance of polymorphism in *Philaenus* (Thompson and Halkka, 1973; Stewart and Lees, 1987; Yurtsever, 2003; Rodrigues *et al.*, 2016). However, few molecular studies are available, focusing only on the most common morphs. The molecular basis of each scheme or colour is still a matter of research (Rodrigues, 2016).

Literature on morphs spreads considerably in time and country of publication. Leaving authors and the publication year *in extenso* will help recognise the corresponding contribution better than a list of numbers (table 1).

Philaenus preservation and imaging

Insects were preserved glued on cards, stored in dry plastic vented vials or Petri dishes or in EtOH (75%, v/v in water) in case of a massive collection. A stereoscope helped the individual scrutiny and identification by slide mounting (Porcelli, 2019). A Zeiss Tessovar® with Olympus Pen camera shot macro pictures. Hitachi TM3000 SEM imaged *Philaenus* cuticular details. An overnight immersion in 10 ml of propyl acetate (95%) (aka propyl ethanoate) followed by air-drying de-greased oily individuals.

Morphs name gender

Finally, we name all the morph in feminine by a change in desinence because of the feminine gender of the "morph" term rooted in the Greek "μορφή- ής" = *morphḗ*, a feminine word of the first declension.

Results and discussion

Several reports of *P. spumarius* exist in North Africa (Algeria, Tunisia, and Morocco) (Puton, 1886; Nast, 1972; Rodrigues *et al.*, 2014). We did not find any specimen of *P. spumarius* in the new collections from Tunisia, supporting a recent study in Morocco (Haddad *et al.*, 2021).

After a study of the available literature on *Philaenus* specimens, we recognise 25 valid morphs in the genus *Philaenus*, namely: *albomaculata* (ALB), *binotata* (BIN), *fasciata* (FAS), *flavicollis* 1 (FLA-1), *flavicollis* 2 (FLA-2), *gibba* (GIB), *giona* (GIO), *hexamaculata* (HEX), *impressa* (IMP), *lateralis* (LAT), *leucocephala* (LCE), *leucophtalma* (LOP), *marginella* (MAR), *melanocephala* (MEL), *praeusta* (PRA), *populi* (POP), *quadrimaculata* (QUA), *rufescens* (RUF), *spumaria* (SPU), *trilineata* (TRI), *typica* (TYP), *ustulata* (UST), *variata* (VAR), *vittata* (VIT), and *vourinos* (VOU) (table 1).

Table 1. *Philaenus* morphs acronym and proper literature.

Acronym	Reference
ALB	Haupt, 1917; Halkka, 1962; Halkka, 1964; Adenuga, 1968; Farish, 1972; Whittaker, 1972; Thompson and Halkka, 1973; Harper, 1974; Klimmaszewski and Kosonocka, 1981; Ossiannilsson, 1983; Berry, 1983; Lees and Dent, 1983; Boucelham and Raatikainen, 1984; Honěk, 1984; Berry and Willmer, 1986; (Halkka and Halkka, 1990, Quartau and Borges, 1997; Yurtsever, 2000b; Kolova, 2009; Biedermann and Niedringhaus, 2009; Mayorova and Kolova, 2015; Silva *et al.*, 2015
BIN	Raatikainen, 1971; Kolova, 2009; Kolova, 2010
FAS	Haupt and Halle, 1911; de Bergevin, 1913; Haupt, 1917; Scholl and Medler, 1947; Weaver and King, 1954; Wagner, 1955; Farish and Scudder, 1967; Farish, 1972; Ossiannilsson, 1981; Biedermann and Niedringhaus, 2009
FLA	Haupt, 1917; Halkka, 1962; Halkka, 1964; Adenuga, 1968; Beregovoi, 1972; Whittaker, 1972; Thompson and Halkka, 1973; Harper, 1974; Klimmaszewski and Kosonocka, 1981; Berry, 1983; Lees and Dent, 1983; Boucelham and Raatikainen, 1984; Honěk, 1984; Thompson, 1984; Berry and Willmer, 1986; Halkka and Halkka, 1990; Loukas and Drosopoulos, 1992; Quartau and Borges, 1997; Hodge and Keesing, 2000; Yurtsever, 2000b; Yurtsever, 2001; Yurtsever, 2004; Zeybekoglu *et al.*, 2004; Kolova, 2009; Tishechkin, 2013; Mayorova and Kolova, 2015; Silva *et al.*, 2015; Mayorova and Kolova, 2019; Tanyeri and Zeybekoğlu, 2019
FLA-1	Raatikainen, 1971; Drosopoulos, 2003; Drosopoulos *et al.*, 2010
FLA-2	Raatikainen, 1971; Drosopoulos, 2003; Drosopoulos *et al.*, 2010
GIB	Haupt and Halle, 1911; Haupt, 1917; Halkka, 1962; Halkka, 1964; Thompson and Halkka, 1973; Whittaker, 1972; Harper, 1974; Ossiannilsson, 1981; Berry, 1983; Lees and Dent, 1983; Boucelham and Raatikainen, 1984; Honěk, 1984; Berry and Willmer, 1986; Halkka and Halkka, 1990; Quartau and Borges, 1997; Yurtsever, 2000b; Remane and Drosopoulos, 2001; Biedermann and Niedringhaus, 2009; Kolova, 2009; Drosopoulos *et al.*, 2010; Mayorova and Kolova, 2015; Silva *et al.*, 2015
GIO	Drosopoulos and Asche, 1991; Drosopoulos *et al.*, 2010
HEX	Beregovoi, 1970; Beregovoi, 1972; Yurtsever, 2000b; Kolova, 2009; Kolova, 2010
IMP	DeLong and Severin, 1950, Severin, 1950; Winkler, 1949
LAT	Haupt and Halle, 1911; Haupt, 1917; Fisher and Allen, 1946; Scholl and Medler, 1947; Weaver and King, 1954; Halkka, 1962; Halkka, 1964; Farish and Scudder, 1967; Adenuga, 1968; Raatikainen, 1971; Beregovoi, 1972; Farish, 1972; Thompson and Halkka, 1973; Whittaker, 1972; Harper, 1974; Klimmaszewski and Kosonocka, 1981; Ossiannilsson, 1981; Berry, 1983; Lees and Dent, 1983; Boucelham and Raatikainen, 1984; Honěk, 1984; Thompson, 1984; Berry and Willmer, 1986; Halkka and Halkka, 1990; Quartau and Borges, 1997; Yurtsever, 2000b; Yurtsever, 2004; Zeybekoglu *et al.*, 2004; Biedermann and Niedringhaus, 2009; Kolova, 2009, Drosopoulos *et al.*, 2010; Mayorova and Kolova, 2015; Silva *et al.*, 2015; Tanyeri and Zeybekoğlu, 2019
LCE	Haupt and Halle, 1911; Haupt, 1917; Doering, 1930; Scholl and Medler, 1947; DeLong and Severin, 1950; Weaver and King, 1954; Halkka, 1962; Halkka, 1964; Farish and Scudder, 1967; Adenuga, 1968; Farish, 1972; Thompson and Halkka, 1973; Whittaker, 1972; Harper, 1974; Klimmaszewski and Kosonocka, 1981; Ossiannilsson, 1981; Berry, 1983; Lees and Dent, 1983; Boucelham and Raatikainen, 1984; Honěk, 1984; Thompson, 1984; Berry and Willmer, 1986; Halkka and Halkka, 1990; Quartau and Borges, 1997; Yurtsever, 2000b; Drosopoulos, 2003; Yurtsever, 2004; Zeybekoglu *et al.*, 2004; Biedermann and Niedringhaus, 2009; Kolova, 2009; Drosopoulos *et al.*, 2010; Silva *et al.*, 2015; Mayorova and Kolova, 2015; Yurtsever, 2018; Mayorova and Kolova, 2019; Tanyeri and Zeybekoğlu, 2019
LOP	Haupt and Halle, 1911; Doering, 1930; Severin, 1950; Weaver and King, 1954; Scholl and Medler, 1947; Winkler, 1949; Halkka, 1964; Farish and Scudder, 1967; Beregovoi, 1972; Farish, 1972; Thompson and Halkka, 1973; Whittaker, 1972; Harper, 1974; Klimmaszewski and Kosonocka, 1981; Ossiannilsson, 1981; Berry, 1983; Lees and Dent, 1983; Boucelham and Raatikainen, 1984), (Honěk, 1984; Thompson, 1984; Berry and Willmer, 1986; Halkka and Halkka, 1990; Quartau *et al.*, 1992; Quartau and Borges, 1997; Drosopoulos, 2003; Kolova, 2009; Drosopoulos *et al.*, 2010; Silva *et al.*, 2015; Yurtsever, 2000a; Biedermann and Niedringhaus, 2009; Mayorova and Kolova, 2019
MAR	Haupt and Halle, 1911; de Bergevin, 1913; Doering, 1930; Scholl and Medler, 1947; Winkler, 1949; DeLong and Severin, 1950; Pielou, 1950; Severin, 1950; Weaver and King, 1954; Wagner, 1955; Halkka, 1962; Owen and Wiegert, 1962; Halkka, 1964; Farish and Scudder, 1967; Adenuga, 1968; Raatikainen, 1971; Beregovoi, 1972; Farish, 1972; Thompson and Halkka, 1973; Whittaker, 1972; Harper, 1974; Klimmaszewski and Kosonocka, 1981; Ossiannilsson, 1981; Berry, 1983; Lees and Dent, 1983; Boucelham and Raatikainen, 1984; Honěk, 1984; Thompson, 1984; Berry and Willmer, 1986; Halkka and Halkka, 1990; Loukas and Drosopoulos, 1992; Quartau and Borges, 1997; Yurtsever, 1999; Yurtsever, 2000a; Remane and Drosopoulos, 2001; Yurtsever, 2001; Drosopoulos, 2003; Yurtsever, 2004; Zeybekoglu *et al.*, 2004; Biedermann and Niedringhaus, 2009; Kolova, 2009; Drosopoulos *et al.*, 2010; Mayorova and Kolova, 2015; Silva *et al.*, 2015; Yurtsever, 2018; Tanyeri and Zeybekoğlu, 2019
MEL	Beregovoi, 1970; Kolova, 2009; Kolova, 2010
PRA	Haupt and Halle, 1911; Haupt, 1917; Halkka, 1964; Whittaker, 1972; Thompson and Halkka, 1973; Harper, 1974; Klimmaszewski and Kosonocka, 1981; Ossiannilsson, 1981; Quartau and Borges, 1997; Kolova, 2009; Drosopoulos *et al.*, 2010; Silva *et al.*, 2015; Yurtsever, 2000a
POP	Haupt and Halle, 1911; de Bergevin, 1913; Haupt, 1917; Doering, 1930; Fisher and Allen, 1946; Scholl and Medler, 1947; Winkler, 1949; Pielou, 1950; Severin, 1950; Weaver and King, 1954; Halkka, 1962; Owen and Wiegert, 1962; Halkka, 1964; Farish and Scudder, 1967; Adenuga, 1968; Farish, 1972; Thompson and Halkka, 1973; Whittaker, 1972; Harper, 1974; Klimmaszewski and Kosonocka, 1981; Ossiannilsson, 1981; Berry, 1983; Lees and Dent, 1983; Honěk, 1984; Thompson, 1984; Berry and Willmer, 1986; Halkka and Halkka, 1990; Drosopoulos and Asche, 1991; Loukas and Drosopoulos, 1992; Quartau *et al.*, 1992; Quartau and Borges, 1997; Hodge and Keesing, 2000; Remane and Drosopoulos, 2001; Yurtsever, 2001; Drosopoulos, 2003; Yurtsever, 2004; Zeybekoglu *et al.*, 2004; Kolova, 2009; Drosopoulos *et al.*, 2010; Tishechkin, 2013; Mayorova and Kolova, 2015; Silva *et al.*, 2015; Borges *et al.*, 2018; Yurtsever, 2018; Tanyeri and Zeybekoğlu, 2019
QUA	Haupt, 1917; Halkka, 1962; Halkka, 1964; Adenuga, 1968; Beregovoi, 1972; Whittaker, 1972; Thompson and Halkka, 1973; Harper, 1974; Klimmaszewski and Kosonocka, 1981; Ossiannilsson, 1981; Berry, 1983; Lees and Dent, 1983; Boucelham and Raatikainen, 1984; Honěk, 1984; Berry and Willmer, 1986; Halkka and Halkka, 1990; Quartau and Borges, 1997; Hodge and Keesing, 2000; Yurtsever, 2000a; Drosopoulos, 2003; Biedermann and Niedringhaus, 2009; Kolova, 2009; Drosopoulos *et al.*, 2010; Mayorova and Kolova, 2015; Silva *et al.*, 2015; Borges *et al.*, 2018; Mayorova and Kolova, 2019; Tanyeri and Zeybekoğlu, 2019
RUF	Melichar, 1896; Adenuga, 1968; Raatikainen, 1971

(Continued)

(Table 1 continued)

Acronym	Reference
SPU	Haupt, 1917; Doering, 1930; Winkler, 1949; DeLong and Severin, 1950; Fisher and Allen, 1946; Scholl and Medler, 1947; Pielou, 1950; Weaver and King, 1954; Owen and Wiegert, 1962; Farish and Scudder, 1967; Adenuga, 1968; Farish, 1972
TRI	Haupt, 1917; Doering, 1930; Fisher and Allen, 1946; Scholl and Medler, 1947; Winkler, 1949; Severin, 1950; Halkka, 1962; Owen and Wiegert, 1962; Halkka, 1964; Farish and Scudder, 1967; Adenuga, 1968; Raatikainen, 1971; Beregovoi, 1972; Farish, 1972; Thompson and Halkka, 1973; Whittaker, 1972; Harper, 1974; Klimmaszewski and Kosonocka, 1981; Ossiannilsson, 1981; Berry, 1983; Lees and Dent, 1983; Boucelham and Raatikainen, 1984; Honěk, 1984; Thompson, 1984; Berry and Willmer, 1986; Halkka and Halkka, 1990; Quartau and Borges, 1997; Yurtsever, 1999; Yurtsever, 2000a; Yurtsever, 2001; Yurtsever, 2004; Zeybekoglu *et al.*, 2004; Kolova, 2009; Drosopoulos *et al.*, 2010; Mayorova and Kolova, 2015; Silva *et al.*, 2015; Yurtsever, 2018; Tanyeri and Zeybekoğlu, 2019
TYP	Haupt and Halle, 1911; Haupt, 1917; Halkka, 1962; Halkka, 1964; Farish and Scudder, 1967; Adenuga, 1968; Beregovoi, 1972; Farish, 1972; Thompson and Halkka, 1973; Halkka and Halkka, 1990; Loukas and Drosopoulos, 1992; Quartau *et al.*, 1992; Quartau and Borges, 1997; Yurtsever, 2000a; Remane and Drosopoulos, 2001; Yurtsever, 2001; Yurtsever, 2004; Kolova, 2009; Mayorova and Kolova, 2015; Mayorova and Kolova, 2019; Tanyeri and Zeybekoğlu, 2019
UST	Haupt and Halle, 1911; de Bergevin, 1913; Haupt, 1917; Halkka, 1964; Beregovoi, 1972; Thompson and Halkka, 1973; Quartau and Borges, 1997; Yurtsever, 2000a; Kolova, 2009
VAR	Drosopoulos, 2003; Drosopoulos *et al.*, 2010
VIT	Haupt and Halle, 1911; Haupt, 1917; Wagner, 1955; Halkka, 1964; Adenuga, 1968; Thompson and Halkka, 1973; Farish, 1972; Thompson and Halkka, 1973; Klimmaszewski and Kosonocka, 1981; Ossiannilsson, 1981; Loukas and Drosopoulos, 1992; Quartau and Borges, 1997; Yurtsever, 2000a; Yurtsever, 2001; Yurtsever, 2004; Zeybekoglu *et al.*, 2004; Kolova, 2009; Drosopoulos *et al.*, 2010; Silva *et al.*, 2015
VOU	Drosopoulos and Asche, 1991; Drosopoulos *et al.*, 2010; Tishechkin, 2013

Morph literature, key, and pictorial representation

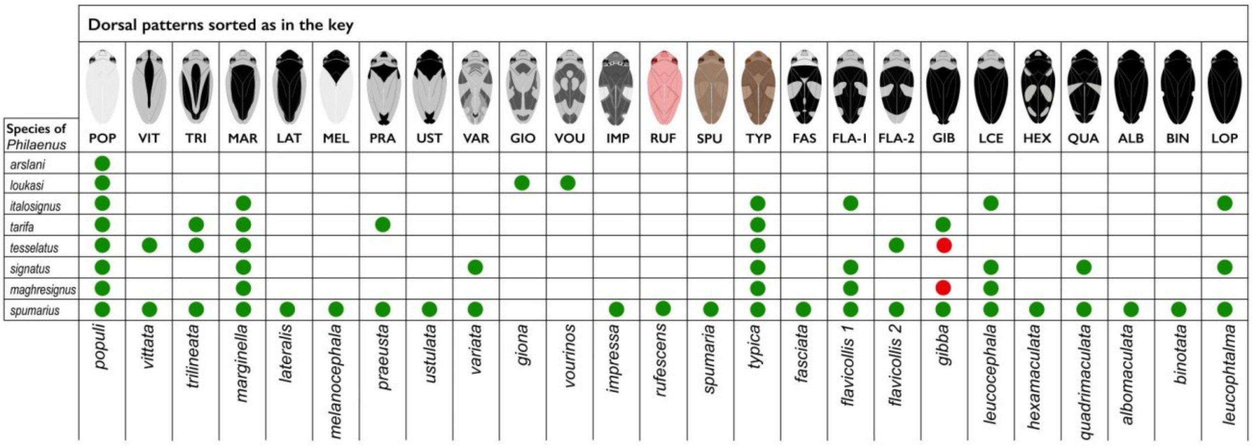
Morphs cluster in melanic and non-melanic, following dorsal pigmentation (Berry, 1983; Honěk, 1984; Yurtsever, 2000a; 2018; Tanyeri and Zeybekoğlu, 2019). However, the classification of certain morphs in melanic or non-melanic is confusing or conflicting. Quartau and Borges (1997) and Yurtsever (2018) classified TYP as a non-melanic morph, but Stewart and Lees (1987) and Drosopoulos (2003) considered TYP a melanic morph.

The major drawback of "melanic vs non-melanic" morphs classification is its pigmentation-only dependence, not considering the shape and the number of the marks. Wagner (1955), Halkka (1964), and Harper (1974) arranged morphs based on the orientation of field pigmentation in two groups of series: *fasciata* and *vittata* and intermingling combinations. The *fasciata* series contains transversely striated forms, while the *vittata* series contains longitudinally striated forms. According to the Wagner (1955), Halkka (1964), and Harper (1974) approaches, we present a *Philaenus* spp. morphs key based on background colour, shape, and extent of dark areas observed from the dorsum.

Key to *Philaenus* morphs

The aphrophorid genus *Philaenus* has eight spurs on the ventral side of the tibial distal end. Tegmina dorsal side has prominent veins. The dorsum, head, prothorax, scutellum and tegmina appear tomentose because of tiny, short seta, apart from an arched series of six hairless areas crossing the prothorax from side to side. Hairless spots exist on the anterior third of the pronotum. The areas are usually concolour with the pronotum, and the two lateral are larger than the sub-medial pair. We expect a certain number of failures in identification because of intermediate morph existence.

The keys in this paper helps identify the *Philaenus* morphs *via* the examination of the adult from the dorsal view.



Dorsal patterns sorted as in the key

Species of *Philaenus*	POP	VIT	TRI	MAR	LAT	MEL	PRA	UST	VAR	GIO	VOU	IMP	RUF	SPU	TYP	FAS	FLA-1	FLA-2	GIB	LCE	HEX	QUA	ALB	BIN	LOP
arslani	🟢																								
loukasi	🟢									🟢	🟢														
italosignus	🟢			🟢											🟢		🟢			🟢					🟢
tarifa	🟢		🟢	🟢			🟢								🟢				🟢						
tesselatus	🟢	🟢	🟢	🟢											🟢			🟢	🔴						
signatus	🟢			🟢					🟢						🟢		🟢			🟢		🟢			🟢
maghresignus	🟢			🟢											🟢		🟢		🔴	🟢					
spumarius	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢			🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢
	populi	*vittata*	*trilineata*	*marginella*	*lateralis*	*melanocephala*	*praeusta*	*ustulata*	*variata*	*giona*	*vourinos*	*impressa*	*rufescens*	*spumaria*	*typica*	*fasciata*	*flavicollis 1*	*flavicollis 2*	*gibba*	*leucocephala*	*hexamaculata*	*quadrimaculata*	*albomaculata*	*binotata*	*leucophtalma*

Figure 1. *Philaenus* morphs sorted in the key order. Green dots mark the presence of the morph in the corresponding species, red dots mark the new morphs hitherto not recorded for that species.

Key to *Philaenus* morphs *via* the examination of adult dorsal view

1	- No mark darker than cuticle	POP
--	- Mark(s) darker than cuticle	2
2	- Non-longitudinal mark(s)	3
--	- Continuous longitudinal mark(s)	4
--	- Discontinuous longitudinal mark(s)	7
3	- Transversal marks	9
4	- Thin mark(s)	5
--	- Broad mark(s)	6
5	- One slender medial mark partially or entirely crossing the head, prothorax, scutellum, and tegmina	VIT
--	- Three slender marks, one medial crossing head (eventually) prothorax, scutellum, and tegmina, two submarginal one per tegmen	TRI
6	- Broad medial to submarginal mark(s) crossing prothorax, scutellum, and tegmina	MAR
--	- Broad medial to submarginal mark(s) crossing head, prothorax, scutellum, and tegmina	LAT
7	- Marks restricted to head, prothorax, and scutellum	MEL
--	- No mark(s) between scutellum and distal tegmina tips	8
8	- Marks on the prothorax, scutellum, and distal tegmina tips	PRA
--	- Marks on head, prothorax, scutellum, humeral angle, and distal tips of tegmina	UST
9	- Four disconnected marks	VAR
--	- Marks connected or disconnected	10
10	- Isolated twin marks at the tip of tegmina	11
--	- No isolated marks at the tip of tegmina, clear scutellum	12
11	- Isolated twin marks at the tip of tegmina, one isolated mark on prothorax	GIO
--	- Isolated twin marks at the tip of tegmina, no isolated mark on prothorax	VOU
12	- Six dark spots marking of each the hairless and micro-sculptured cuticle areas of the pronotum near the head	IMP
--	- No dark spot on pronotum near the head	13
13	- Orange-pink background colour, head concolour	RUF
--	- Middle to light brown background, two large marginals to sub-median pale chevrons marks	14
--	- Background dark brown to black	15
14	- Background rather uniform	SPU
--	- Background mottled; darker margins create a diamond-shaped dorsal mark	TYP
15	- Background dark brown to black, head and pronotal margin clear	16
--	- Background dark brown to black, head concolour	19
16	- Four large marginal to submedian white marks on tegmina	FAS
--	- Four white marks on tegmina discrete or confluent at the tegmina tip	17
--	- Two or no white spots on tegmina tip	18
17	- Four main discrete white marks on tegmina	FLA-1
--	- Posterior white marks confluent at tegmina tip	FLA-2
18	- Two small apical white spots on tegmina	GIB
--	- Tegmina black, no white marks	LCE
19	- Six to four white spots on tegmina	20
--	- Two or no white spots on tegmina	21
20	- Six white spots on tegmina	HEX
--	- Four white spots on tegmina	QUA
21	- Two subapical white spots on tegmina	ALB
--	- Two medial or no white spots on tegmina	22
22	- Two medial white spots on tegmina	BIN
--	- No white spots on tegmina	LOP

Pictorial representation

Figure 1 shows the available morphs reported worldwide sorted following the key. Each green spot marks the presence of that morph in the corresponding species list on the left. Each red dot marks a morph not found hitherto for that species.

Morphs in *P. maghresignus* and *P. tesselatus*

P. maghresignus and *P. tesselatus* show the morphs cited in the literature (Drosopoulos, 2003; Drosopoulos *et al.*, 2010), plus the morph GIB each on a female individual. Endocytobionts study (Kapantaidaki *et al.*, 2021) will possibly mitigate the female identification gap and the consequent difficulties in morph attribution.

Morphs in *P. spumarius*

P. spumarius shows twenty-three morphs; among them, only the morphs *impressa*, *flavicollis*, *rufescens*, and *spumaria* require further discussion.

Figure 2. *P. spumarius* morph *impressa* head and pronotum with the dark minute decoration on the concave hairless cuticle impressions, collected in Bitritto (Bari, Apulia). Measure segment = 1 mm.

Morph *impressa* (figures 2 and 7: IMP)

In 1950 DeLong and Severin (1950) named, described and illustrated the Nearctic morph *impressa* (IMP), including individuals with "a row of four impressed dark pigment spots arranged transversely across the pale anterior portion of the pronotum". It is unclear why the authors report "four impressed dark pigment spots" showing six dark spots in 22 over 24 displayed individuals.

Farish (1972) considered the *impressa* of DeLong and Severin (1950) an available morph. However, Thompson and Halkka (1973) did not list Farish (1972) in their references and refused the validity of DeLong and Severin's (1950) *impressa* morph concept.

We accept the *impressa* morph based on the arguments below.

Farish (1972) wrongly associates the four impressed spots on the Palaearctic *praeusta* morph pronotum with the "four impressed dark" spots on the Nearctic "*impressus*" morph pronotum of DeLong and Severin (1950), thus wrongly concluding that *impressa* morph occurs in the European *Philaenus* population also.

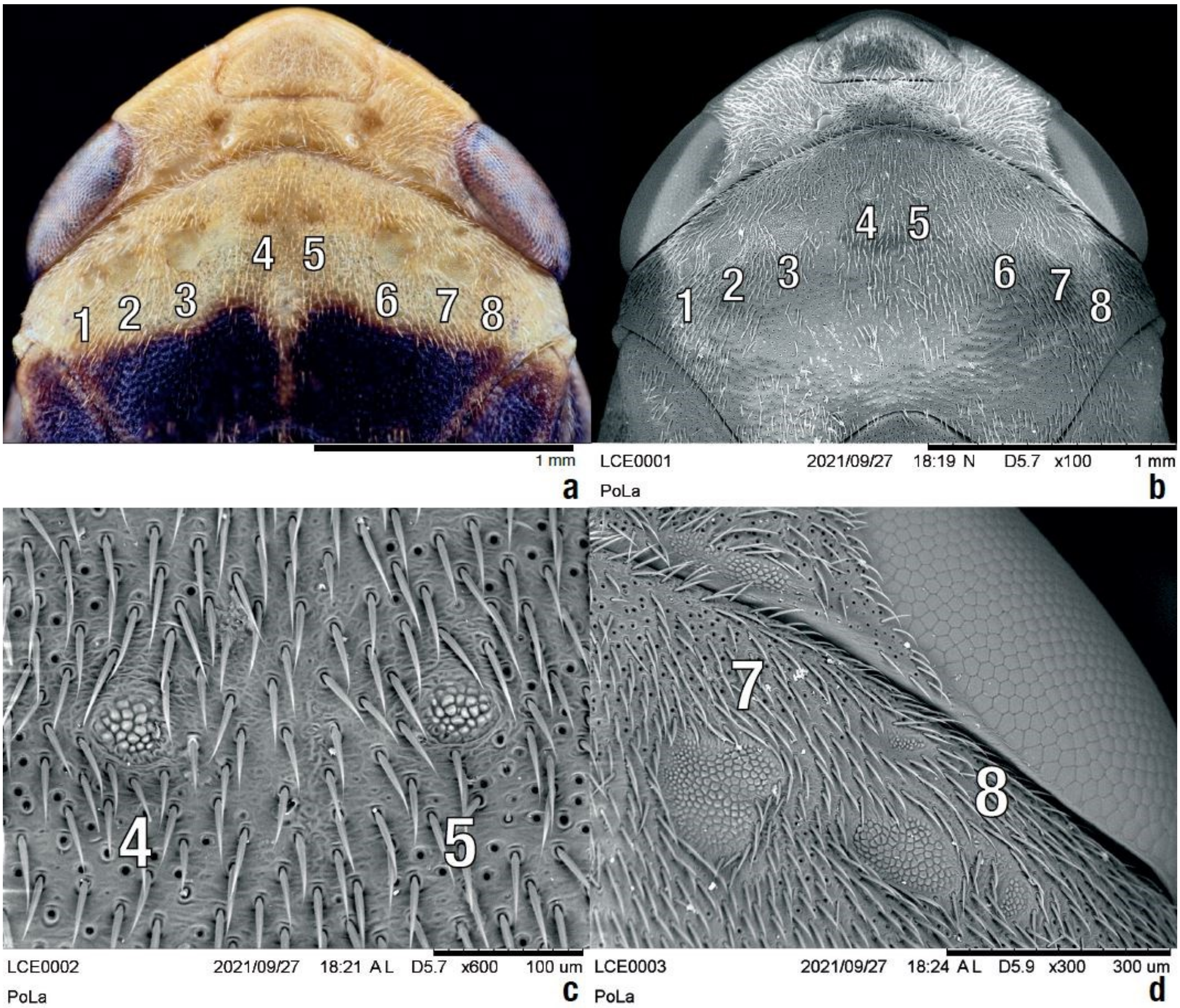


Figure 3. *P. spumarius*, morph *flavicollis* hairless concave areas on the cephalic half of pronotum of the same individual imaged by **(a)** Zeiss Tessovar and **(b**, **c**, **d)** HITACHI TM3000 SEM.

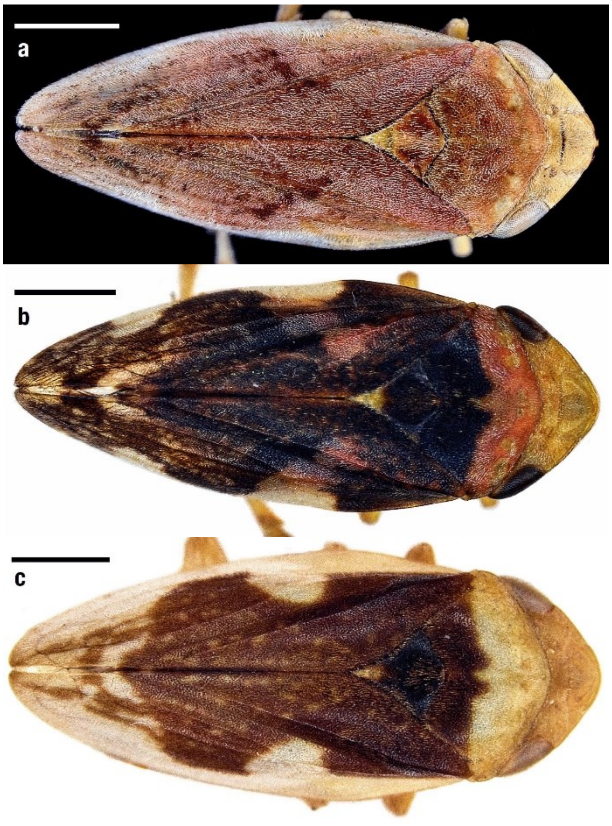


Figure 4. *P. spumarius* **(a)** *rufescens*; **(b)** intermediate morph between *rufescens* and *flavicollis*; **(c)** intermediate morph between *marginella* and *flavicollis*, individuals collected in Bitritto **(a, b)** and Valenzano **(c)** (Bari, Apulia). Measure segment = 1 mm.

Based on DeLong and Severin's (1950) descriptions and illustrations of *impressa*, we found eight individuals in the DiSSPA collection of *Philaenus* that are easily referable to *impressa* morph. The pronotum of each individual shows a sequence of dark brown to black spots corresponding to concave pronotal areas (figure 2).

The four lateral hairless areas are large, and the two median hairless areas are small. Spots counting may shift from five to six because the two median spots are so near each other to join in a single spot if cuticle blackening exceeds the intermingled cuticle area. The position of impressions corresponds to the dark spots place of DeLong and Severin's (1950) *impressa* morph.

Here, we report the presence of the *impressa* morph in Italy. Two of the eight available individuals are in figures 2 and 7: IMP. A comparison with the illustrations provided in DeLong and Severin (1950) supports the *impressa* morph pertinence.

Winkler (1949) and Severin (1950) reported that *P. spumarius* morph *impressa* is a vector of the causal agent of Pierce's disease in North America.

Given the detection of the Xf in Apulia, we guess that a possible further route of Xf introduction *via* imported American infected adults of *P. spumarius*.

Morph *flavicollis* (figure 3)

Haupt (1917) described in Germany a *flavicollis* morph with "five" concavities but no dark marks on the anterior part of the thorax: "*Cicada spumaria, capite, thoraceque antice flavis; in thorace antice puncta quinque impressa*". A misinterpretation may arise from the meanings of the Latin verb "*imprimere*" and its past participle "*impressus*", which means "pressed into or upon", to engrave a concavity or "stamped", "printed by a stain". Haupt (1917) described the morph *flavicollis* with "five" impressed, concave, but "not stained areas".

Scrutinising *Philaenus* individuals, we rediscover the "five" concave areas on the pronotum previously noted in Haupt (1917). The actual macro-photography and SEM allow us to recognise six concave areas deprived of setae in *Philaenus* pronotum (figure 3), nor four, not five, as reported in Haupt (1917) and DeLong and Severin (1950), possibly because the two medial spots are so near and small to be easily misinterpreted as a single one. We suggest that those concave areas correspond to the impressions previously indicated in Haupt (1917) in the morph *flavicollis*.

Morph *rufescens* (figure 4a)

Melichar (1896) and Raatikainen (1971) named a "*rufescens* morph" for *Philaenus* specimens with a red background. Farish (1972) ignored the RUF morph, proposing that the red colouration depended on bad storage, host plant effect, or a genetic mutation, and that reddish colour occurs in morphs with and without patterns.

We consider the morph *rufescens* valid (figure 4a). In Italy, we found *P. spumarius rufescens* individuals with red coloured dorsum and symmetric dark red patterns on tegmina, hairless areas, and pale brown vertex. Moreover, we observed several specimens with intermediate morphs like *rufescens* with *flavicollis* (figure 4b) and *marginella* with *flavicollis* (figure 4c).

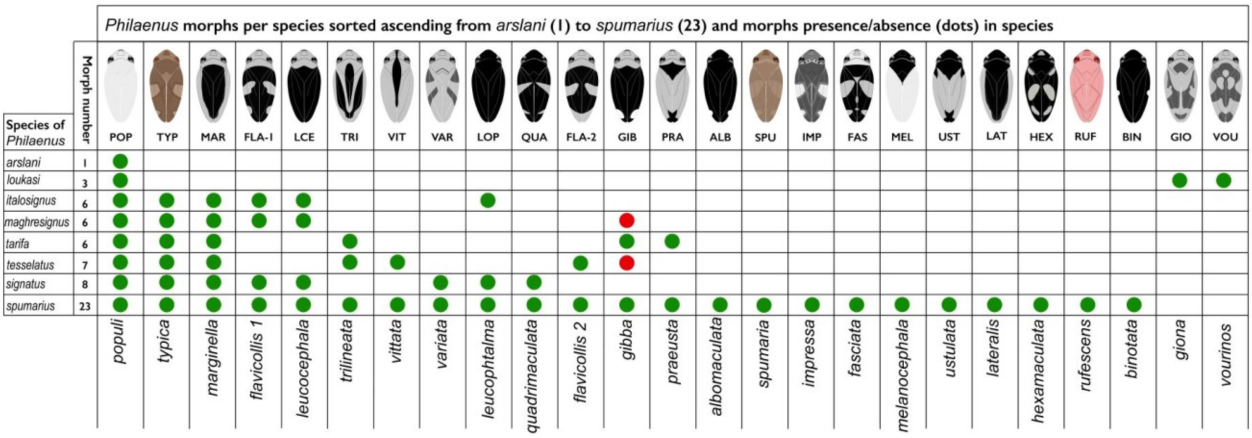
Morph *spumaria*

The morph *spumaria* is known from the Nearctic (Fisher and Allen, 1946; Scholl and Medler, 1947; DeLong and Severin, 1950; Pielou, 1950; Weaver and King, 1954; Owen and Wiegert, 1962; Farish and Scudder, 1967; Adenuga, 1968; Kolova, 2009) whereas the same morph was named *graminis* in Europe (Haupt, 1917; Weaver and King, 1954). We maintain *spumaria* because it is distinguishable from other morphs with pale background by two large marginals to sub-median soft chevrons marks.

Number of morphs and *Philaenus* abundance

The number of morphs is variable among *Philaenus* species (figure 5). The meadow spittlebug *P. spumarius* has the highest number of morphs with 23 different dorsal patterns. *P. signatus* is octomorphic, *P. tesselatus* is heptamorphic, *P. italosignus*, *P. maghresignus* and *P. tarifa* are hexamorphic, *P. loukasi* is trimorphic, and *P. arslani* is monomorphic.

P. spumarius is a widespread and abundant insect in the Holarctic region. Although *P. spumarius* and *P. tesselatus* are genetically similar, the dorsal pattern varies less



Philaenus morphs per species sorted ascending from *arslani* (1) to *spumarius* (23) and morphs presence/absence (dots) in species

Species of *Philaenus*	Morph number	POP *populi*	TYP *typica*	MAR *marginella*	FLA-1 *flavicollis 1*	LCE *leucocephala*	TRI *trilineata*	VIT *vittata*	VAR *variata*	LOP *leucophtalma*	QUA *quadrimaculata*	FLA-2 *flavicollis 2*	GIB *gibba*	PRA *praeusta*	ALB *albomaculata*	SPU *spumaria*	IMP *impressa*	FAS *fasciata*	MEL *melanocephala*	UST *ustulata*	LAT *lateralis*	HEX *hexamaculata*	RUF *rufescens*	BIN *binotata*	GIO *giona*	VOU *vourinos*
arslani	1	🟢																								
loukasi	3	🟢																							🟢	🟢
italosignus	6	🟢	🟢	🟢	🟢	🟢				🟢																
maghresignus	6	🟢	🟢	🟢	🟢	🟢							🔴													
tarifa	6	🟢	🟢	🟢			🟢						🟢	🟢												
tesselatus	7	🟢	🟢	🟢			🟢	🟢				🟢	🔴													
signatus	8	🟢	🟢	🟢	🟢	🟢			🟢	🟢	🟢															
spumarius	23	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢	🟢		

Figure 5. Number of morphs for each *Philaenus* species. Green dots mark the presence of the morph in the corresponding species, red dots mark the new morphs hitherto not recorded for that species.

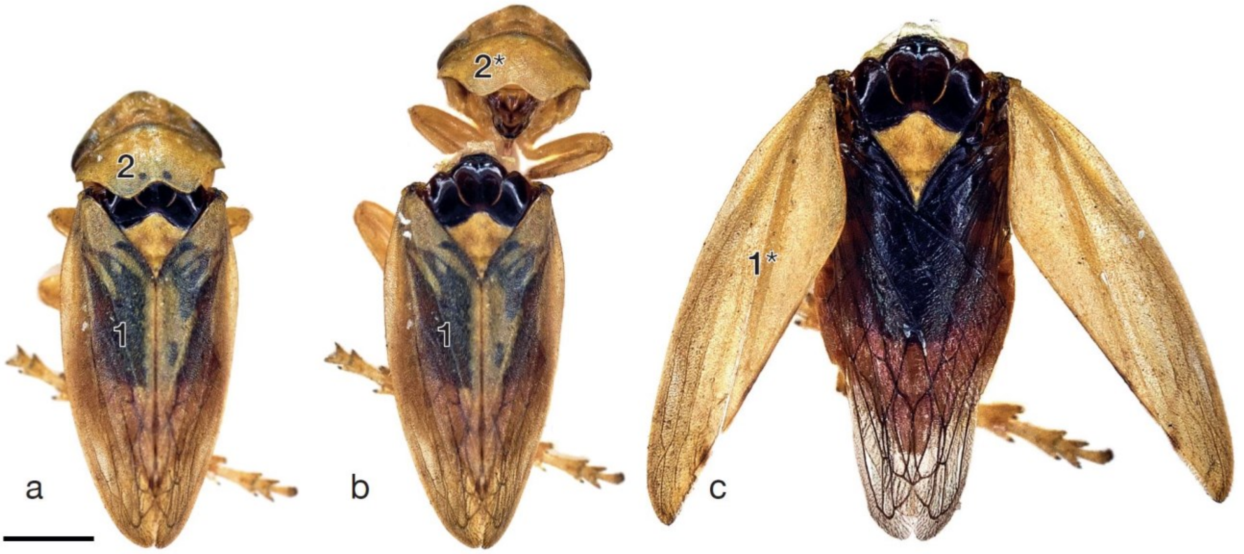


Figure 6. A badly stored and greasy *P. spumarius* **(a)** 1. Blackened area on tegmina, 2. Two black spots of oil on the partially separated head and prothorax, **(b)** 2*. The two black spots disappear on the fully detached head and prothorax, **(c)** 1*. Blackened areas disappear from the separated tegmina. Measure segment in "a" = 1 mm.

in *P. tesselatus* than in *P. spumarius*, which exhibits all the available morphs except the recently described morphs GIO and VOU.

P. arslani was described in 1996 from Lebanon on only 20 collected individuals (Abdul-Nour, 1996). It has only the POP morph. This species appears rare in Lebanon and absent in any other country. Hence, the low number of morphs for the species. The morph POP occurs in all *Philaenus* species. TYP and MAR morphs happen in all species except *P. arslani* and *P. loukasi*. GIO and VOU morphs are reported only in *P. loukasi* (Drosopoulos and Asche, 1991). Morphs such as BIN, FAS, HEX, IMP, LAT, MEL, RUF, SPU, and UST exist only in *P. spumarius*.

The number of morphs per species is subject to the abundance of individuals per species. We cannot wholly accept the argument that some morphs are species-specific in *Philaenus* until many catching exist for each *Philaenus* species.

The population size of *Philaenus* species depends on available host plant biomass in a given area. In Tunisia, both *P. tesselatus* and *P. maghresignus* are present. Still, adults of *P. tesselatus* are polyphagous (Drosopoulos *et al.*, 2010), while adults of *P. maghresignus* thrive "exclusively on *Asphodelus*, mostly *Asphodelus ramosus*, growing under and between oaks" (Drosopoulos, 2003; Drosopoulos *et al.*, 2010). Given the relative host plant abundance, we expect a *P. tesselatus* population larger than a *P. maghresignus* population.

Tunisia hosts nine *Asphodelus* species (Le Floc'h *et al.*, 2010; Osman *et al.*, 2019). An accurate search on *Asphodelus* for *Philaenus* juveniles will result in a census study, supporting a population size estimation. Such a study will also give valuable data for appropriate Xf candidate vector control timing.

Specimen storage and preservation may influence the morphs appearance

Stored, dry-preserved but greasy specimens (Banks, 1909; Upton and Mantle, 2010) can exhibit unconventional dark brown to black-blue lobate spots in the pronotum (Adenuga, 1968; Yurtsever, 2018) (figures 6 and 7). Unusual patterns and unconventional marks can appear during storage because greasing makes the cuticle translucent and more diaphanous. The insect body fat leaks as oil during storage. The oil fills the gap between pronotum and mesonotum, making the black mesonotum apparent as false dorsal patterns, simulating marks through the translucid pronotal posterior flap. It is strongly suggested to de-greasing individuals before morphs examination for a correct morph identification.

Figure 7 shows selected cases with propyl acetate's cleaning, aka propyl ethanoate, effect.

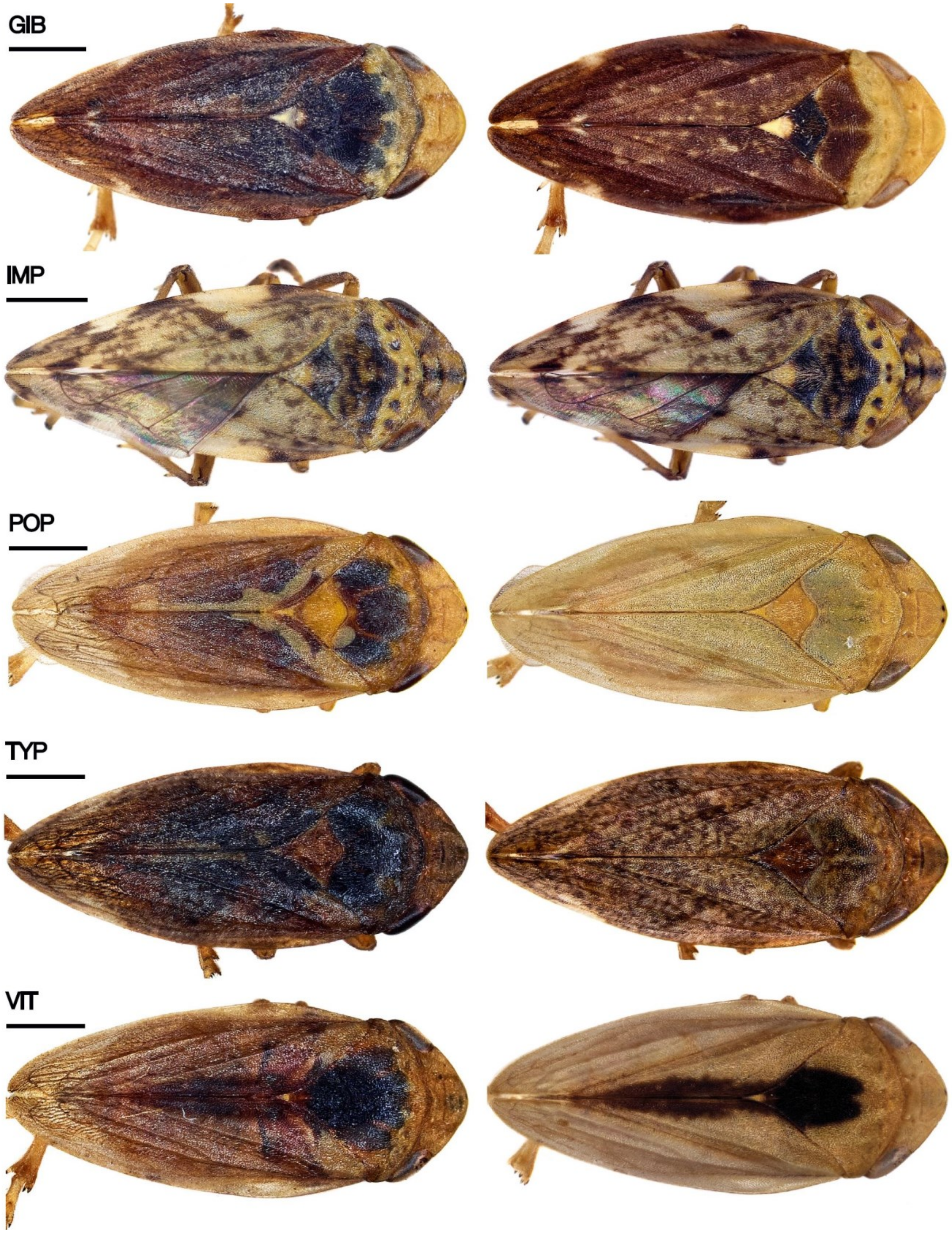


Figure 7. Morphs GIB, IMP, POP, TYP and VIT: left greasy, right: after cleaning. Measure segment = 1 mm.

Conclusions

All the xylem-sap feeders are candidates or vectors of Xf (EFSA, 2018), and Aphrophoridae are all included. *P. spumarius* and *P. italosignus* are Xf vectors in Apulia region. *X. fastidiosa* transmission via other *Philaenus* species is still not proven, but they are considered Xf candidate vectors.

Philaenus species adults show misleading colour patterns polymorphism on tegmina, pronotum and head dorsum. The different *Philaenus* species names and morphs changed considerably among countries and through time, creating confusion in morphs discrimination and specimen identification. Polymorphism makes identification difficult for common species such as *P. spumarius* but easier for scarce entities such as *P. loukasi*. The revision and assemblage of the existing morphs in the genus *Philaenus* and the pictorial key help a first gross identification attempt.

According to the present study on *Philaenus* morphs, *P. spumarius* has 23 morphs, *P. signatus* has eight morphs, *P. tesselatus* has seven morphs, *P. italosignus, P. maghresignus* and *P. tarifa* have every six morphs, *P. loukasi* has three, and *P. arslani* has only one morph.

The morph GIB was observed for the first time in a female of *P. maghresignus* or *P. tesselatus*.

The study of *Philaenus* individuals with Light and Scanning Electron Microscopy revealed six concave hairless and concolour areas in the prothorax. The hairless areas are dark brown to black in *impressa* morph.

Previously known only from North America, the morph "*impressa*" is now in Europe on *Philaenus* collected from Apulia, southern Italy. The morph IMP currently occurs only on *P. spumarius*, the main vector of Xf. The presence of IMP in the Apulian *Philaenus* population suggests that a further entrance route of the bacterium to Europe has been possible by the entrance of infected adult *P. spumarius* from North America.

The morphs species-restriction may help relevant Xf vector candidates identification, supporting single morph wide-area vector surveys for Xf risk management.

Colour pattern schemes could provide the ground for future Xf-vectors' species recognition, following different morphs in different sympatric aphrophorid species populations.

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