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# Differences in wing venation between parthenogenetic and bisexual species of *Empoasca* leafhoppers from Madeira Island

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## Abstract

*Empoasca* is a large worldwide distributed genus of about 400 species many of which are pests to agricultural plants. Species of *Empoasca* are bisexual but recently three parthenogenetic morphotypes (A, B and C) of various degrees of polyploidy have been reported from Madeira Island. Females of *Empoasca* are difficult to identify because they show insufficient morphological diagnostic characters. In this work, we evaluate the utility of wing venation pattern for the identification of the three bisexual and three unisexual taxa of *Empoasca* present in Madeira. Our main motivation is to test whether the wing venation pattern is a stable character and to assess whether it could be suitable to develop an identification key for females of taxa present in Madeira Island. We analysed 107 categorical characters of wing patterns and vein shape in the forewings and hind wings of 677 females. The results showed that wing venation may provide useful characters to identify species of *Empoasca* despite a considerable amount of intraspecific variation. The variation within each species in wing pattern was analysed and several modifications in vein number such as additional or missing veins and in vein shape as bifurcations or incomplete veins are reported.

**Key words:** Hemiptera, Auchenorryncha, Typhlocybinae, parthenogenesis.

## Introduction

The genus *Empoasca* is one of the most specious and economically important genera of the family Cicadellidae (Southern and Dietrich, 2010). It was established by Walsh in 1862 and currently comprises about 400 species grouped in 11 subgenera (Oman *et al*., 1990). Several species of *Empoasca* are relevant pests to agricultural plants such as potatoes, alfalfa, beans, citrus or grapes (Baspinar, 1994; Lamp *et al*., 1994, 2011; Egwurube *et al*., 2005; Kaplan *et al*., 2008; Naseri *et al*., 2009). Nymphs and adults of most species feed on the plant phloem (Poos and Wheeler, 1943) that they suck from the under surfaces of plant leaves (Lamp *et al*., 2004) but some species feed also on parenchyma (mesophyll) cells (Hunt and Backus, 1989; Günthardt and Wanner, 1981). The feeding damage is responsible for the decline in vigour and reduced vitality of infested plants. During the process of feeding, the hoppers inject a toxin that causes stunting of growth, leaf curl, and a complex array of symptoms known as "hopper burn". This malady is characterized by a yellowing of the tissue at the tip and margin of the leaves (DeLong, 1938; Backus *et al*., 2005). In heavy infestations, the leaves turn yellow or brown and most of them fall off, which may reduce the photosynthetic capacity of the plant, but also may affect the quality and quantity of its production. Some species of leafhoppers can act also as vectors of infectious diseases of plants (e.g. *Empoasca stevensi* Young) (Haque and Parasram, 1973). As defended by Poos and Wheeler (1943), information on the identity, distribution, and host plants are of great significance to outline appropriate control measures against those species that act as pests.

Due to its great diversity, large distribution, and external similarity of the species, *Empoasca* is considered a taxonomically difficult genus (Young, 1952). Although males and females are easy to separate from species of other genera due to the reduction of the wing venation pattern, most species of *Empoasca* are very similar to each other (DeLong, 1931; Cunningham and Ross, 1965). Males can be identified according to the shape of the body, abdominal apodemes and the highly specialized male genital structures but females lack good diagnostic characters. In some species, females may differ in the shape of the last abdominal sternite (Cunningham and Ross, 1965) and ovipositor (Balduf, 1934; Demichelis *et al*., 2010; Readio, 1922) but in most cases their identification is only possible when there is an obvious association to males of the same sample. When samples are large, include more than one species or males are absent, the identification of females is usually impossible. Failure in female identification, however, limits the understanding of population dynamics and hampers the selection and operationalization of suitable management of culture practices.

Molecular characters have been proven to be useful for the identification of *Empoasca* females (Demichelis *et al*., 2010; Loukas and Drosopoulos, 1992; Taylor *et al*., 1995) but are still not accessible or economically affordable for routine work. On the other hand, several attempts have been made to find suitable morphological characters of external female genitalia for species identification. Ovipositor structures like tooth morphology, sculpturing and sensilla have been found to be valuable for identification (Balduf, 1934; Cunningham and Ross, 1965; Demichelis *et al*., 2010; Habib *et al*., 1975; Readio, 1922). In addition, morphometric characters of the external anatomy have been used to separate males and females of *Empoasca* from closely allied genera (Quartau and Simões, 1995). The chief inconvenience in using the shape of ovipositional structures and morphometric characters in female identification is that the

observation of these characters under the light stereomicroscope is difficult and time consuming, and therefore, not suitable to apply to the identification of a large number of individuals. Other characters like patterns of wing venation could be more convenient and practical for species identification, if proven useful. Wing shape, texture, and venation are quite distinctive in insects and usually are useful as aides for identification from family to genus and, less often, to species. In Typhlocybinae, wing venations are quite characteristic often down to the generic level (Dworakowska, 1993). Although differences in wing venation have been reported for many *Empoasca* species mainly as part of taxonomic studies and species descriptions (e.g. Lower, 1952; Al-Asady, 2002; Vidano, 1958), the utility of these characters in species recognition has not been carefully determined. In this work, we evaluate the convenience of these characters to separate females of six unisexual and bisexual *Empoasca* taxa present in Madeira Island. Recently, three parthenogenetic morphotypes of *Empoasca* with various degrees of polyploidy have been reported from this island (Aguin-Pombo *et al*., 2006) together with three bisexual *Empoasca* species (Aguin-Pombo and Freitas, 2008; Freitas and Aguin-Pombo, 2004; 2005).

## Materials and methods

### Sampling of specimens

The specimens of *Empoasca* examined consisted in three bisexual species: *Empoasca decedens* Paoli 1932, *Empoasca fabalis* DeLong 1930 and *Empoasca alsiosa* Ribaut 1933 and three unisexual species known to reproduce parthenogenetically (Aguin-Pombo *et al*., 2006) referred here as morphotype A, B and C. In agreement with Dworakowska (1971), we consider here the genus *Asymmetrasca* as a junior synonym of *Empoasca* i.e. *E. decedens* instead of *Asymmetrasca decedens.* All specimens studied were sampled with a net, mainly associated to ornamental and agricultural plants and herbaceous vegetation, in the years 2010 and 2011. Most samples were kept in absolute alcohol but some were fixed in carnoy (1 acetic acid: 3 ethanol). Representatives of these species were sampled in five islands of Macaronesia, namely the archipelago of Madeira (Madeira and Porto Santo) and the Canary Islands (La Palma, Gran Canaria and La Gomera) but also in the European mainland in Greece, Spain (Cádiz, Granada and Córdoba) and Portugal (Portimão). The number of specimens collected, as well as the sampling sites and food plants are shown in table 1.

### Observation and drawings of wing structures

Forewings and hind wings were observed in the whole specimens through a stereomicroscope Olympus SZ-60. For illustrations, wings were removed gently with forceps and then placed in a microscopic slide in a drop of glycerol and covered with a cover slide. The forewing and hind wing vein patterns were observed using phase-contrast and illustrated using a drawing device attached to an Olympus B×50 transmission microscope with plan lenses. The plan lenses adjust focusing errors caused by

**Table 1.** List of taxa included in this study indicating the number of specimens studied and details of sampling regions and food plants. Values correspond to those included in the original data matrix (Matrix 1).

| Taxa | Country | Island/Locality | Plant species in the field book | Nº females |
|---|---|---|---|---|
| Morphotype A | Portugal | Porto Santo Isl. | *Solanum tuberosum*, *S. muricatum*, *Lycopersicon* sp., several herbaceous plants | 14 |
| Morphotype B | Portugal | Porto Santo Isl. | *Solanum tuberosum*, *S. muricatum*, *Ipomoea batatas*, *Prunus* sp., several herbaceous plants | 78 |
| Morphotype C | Portugal | Madeira Isl.<br>Porto Santo Isl. | *Ricinus communis*, *Schinus molle*, *Vicia faba*, Compositae sp. | 212 |
| *Empoasca decedens* | Greece | Gerakari<br>Neochori | *Medicago sativa*, *Citrullus lanatus* | 207 |
| | Portugal | Madeira Isl.<br>Portimão<br>Porto Santo Isl. | *Ricinus communis*, *Vicia faba*, *Rumex* sp. | |
| | Spain | Cádiz<br>Córdoba<br>Granada | *Prunus* sp., *Citrullus lanatus*, *Prunus* sp., *Lycopersicon* sp. | |
| *Empoasca fabalis* | Portugal | Madeira Isl.<br>Portimão<br>Porto Santo Isl. | *Ipomoea batatas*, *I. purpurea*, *Ipomoea* sp., *Rumex* sp. | 141 |
| | Spain | Cádiz<br>Gran Canária Isl.<br>Gomera Isl.<br>La Palma Isl. | *Ipomoea acuminata*, *I. batatas*, *I. purpurea* | |
| *Empoasca alsiosa* | Portugal | Madeira Isl. | *Coprosma repens* | 24 |
| | Spain | Gran Canária Isl.<br>La Palma Isl. | *Phaseolus vulgaris* | |

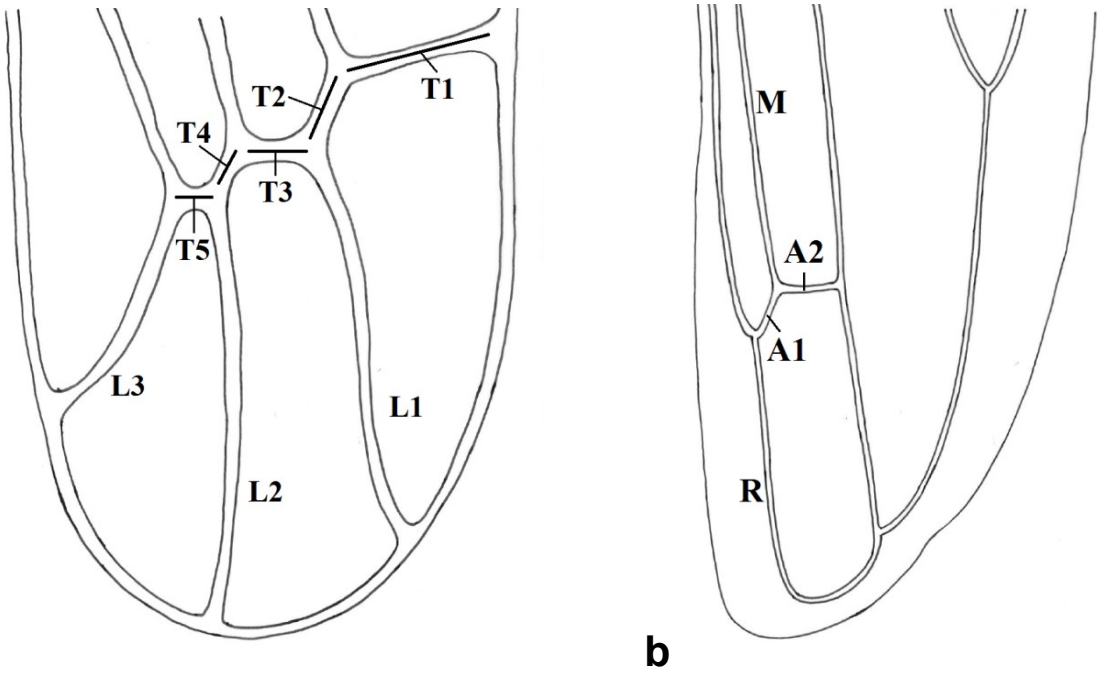


**Figure 1.** Apical part of the left forewings (a) and hind wings (b) indicating the veins studied with bars and the respective codes. FORE WINGS. *Apical transverse veins*: T1 - cubitus anterior; T2 - cubitus posterior; T3 - media anterior; T4 - media posterior; T5 - radius. *Apical longitudinal veins*: L1 - cubitus; L2 - media; L3 - radius. HIND WINGS. A1 - transversal radius; A2 - tranversal media; R - apical radius media; M - media.

field curvature, so focusing was the same throughout the view field. Drawings were performed at a magnification of 200× and 100× for forewings and hind wings respectively. For each specimen, left and right forewings were observed and the shape and relative position of apical veins and apical cells has been numbered from inner to radial margin, for convenience sake (figure 1).

### Variable selection and codification of wing information

The nomenclature of wing veins was intuitively adapted to facilitate the comprehension of the characters studied as indicated in table 2 and figure 1. To characterize the wing pattern of the four wings of each taxon, two main types of structures were studied: the whole wing shape pattern and the shape of each vein separately. The whole wing shape pattern was characterized according to the type of wing pattern (Type) into normal or abnormal and according to the form of the whole wing variation (Var). The wings with abnormal type pattern were those that have rare vein modifications such as incomplete and missing veins or additional and bifurcated veins. The form of the wing variation was classified according to the variation in the relative position of veins. Specimens with the same relative length, position and curvature of veins were considered to have the same form of wing variation. The wings of the individuals of all species were classified in 119 different forms being numbered from 1 up to 119. However, each species has a vein pattern that was the most common and this is referred in the text as the *common wing pattern* or *common pattern*.

In addition, veins of forewings and hind wings were studied independently both in the right and left wings according to the type of vein variation. The characters vein presence or absence (Pr), relative vein length to other veins (Le), curvature of the vein apically (Cu), relative vein position of veins T3 and T5 (Rp) and type of vein variation (Va) were recorded for 8 veins of forewings and 4 veins in hind wings (figure 1). These represented per specimen 66 variables for the two forewings and 32 for the two hind wings.

### Variable reduction procedure

The initial data matrix included 107 variables (9 nominal and 98 ordinals) for 677 female specimens. Of these, 21 variables which showed no variation were discarded remaining 86 variables (Matrix 1). After this preliminary analysis, characters had to be re-codified: 4 as nominal (Var, Type, Cu, Va) and 3 as ordinal (Pr, Le, Rp) as it is shown in table 2. From the Matrix 1, two additional matrices were obtained, one in which were exluded individuals with missing values (Matrix 2) and one other similar to this but without specimens that had one or more veins absent (Matrix 3). These specimens were removed because when a vein was absent, the information of some characters (Cu, Rp, Va, Le, Pr) could not be codified for a large number of other variables. Matrix 2 included 648 individuals and 85 categorical variables (45 nominal and 40 ordinals); one variable was reduced because it showed no variation after removing the specimens mentioned above. Matrix 3 included 79 categorical variables (43 nominal and 35 ordinals) and 472 individuals. Again in this case 7 variables were removed for the same reason as in Matrix 2. From Matrix 2 was obtained a reduced matrix used for cluster analysis, which included the characters Var, Le,

**Table 2.** Structures studied in wings and veins and characters studied in each with respective codes. The codification is the same used in data of Matrix 1.

| **Structures**<br>Structures studied (codes) | Characters studied per structure | Character Code | Character Classification | Structure codes (Nº of variables per specimen) |
|---|---|---|---|---|
| **Wings** | | | | |
| Left forewing (FL)<br>Right forewing (FR)<br>Left hind wing (HL)<br>Right hind wing (HR) | Type of whole wing pattern | Type | 0 = normal pattern<br>1 = abnormal pattern | FL, FR, HL, HR<br>(4) |
| Left forewing (FL)<br>Right forewing (FR)<br>Left hind wing (HL)<br>Right hind wing (HR) | Form of whole wing pattern | Var | 1 = wing pattern 1<br>2 = wing pattern 2<br>...<br>119 = wing pattern 119 | FL, FR, HL, HR<br>(4) |
| **Veins** | | | | |
| Transversal cubitus anterior (T1)<br>Transversal cubitus posterior (T2)<br>Transversal media anterior (T3)<br>Transversal media posterior (T4)<br>Transversal radius (T5)<br>Apical cubitus (L1)<br>Apical media (L2)<br>Apical radius (L3)<br>Apical radius media (R)<br>Media (M)<br>Transversal radius (A1)<br>Transversal media (A2) | Presence of vein | Pr | 0 = vein absent<br>1 = vein present but incomplete<br>2 = vein present | FL & FR<br>(T1, T2, T3, T4, T5, L1, L2, L3)<br>(16)<br><br>HL & HR<br>(R, M, A1, A2)<br>(8) |
| Transversal cubitus anterior (T1)<br>Transversal cubitus posterior (T2)<br>Transversal media anterior (T3)<br>Transversal media posterior (T4)<br>Transversal radius (T5)<br>Apical cubitus (L1)<br>Apical media (L2)<br>Apical radius (L3)<br>Apical radius media (R)<br>Medial (M)<br>Transversal radius (A1)<br>Transversal media (A2) | Relative vein length | Le | 0 = shorter than in most common wing form pattern<br>1 = length as in most common wing form pattern<br>2 = longer than in most common wing form pattern | FL & FR<br>(T1, T2, T3, T4, T5, L1, L2, L3)<br>(16)<br><br>HL & HR<br>(R, M, A1, A2)<br>(8) |
| Transversal cubitus anterior (T1)<br>Transversal cubitus posterior (T2)<br>Transversal media anterior (T3)<br>Transversal media posterior (T4)<br>Transversal radius (T5)<br>Apical cubitus (L1)<br>Apical media (L2)<br>Apical radius (L3)<br>Apical radius media (R)<br>Media (M)<br>Transversal radius (A1)<br>Transversal media (A2) | Vein curvature | Cu | 0 = without curvature<br>1 = with curvature | FL & FR<br>(T1, T2, T3, T4, T5, L1, L2, L3)<br>(16)<br><br>HL & HR<br>(R, M, A1, A2)<br>(8) |
| Transverse media anterior (T3)<br>Transverse radius (T5) | Relative position of T3 and T5 veins | Rp | 0 = T3 much higher than T5<br>1 = T3 higher than T5<br>2 = T3 slightly higher than T5<br>3 = T3 at the same level than T5<br>4 = T3 slightly lower, lower or much lower than T5 | T3, T5<br>(2) |
| Transversal cubitus anterior (T1)<br>Transversal cubitus posterior (T2)<br>Transversal media anterior (T3)<br>Transversal media posterior (T4)<br>Transversal radius (T5)<br>Apical cubitus (L1)<br>Apical media (L2)<br>Apical radius (L3)<br>Apical radius media (R)<br>Medial (M)<br>Transversal radius (A1)<br>Transversal media (A2) | Type of vein variation | Va | 0= no variation<br>1= vein with bifurcation<br>2= additional vein | FL & FR<br>(T1, T2, T3, T4, T5, L1, L2, L3)<br>(16)<br><br>HL & HR<br>(R, M, A1, A2)<br>(8) |

**Table 3.** Form of the most common wing pattern (Var) and percentage of specimens of each taxon showing the most common wing pattern in the fore and hind wings. Codes as in table 2.

| Taxon | F L | | F R | | H L | | H R | | Nº of specimens with |
|---|---|---|---|---|---|---|---|---|---|
| | Var | % | Var | % | Var | % | Var | % | common wing pattern |
| Morphotype A | 2 | 62.5 | 2 | 87.5 | 20 | 100 | 20 | 100 | 8 |
| Morphotype B | 23 | 63.5 | 35 | 60.0 | 35 | 100 | 35 | 100 | 50 |
| Morphotype C | 40 | 65.0 | 40 | 64.7 | 63 | 100 | 63 | 100 | 184 |
| *Empoasca decedens* | 75; 68 | 54.1 | 68; 75 | 59.3 | 87 | 90.4 | 87 | 100 | 135 |
| *Empoasca fabalis* | 92 | 86.4 | 92 | 84.1 | 108 | 100 | 108 | 100 | 44 |
| *Empoasca alsiosa* | 112 | 53 | 112; 111 | 58.8 | 118 | 100 | 118 | 100 | 17 |

Cu and Rp for the left forewing and hind wing (27 variables as a whole). This data matrix comprised the wing patterns more frequently found in each species and were found in 403 female specimens. These common patterns added up to 21 being all included in the cluster analysis.

### Statistical analysis

The differences between species in mean values of all morphological characteristics were compared for each species by the Kruskal-Wallis test and pairs of species were compared by the Wilkoxon test. The chi-square test for goodness-of-fit was used but when the expected numbers were lower than 5, as an alternative, the Fisher's exact test was performed (http://www.langsrud.com/fisher.htm). Significant levels of p-value are shown as follows n.s. = non-significant; (*) $0.5 > p \geq 0.01$; (**) $0.01 > p \geq 0.001$; (***) $p < 0.001$. Statistical analyses were performed by SPSS 10.0 software (SPSS Inc., Chicago, IL).

The cluster analysis of morphological data included the most common wing patterns of all taxa which as a whole are 21 patterns. Cluster analyses based on data of Matrix 2 (see above) were conducted using NTSYSpc version 2.01a (Rohlf, 1998). First it was computed a distance matrix based on the Average taxonomic distances, Manhattan, chi-squared and Euclidean distances with the SIMINT function, prior to being subjected to the unweighted pair-group method (UPGMA) with an arithmetic average clustering algorithm with SAHN function. In order to estimate how well the phenogram represents its corresponding pair-wise distance matrix, the co-phenetic correlation coefficients were calculated for each phenogram and data matrix pair. This was done with the help of COPH and MXCOMP functions of NTSYSpc (Rohlf, 1998). The higher the correlation, the better was the correspondence. If values are larger than 0.9, the correlation is high, while in values lower than 0.74, the correlation is not significant (Sneath and Sokal, 1973).

## Results

### Differences in wing pattern between taxa

The differences in the form of the whole wing pattern (Var), both in the fore and hind wings, allowed the recognition of the six taxa present in Madeira. The general pattern of these species differed in the curvature (Cu), the relative length (Le) and the relative position of the T3 and T5 veins of the forewings (Rp) (figure 2). Each species has a single most common pattern for forewings and hind wings, except *E. decedens* that has two common patterns of the forewings, the pattern number 68 and 75 (table 3). The forewings differed significantly for the 19 variables examined except for the character Cu of the T4 vein of the right wing ($H = 2.667$, 5 d.f., n.s.). Significant differences in the hind wings were observed also in the Cu and Le veins in the seven variables examined, except for relative length vein (Le) of the L2 vein ($H = 9.401$, 5 d.f., n.s.).

The main differences between taxa were observed in the shape of the apical cubital cell (morphotype C), in the shape of the apical media vein (morphotype A), in the shape of the apical radius vein (*E. fabalis*) and in the almost parallel position of the apical media and apical radius veins (*E. decedens*). *E. alsiosa* was the only species that did not show prominent distinctive characters. Morphotype A differed from morphotype B in regards to the shape of the medial cell and apical shape of the veins L1 and L2 (Cu). Morphotype C has a comparatively thinner cubital cell and a more curved T1 vein. *E. decedens* could be identified because the median cell was very regular in shape, due to the almost parallel position of L1 and L2. In the case of *E. fabalis*, L3 was comparatively shorter and more curved than in other taxa. In contrast to forewings, the variation observed in hind wing venation was not as useful but the differences in the transverse apical veins that join the radius and media veins were useful to separate morphotype C, *E. decedens*, and *E. fabalis* (figure 3). In morphotype C, T1 was approximately 1/3 smaller than T2. In *E. decedens*, T2 is almost straight, while in *E. alsiosa*, T1 and T2 form an angle smaller than 90º. The differences between species in the characters Cu, Le and Rp were also large (table 4).

### Differences in wing pattern within taxa

The pattern of wing venation allowed the identification of taxa but the intraspecific variation impairs the identification of a relevant number of individuals. In fact, the percentage of individuals with the common pattern in forewings varies, depending on the species, from 53 to 87%. The number of individuals with variations in the common pattern varied (table 5). In *E. fabalis*, the number of individuals with common pattern was higher (85%) but in *E. alsiosa* and *E. decedens*, the common

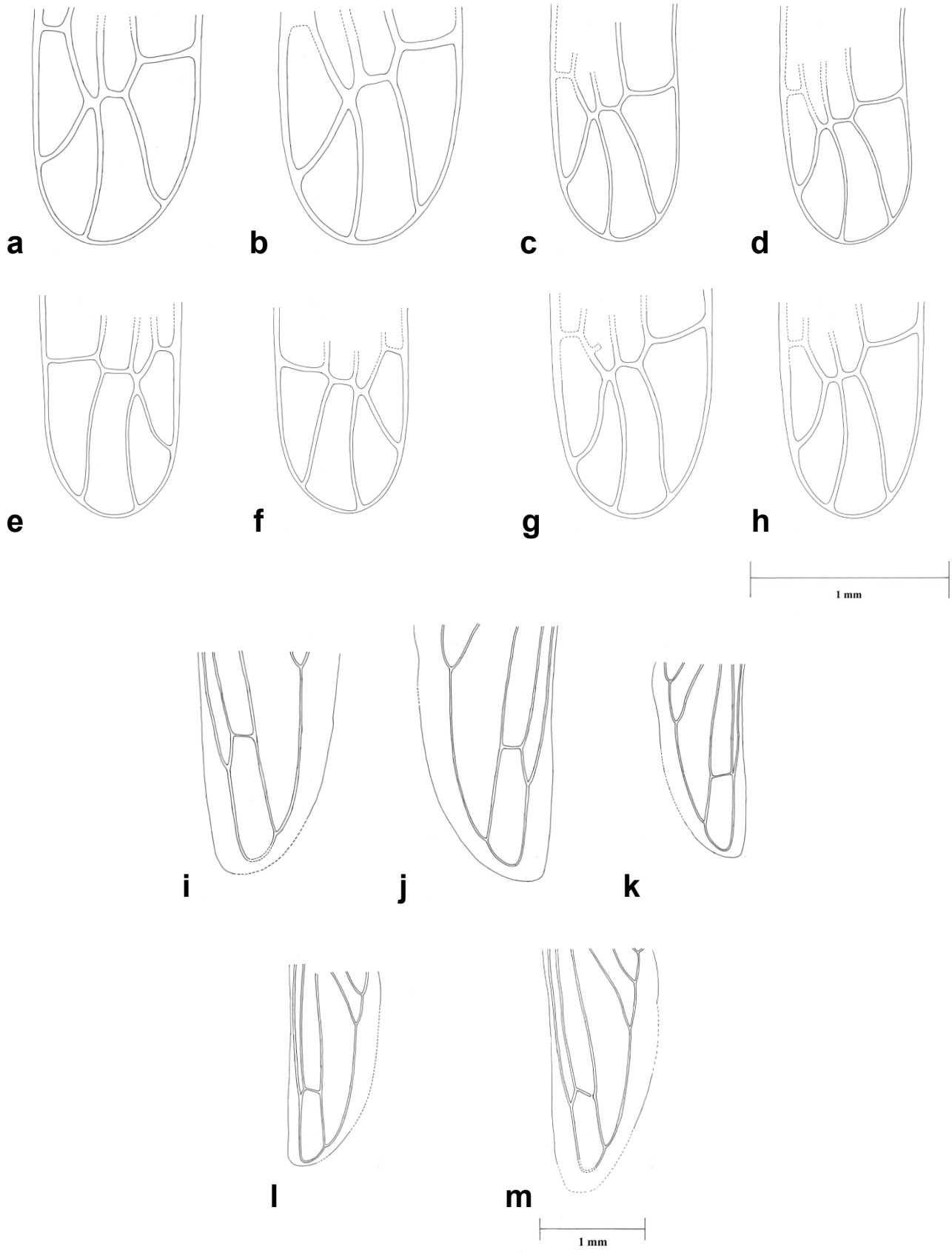


**Figure 2.** Most frequent variations of the most common wing pattern observed in the forewings and hind wings in each taxon. Morphotype A (a, i), Morphotype B (b, j), Morphotype C (c-d, k), *E. decedens* (e, l), *E. fabalis* (f, m) and *E. alsiosa* (g-h).

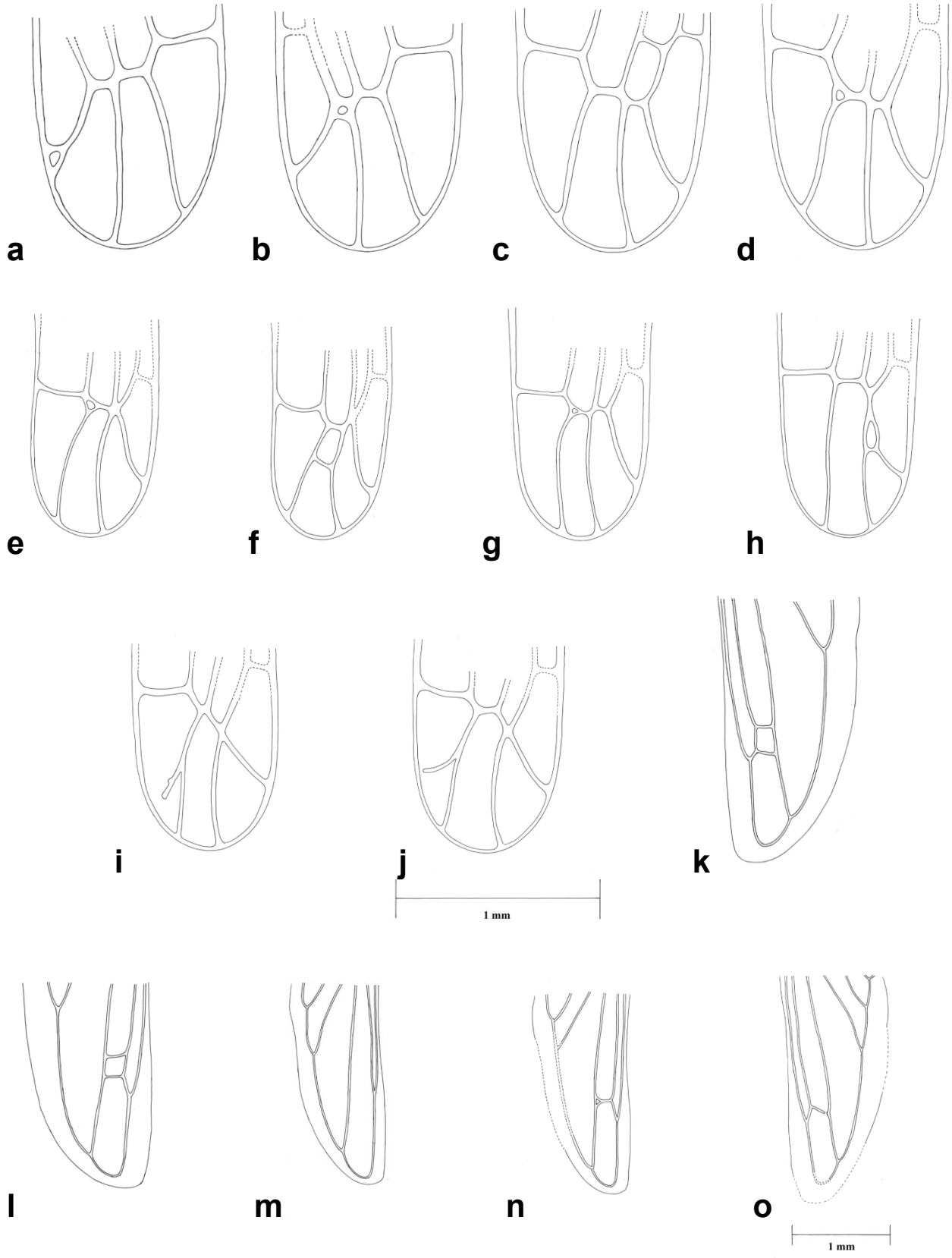


**Figure 3.** Examples of abnormal variations found in the forewings and hind wings of Morphotype A (a, b, k), Morphotype B (c, d, l), Morphotype C (e, f, m), *E. decedens* (g, h, n) and *E. fabalis* (i, j, o). Veins modifications: bifurcations (a, d-e, g-j, n), incomplete veins (i-j, o), additional veins (b-c, f, k-l) and absent veins (m).

**Table 4.** Significant results of the comparisons between taxa for the characters of left fore wing (FL) and hind wings (HL) by Wilcoxon text. The taxa abbreviations are indicated as: A = morphotype A; B = morphotype B; C = morphotype C; D = *E. decedens*; E = *E. fabalis*; F = *E. alsiosa*.

| Structure (Character) | A *vs* B | A *vs* C | A *vs* D | A *vs* E | A *vs* F | B *vs* C | B *vs* D | B *vs* E | B *vs* F | C *vs* D | C *vs* E | C *vs* F | D *vs* E | D *vs* F | E *vs* F |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FL | | | | | | | | | | | | | | | |
| T1 (Le) | * | * | * | ** | | ** | | *** | *** | *** | *** | *** | ** | *** | *** |
| T1 (Cu) | *** | *** | | | * | *** | | *** | * | *** | *** | *** | *** | | *** |
| T2 (Le) | *** | *** | *** | *** | | ** | * | * | *** | | | *** | | *** | *** |
| T2 (Cu) | *** | *** | | ** | | *** | * | *** | | *** | *** | *** | *** | | *** |
| T3 (Le) | * | * | | *** | ** | *** | *** | *** | | | | ** | | | *** |
| T3 (Cu) | * | * | | | | *** | | | | *** | *** | * | | | |
| T3 (Rp) | *** | *** | * | ** | | *** | *** | *** | | *** | *** | *** | *** | ** | ** |
| T2 (Le) | * | * | *** | ** | | *** | * | *** | *** | *** | * | *** | *** | *** | ** |
| T2 (Cu) | * | * | | *** | | * | * | * | ** | | *** | * | *** | * | *** |
| T4 (Le) | *** | *** | *** | *** | | *** | *** | *** | | | *** | *** | *** | *** | ** |
| L1 (Le) | * | * | *** | | ** | ** | *** | | *** | *** | *** | *** | *** | | *** |
| L1 (Cu) | | | *** | | | *** | *** | *** | | | ** | *** | *** | *** | * |
| L2 (Le) | * | * | ** | ** | *** | *** | *** | | *** | *** | *** | *** | *** | * | *** |
| L2 (Cu) | *** | *** | *** | *** | * | | | | | | | | | | |
| L3 (Le) | | | *** | *** | ** | * | *** | *** | *** | *** | *** | *** | *** | ** | |
| L3 (Cu) | * | * | | | | *** | | | | *** | *** | * | | | |
| HL | | | | | | | | | | | | | | | |
| L1 (Le) | | | | *** | | * | *** | | | *** | | | *** | | |
| L1 (Cu) | | | *** | *** | | *** | | | *** | *** | *** | | | *** | *** |
| L2 (Le) | | | | | | * | * | | | * | | | * | | |
| L2 (Cu) | | | *** | | | * | *** | * | | *** | | | *** | *** | |
| T1 (Le) | | | | | | | | | | ** | | | * | | |
| T1 (Cu) | *** | *** | *** | *** | *** | * | ** | | | *** | | | *** | | |
| T2 (Le) | | | | | | | ** | | | *** | | | *** | | |
| T2 (Cu) | *** | *** | | *** | *** | | *** | | | *** | | | *** | *** | |

**Table 5.** Number of individuals which show normal (N) and abnormal (A) wing pattern in the right (FR) and left forewings (FL) and right (HR) and left hindwings (HL). The percentage of individuals with abnormal wing pattern for each taxon is indicated in parenthesis. The values corresponded of those included in data Matrix 2.

| Taxon | F L | | F R | | H L | | H R | | Total nº of specimens observed |
|---|---|---|---|---|---|---|---|---|---|
| | N | A | N | A | N | A | N | A | |
| Morphotype A | 12 | 2 (14.3) | 13 | 1 (7.1) | 14 | 0 | 14 | 0 | 14 |
| Morphotype B | 72 | 2 (2.7) | 69 | 5 (6.8) | 71 | 3 (4.1) | 74 | 0 | 74 |
| Morphotype C | 196 | 8 (3.9) | 195 | 9 (4.4) | 201 | 3 (1.5) | 204 | 0 | 204 |
| *Empoasca decedens* | 195 | 7 (3.5) | 198 | 4 (2.0) | 202 | 0 | 201 | 1 (0.5) | 202 |
| *Empoasca fabalis* | 135 | 0 | 133 | 2 (1.5) | 135 | 0 | 135 | 0 | 135 |
| *Empoasca alsiosa* | 19 | 0 | 19 | 0 | 19 | 0 | 19 | 0 | 19 |
| Total nº of wings | 629 | 19 | 627 | 21 | 642 | 6 | 647 | 1 | |

pattern was present in only about half (53-59%) of the specimens studied. In contrast to forewings, the common pattern in hind wings was very stable for all species, showing almost no variation. In each species the variations in the common pattern of forewings were mainly related to the shape of the cubital, medial, and radial apical cells. This variation was due mainly to differences in the relative length and curvature of the L1, L2 and L3 veins (figure 2), but also was related to the shape and relative position of the transverse T3 and T5 veins. Both veins could be at the same level or one above the other.

## Phenotypic relationships

The most common pattern for each taxon represented about 95-98% of all females (table 3). The most frequent wing patterns in each taxon based on the characters Pr, Le, Cu, Rp and Va were selected to construct a phenogram of similarities using the Average taxonomic distances, Manhattan distances, the chi-squared distance and Euclidean distances. All the clusters obtained showed similar results. The chi-squared distance showed the least distortion with a co-phenetic correlation coefficient of r = 0.974. The phenograms with the Average taxonomic distances (figure 4), Manhattan distances, and

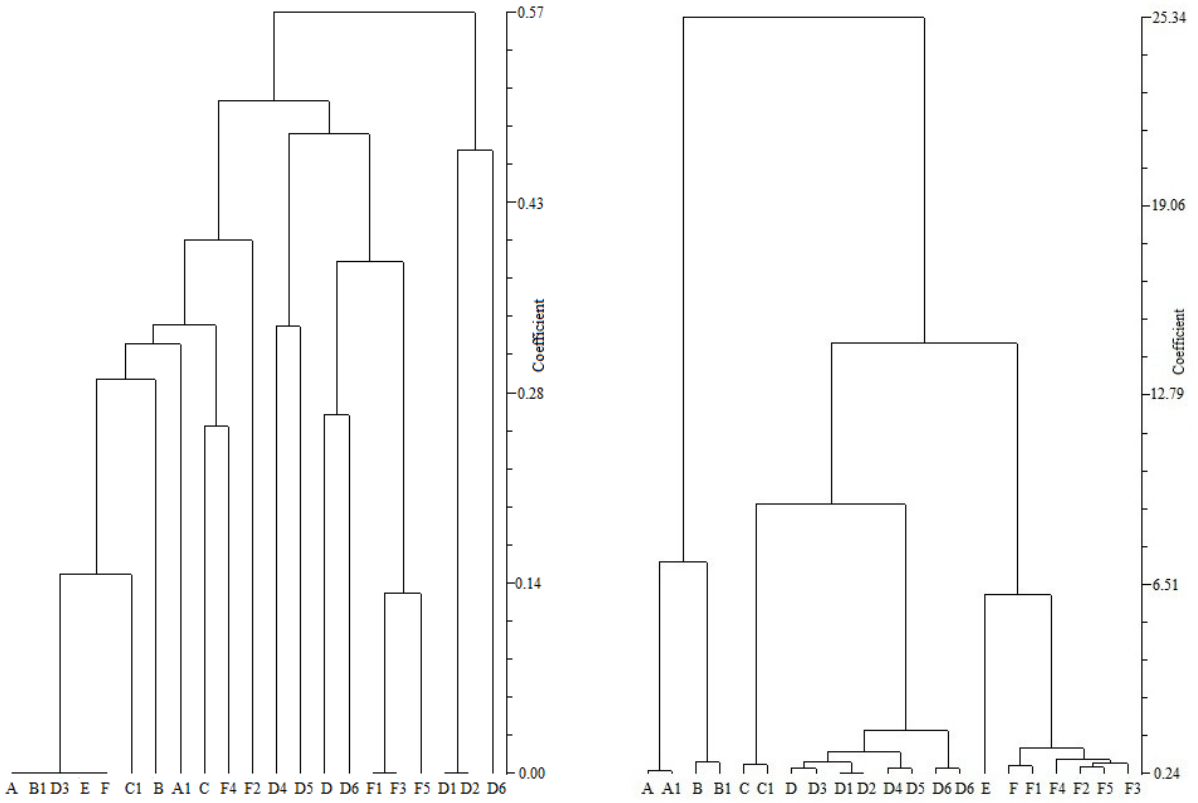


**Figure 4:** UPGMA phenograms of the 21 most frequently wing patterns present in the six different taxa, obtained with the Average taxonomic distance. The variable Var was included in the left phenogram (A) (r = 0.858) and excluded in the right phenogram (B) (r = 0.855). The taxa abbreviations are indicated as: A (A, A1) = morphotype A; B (B, B1) = morphotype B; C (C, C1) = morphotype C; D (D, D1-D6) = *E. decedens*; E = *E. fabalis*; F (F, F1-F5) = *E. alsiosa*.

Euclidian distances, also have large correlation coefficients, being respectively r = 0.855, r = 0.800 and r = 0.854. In all the phenograms, except in the one with chi-squared distance, morphotypes A and B were grouped together and clearly separated from the remaining taxa. The morphotype C was grouped with *E. decedens*. In the three phenograms *E. alsiosa* and *E. fabalis* were grouped together. A similar result was obtained when the cluster analysis was performed using only the form of whole wing pattern (Var) of the left hind wing and forewing (4 variables). However, when the Var was removed from the analysis, individuals of the same taxon with different patterns were grouped with different species (figure 4) decreasing greatly the resolution of the phenogram even though the correlation coefficients were large (r = 0.76 to r = 0.88). The inclusion of Var in the analysis was always determinant to increase the power of resolution. Without Var the resolution was always smaller even when all variables of the four wings were included.

### Abnormal wing patterns and vein modifications

In addition to the variation in the normal pattern, all species showed wings with abnormal patterns. As a whole, forewings showed significantly more modifications than hind wings ($\chi^2_1$ 23.598 ***). Right and left wings did not show significant differences in abnormal patterns both in the forewings ($\chi^2_1$ 0.103, n.s.) and hind wings (Fisher's test, 1 df. n.s.). The percentage of individuals with abnormal wing patterns varied between 0 and 7% in all species, except for morphotype A, which was greater (14.3%) (table 5). These abnormal patterns were related to the presence of three main types of vein modifications: bifurcations (figure 3 a, d-e, g-j, n), incomplete veins (figure 3 i-j, o) and additional (figure 3 b-c, f, k-l) or absent veins (figure 3 m). Additional longitudinal (L1, L2, L3) and transverse (T1 but also T5) veins were present in the left and right forewings while bifurcations were more common in the transverse (T2) and longitudinal (L1, L3) veins. Absent veins were more common in the forewings, particularly in the T4 (6.6-7.9%) and T5 veins (12.5-13.4%). Overall, it was observed that, despite the abnormal wing variation, in some specimens, the common pattern of each species was sufficiently clear to identify them.

## Discussion

The morphological identification of leafhopper species relies essentially on the recognition of males. Differences in the shape of abdominal apodemes and genital structures are the characters most commonly used (Ri-

baut, 1936; Ossiannilsson, 1949; 1981; della-Giustina, 1989). Although intra and interspecific differences in leafhoppers have been recognized using morphometric characters (Downham and Cooter, 1998; Larsen and Walter, 2007; Gillham and Claridge, 1994), these structures were not proven to be useful for the identification of some species of *Empoasca* present in Portugal. Quartau and Simões (1995), based on 13 morphometric characters of four species of Empoascini associated to vineyards, were able to separate male and females of 3 *Empoasca* species from those of *Jacobiasca lybica* (Bergevin et Zanon) but were not able to distinguish the 3 species of *Empoasca*. However, in our study we could identify through wing patterns the six taxa of *Empoasca* included, despite a considerable amount of intraspecific variation.

Wing shape has proven to be a suitable character to discriminate even sibling species such as *Drosophila mercatorum* Patterson et Wheeler and *Drosophila paranaensis* Barros (Moraes *et al*., 2004). Although these two species have a similar pattern of morphological variation in the wings, 95 to 98% of the females were successfully classified. Their wing differences were due to the placement of the posterior transverse vein. A similar case seems to hold to *Empoasca* in which the relative position of the T3 and T5 veins, as well the vein curvature of the apical cells seem to be the most relevant characters for the identification of species. The overall shape is similar but the relative position of some veins varies and this is apparent in some drawings of the wing veins reported in taxonomic works of *Empoasca*. The usefulness of wing shape in species identification comes as no surprise, especially if taking into account the ecological and evolutionary functions that are related to the insect wings such as mating (e.g. location and recognition of mates, courtship rituals), food searching or migration. Moraes *et al.* (2004) proved that wing characters in *D. mercatorum* and *D. paranaensis* have a significant natural heritability and the differences are probably controlled by genes acting additively. A similar case may also be true in *Empoasca*.

The large amount of intraspecific variation in wing patterns agrees with the previous observation made by Young (1952). However, despite this variation, it is still possible to use wing veins in the recognition and identification of species. To understand the differences between species it is necessary to characterize the intraspecific variation in large samples from different geographic areas. In this study, although the whole number of specimens examined represented large samples from different areas, for some species the number of individuals examined was considerably low. A larger number of individuals, particularly of *E. alsiosa* and morphotype B, should be analyzed to validate the extent of intraspecific differences observed.

The particular ease with which the wing venation can be observed under the stereomicroscope and, the probable utility of this character further suggest that wing venation deserves additional research. Other characters such as texture of wings could be also useful in taxonomic studies. Vidano (1958) showed that *Empoasca vitis* (Goethe) has a totally transparent median cell in the forewing of mature adults. Additionally, other measures of wing shape using more recent techniques such as geometric morphometrics, already applied successfully in the identification of Hemiptera species (Campos *et al*., 2011), could also help to explore further the utility of wings in taxonomic studies. Insect wings provide excellent landmarks to study morphometric variation of quantitative characters, although these should be used with caution, at least in *Empoasca,* because they can be affected by environmental temperature (Simonet and Pienkowski, 1980). Compared to size, shape is believed to be controlled by a higher degree of genetic factors and affected more by sex and environment (Birdsall *et al.*, 2000). Therefore, the comparatively low plasticity in wing shape suggests that shape may be more useful in separating species than size. Although wing venation patterns have been used extensively in taxonomic studies of Cicadellidae to classify subfamilies, tribes and genera (Dietrich, 2005; Oman, 1949; Young, 1952), little attention has been given to the use of these characters in species identification. This study shows that wing venation patterns may be suitable characters to separate species within the same genus and among closely related genera.

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