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A preliminary genetic study of Mediterranean species of *Philaenus* based on COI and ITS2 DNA sequences

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Abstract

The monophyly of Mediterranean species of *Philaenus* was supported with a 1.00 BPP value and with a bootstrap value of 100%. Four separate groups were clearly defined. *Philaenus loukasi* Drosopoulos et Asche appeared as basal to all remaining species, also *Philaenus arslani* Abdul-Nour et Lahoud showed distinct genetic differences. The analyses showed the presence of two distinct clades, one comprising *Philaenus maghresignus* Drosopoulos et Remane, *Philaenus tarifa* Remane et Drosopoulos, *Philaenus signatus* Melichar, and *Philaenus italosignus* Drosopoulos et Remane. The other species group was formed by *Philaenus tesselatus* Melichar and *Philaenus spumarius* (L.).

Key words: Phylogeny, *Philaenus*, COI, ITS2.

Introduction

The genus *Philaenus* (Auchenorrhyncha Aphrophoridae) is known for its unique complex colour polymorphism (Halkka and Halkka, 1990; Stewart and Lees, 1996; Drosopoulos, 2003). Until recently only 3 species were recognized in the genus: *Philaenus spumarius* (L. 1758) (a widely distributed Holarctic species), *Philaenus signatus* Melichar 1896 and *Philaenus tesselatus* Melichar 1889, being Mediterranean species (the latter often synonymized under *P. spumarius* or treated as its subspecies). The relationships between species and populations of the genus *Philaenus* have been studied only based on morphology (Halkka and Halkka, 1990; Stewart and Lees, 1996; Remane and Drosopoulos, 2001; Drosopoulos, 2003). In the 1990s intensive studies of the genus *Philaenus* were made in the Mediterranean region (Drosopoulos and Asche, 1991; Loukas and Drosopoulos, 1992; Drosopoulos and Loukas, 1993; Abdul-Nur and Lahoud, 1996; Drosopoulos and Remane, 2000; Remane and Drosopoulos, 2001; Drosopoulos and Quartau, 2002). These studies showed that beside the very widely spread species *P. spumarius*, there is a group of six other species, sympatric with *P. spumarius*, though allopartic to each other (*P. signatus*, *Philaenus loukasi* Drosopoulos et Asche 1991; *Philaenus arslani* Abdul-Nour et Lahoud 1996, *Philaenus maghresignus* Drosopoulos et Remane 2000; *Philaenus italosignus* Drosopoulos et Remane 2000 and *Philaenus tarifa* Remane et Drosopoulos 2001). Also *P. tesselatus*, a species of still unclear taxonomic status, occurring in the southern part of the Iberian Peninsula and North Africa, belongs to this group. The enzyme loci analyses were used for resolving population genetics of *Philaenus* in Greece only (Loukas and Drosopolous, 1992).

The main aim of this project is to describe phylogeny and revise taxonomy of the Mediterranean species of the genus *Philaenus* (*P. tesselatus*, *P. loukasi*, *P. arslani*, *P. signatus*, *P. italosignus*, *P. maghresignus*, *P. tarifa*) and understand their affinity to the widely distributed *P. spumarius* using molecular genetic methods. The study may be also important for identification and description of new species not recognized on the basis of morphological features.

Materials and methods

Total genomic DNA from 4 specimens of *P. spumarius*, *P. tesselatus*, *P. loukasi*, *P. arslani*, *P. signatus*, *P. italosignus*, *P. maghresignus* and *P. tarifa* was extracted with Dneasy Tissue Mini Kit (Qiagen, Valencia, USA). The mitochondrial Cytochrome Oxidase I (COI) region was amplified using primers: F' – TTGATTTTTTGG TCATCCAGAAGT, R' – TTGGTTTAAGAGACCAT TAC and Internal Transcribed Spacer 2 (ITS2) with primers: F' – GCATCGATGAAGAACGCAGC, R' – TCCTCCGCTTATTGATATGC (Simon *et al*., 1994; White *et al*., 1990). The target bands were excised from the gel and recovered using a QIAquick Gel Extraction Kit (Qiagen). Purified DNA was used for sequencing in two directions using the same primers and BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, USA) following manufacturer's instructions. The DNA sequences were edited using the BioEdit Sequence Aligment Editor (v. 5.0.9.) (Hall, 1999). Consensus sequences from all species were aligned and compared using ClustalX (Thompson *et al*., 1997). Both data sets were analyzed separately and combined. Phylogenetic trees were reconstructed using Bayesian methods with MrBayes 3.1 (Ronquist and Huelsenbeck, 2003), Maximum Parsimony and Maximum Likelihood using PAUP*4.0b (Swofford, 2002). For the analyses, the appropriate nucleotide substitution model was first determined using Modeltest 3.06 (Posada and Crandall, 1998) in conjunction with PAUP*.

Results and discussion

A 469 bp segment of the mtDNA COI gene was obtained and aligned for all species as well as for the outgroup [*Cacopsylla nigrita* (Zetterstedt 1828), Psylloidea]. In this gene segment 16.6% of nucleotide sites were variable, most nucleotide substitutions are synonymous because they occur predominantly in the third positions of codons (22%). With 28% A, 40% T, 16% G, and 16% C, the sequenced genes showed the typical AT bias of insect mitochondrial DNA and cytochrome oxidase genes in particular. The ITS2 dataset consisted of 722 characters, 43 of which were gaps coded as alternative presence or absence character state. The average percentages of base compositions were A 18%, T 23%, G 29%, C 29%. The maximum corrected divergence within species was 5.7% between *P. signatus* and *P. spumarius*. The HKY=G model was the best-fit substitution model obtained under the Akaike information criterion for COI genes and the HKY for ITS2 genes. The strict consensus tree of the combined analysis resulted in two MP trees of 810 steps (CI 0.944, HI 0.056, RC 0.81) with strong bootstrap support for all nodes. Out of 1178 aligned positions from the COI and ITS2 regions 376 sites were variable, 235 of which were phylogenetically informative. Trees obtained by ML and Bayesian inference were congruent with the MP topology. The monophyly of Mediterranean species of *Philaenus* was supported with a 1.00 BPP value and with a bootstrap value of 100%. Four separate groups were clearly defined. Two species *P. loukasi* and *P. arslani* showed distinct genetic differences and represent independent groups. The third group comprises *P. maghresignus, P. tarifa, P. signatus*, and *P. italosignus* also well defined from a morphological point of view. The fourth group includes *P. tesselatus* and *P. spumarius.* The results confirmed the electrophoretic studies of enzyme loci indicating that *P. spumarius* and *P. signatus* are more closely related while *P. loukasi* represents a separate group (Loukas and Drosopoulos, 1992).

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