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Morphological and genomic assessment of divergence between closely related species of the genus *Philaenus* (Hemiptera, Aphrophoridae)

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Introduction

Philaenus spumarius is a widespread and polyphagous xylem-feeding insect. It is also the main vector of *Xylella fastidiosa* in Europe [1]. *Philaenus. spumarius* has a sister species, *P. tesselatus*, and they are differentiated on the male genitalia but not at karyological, mitochondrial DNA and some nuclear genes' levels [2, 3, 4]. Genome-wide markers revealed previously unreported genetic differentiation. Here we aim at characterising morphological variation in the male genitalia in both species. Integrating genomic data and more traditional approaches will allow to better define species and distribution boundaries, action-plans for the control of *X. fastidiosa* and conservation of natural ecosystems.

Methods

Male genitalia were extracted and placed in a KOH 10% (w/v) solution for 24 hours. Habitus and aedeagus images were acquired either with a Keyence VHX 5000 system or with an Olympus BX51 microscope, equipped with a The Imaging SDFK 23U274 industrial colour camera controlled with Micro-Manager 2.4 software. Images were focus-stacked, scaled and measured in ImageJ 1.52p. Outline analysis and semi-landmarks analysis were performed on **habitus** images of **76 specimens**. Classic morphometric analysis was performed on **aedeagus** images of **170 specimens**.

Results

- **No differentiation** between *P. spumarius* and *P. tesselatus* in geometric morphometric analyses of **habitus** (data not shown).
- **Clear differentiation** between species in **aedeagus** morphology in PC1.
- **Total length** of aedeagus, mean lengths of **lower** and **upper appendages** are the best characters to discriminate species. *Philaenus tesselatus* tends to have larger values for these measurements.
- **Intra-specific morphological variation** detected in PC2 and PC3 associated with mean curvature of upper appendages and mean length of middle appendages.
- *Philaenus tesselatus* was only found in Portugal, Spain and Morocco in this study. **Both species** occur in **Iberian Peninsula** where *P. spumarius* is widespread but *P. tesselatus* seems to be restricted to the south where it is the dominant species.
- **No differentiation** between *P. spumarius* and *P. tesselatus* at **mtDNA** (same haplotypes in both).
- **Clear differentiation** between *P. spumarius* and *P. tesselatus* at **genome-wide markers**.

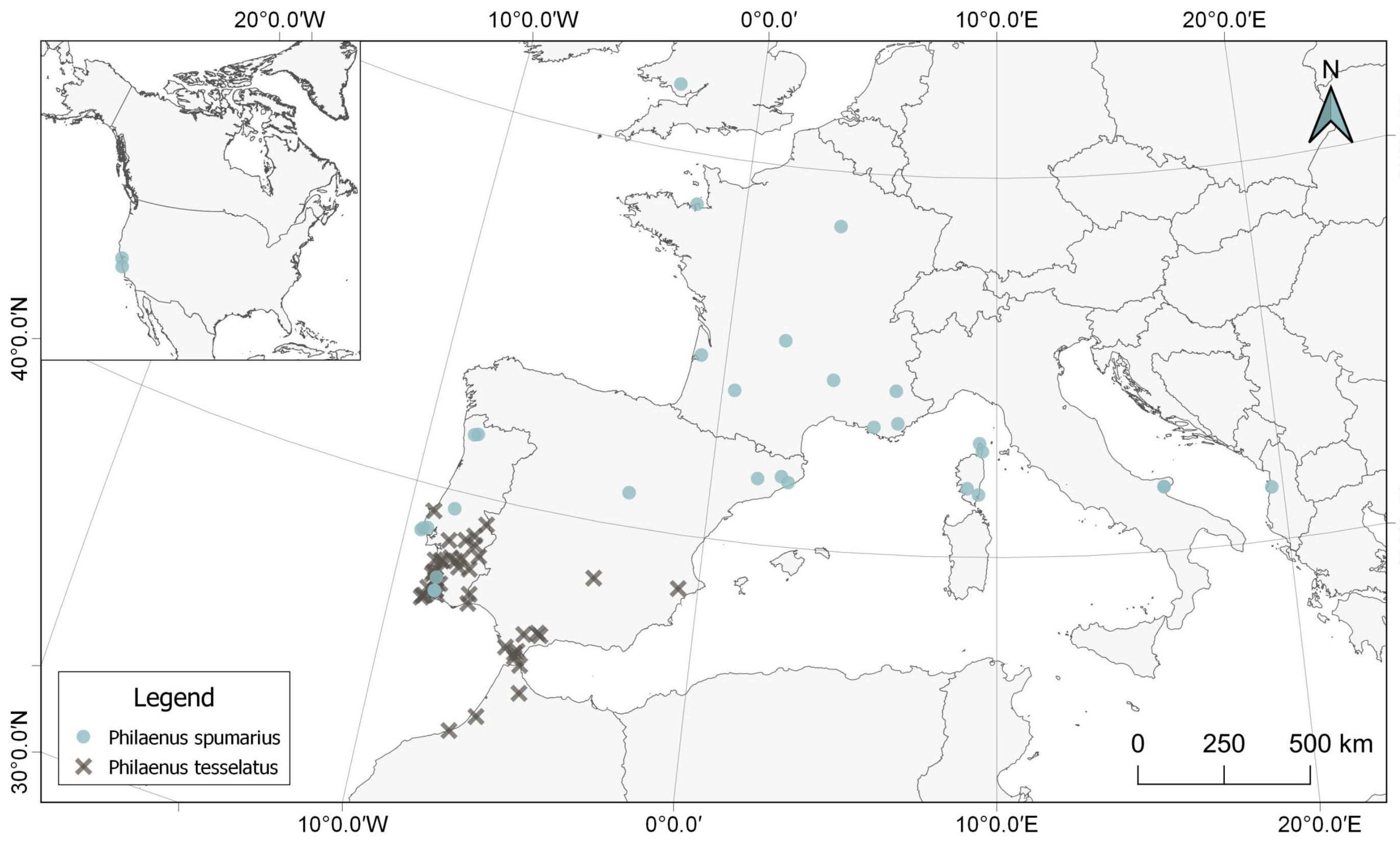
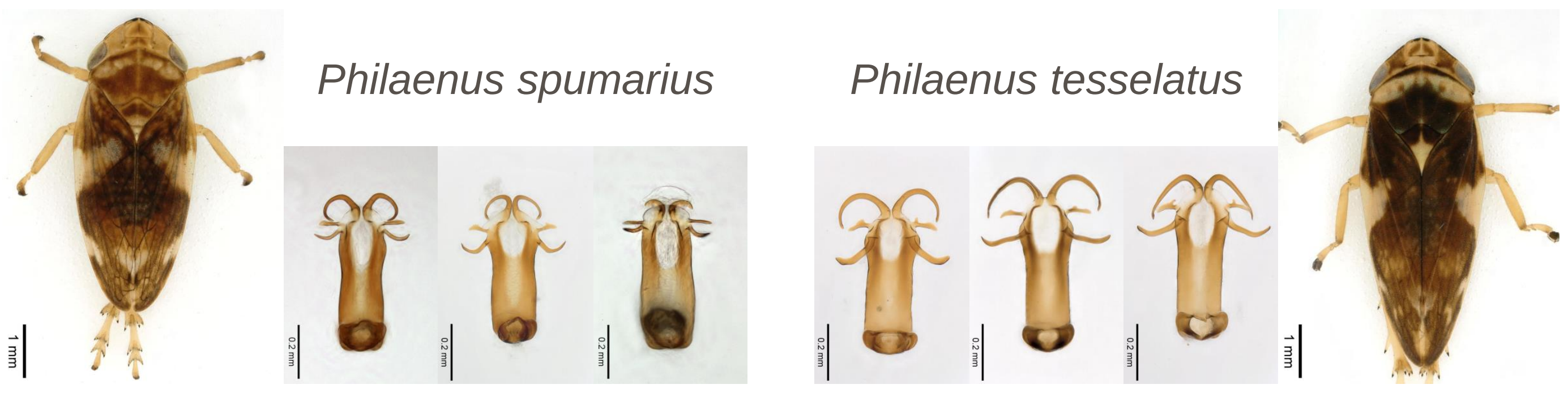


Figure 1 – Sampling locations of *P. spumarius* and *P. tesselatus* for morphological analyses (N = 79). Four sampling points from Morocco not shown.



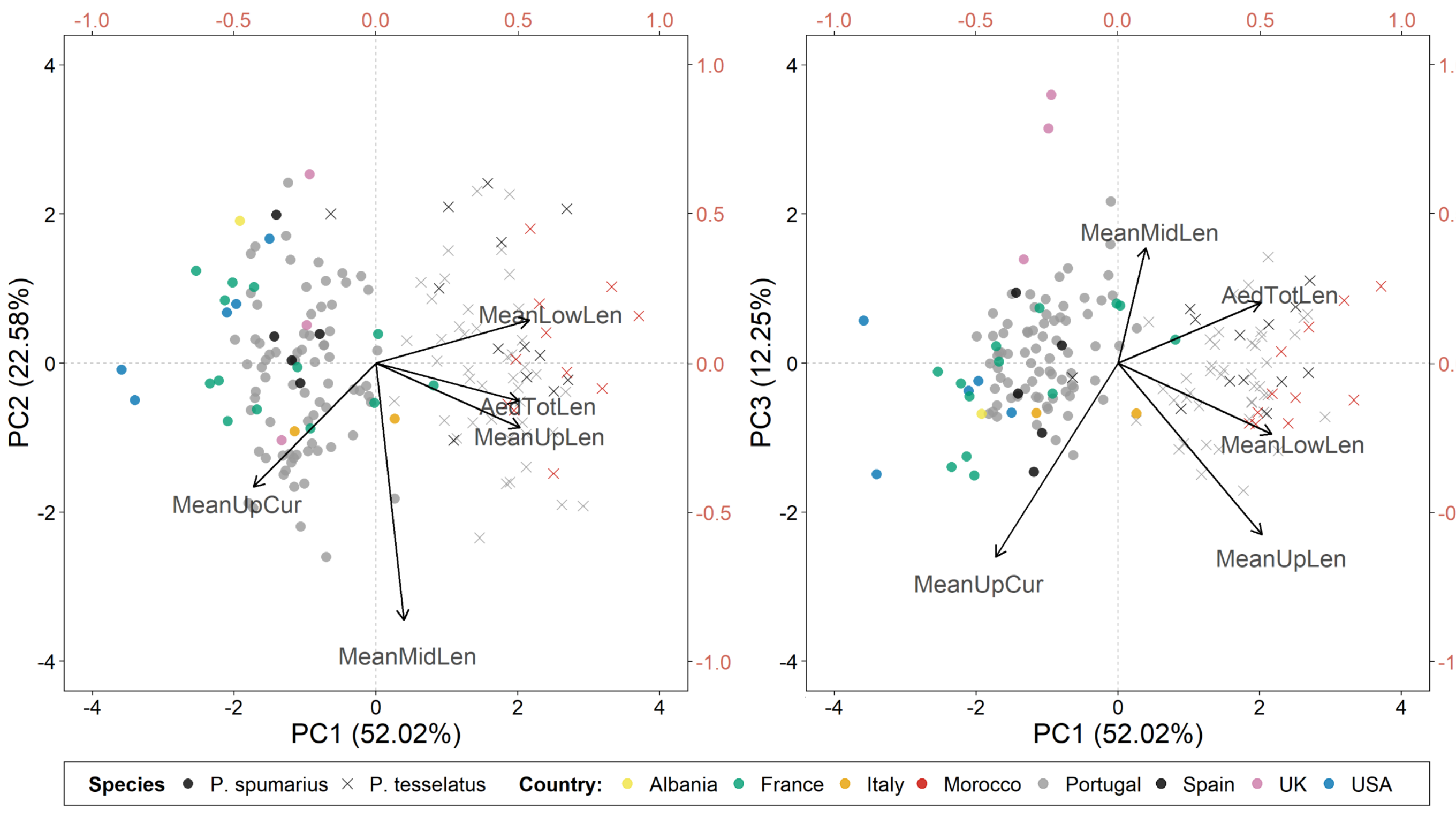


Figure 3 – Principal component analysis (PCA) of morphometric measurements of aedeagus from *P. spumarius* and *P. tesselatus*.

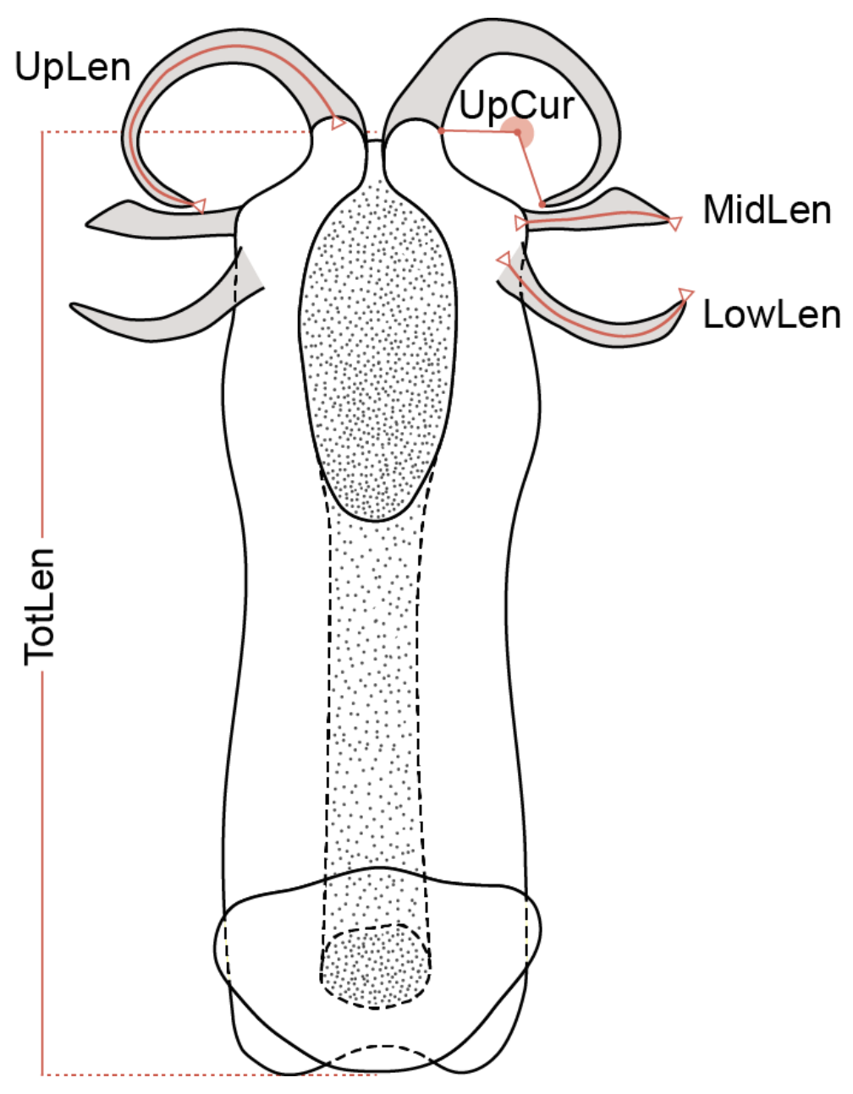


Figure 2 – Measurements on *Philaenus* aedeagus: TotLen - total length of aedeagus; LowLen - length of lower appendages; MidLen - length of middle appendages; UpLen - length of upper appendages; UpCur - curvature of upper appendages. Curvature determination based on Feduccia [5].

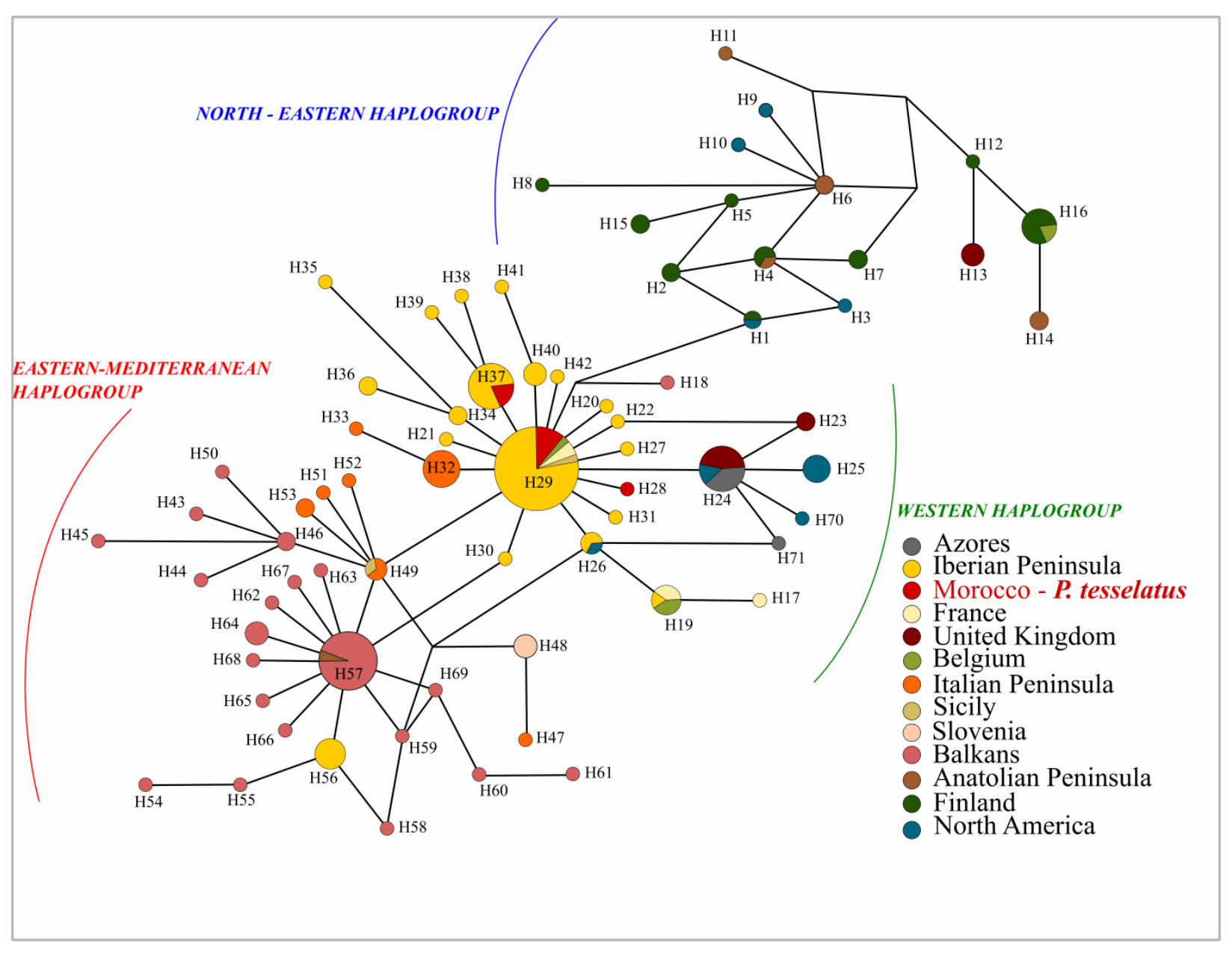


Figure 4 – Median-joining haplotype network based on mitochondrial gene cytochrome oxidase I sequences for *P. spumarius*. *P. tesselatus* haplotypes from Morocco are shown in red. Adapted from Rodrigues *et al.* [4].

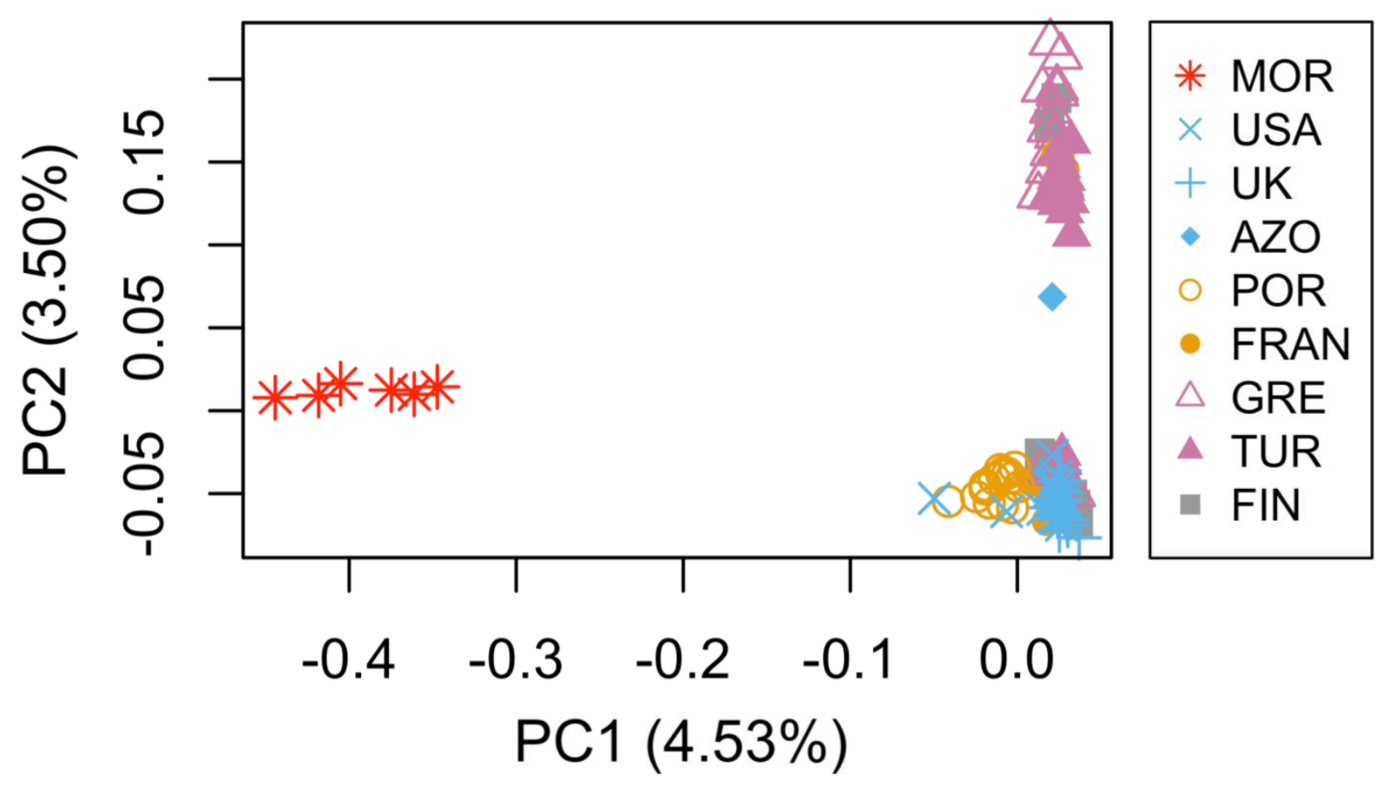


Figure 5 – Principal component analysis (PCA) of 2796 Single Nucleotide Polymorphisms for 133 individuals of *P. spumarius* (from several parts of Europe) and *P. tesselatus* (from Morocco, in red). Adapted from Seabra *et al.* (in prep).

20:19

Take home messages

Total length of aedeagus and lower appendages provide the best morphological characters to distinguish *P. spumarius* from *P. tesselatus*.

SNP markers will provide more accessible genotyping of specimens to discriminate these two closely related species (not distinguished by mtDNA).

The ability to correctly identify *P. spumarius* and *P. tesselatus* will allow a more comprehensive knowledge on their distribution, population dynamics and ecological requirements. These are known important drivers of *X. fastidiosa* spread by other vector species and are relevant for disease management.

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