Morphological identification and molecular characterization of *Anopheles algeriensis* Theobald, 1903 (Diptera: Culicidae) from Apulia region

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INTRODUCTION. *Anopheles algeriensis*, formerly considered competent for *Plasmodium* parasites, is a species distributed in the Mediterranean area and more rarely found in Central and Northern Europe. In Italy, the disappearance of suitable breeding sites has been affecting the occurrence of this species, once common along the Southern coasts and islands. In 2020, in the framework of monitoring activities of the IZSPB 1/18 RC Project on residual anophelism, adults of *An. algeriensis* were collected in sites close to two different brackish lake basins in Foggia province, Apulia region. Given the noteworthy record of such a rare *Anopheles* species, an in-depth molecular investigation was performed.

MATERIALS AND METHODS. Mosquitoes were collected in two farms located near Lesina and Salso lakes, to the north and south of Gargano Promontory respectively (Fig. 1), using CDC and BG sentinel® traps. All specimens were morphologically identified as *An. algeriensis* (Fig. 2), using keys of Severini *et al.* (Severini et al., 2009 Fragm Entomol; 41:213-372).

A sub-sample (15% of the total) from the two study areas was amplified and sequenced for COI (Folmer et al., 1994 Mol Mar Biol Biotechnol 3:294-9) and ITS2 markers (Marinucci et al., 1999 Insect Mol Biol 8:469-80). GenBank dataset of *An. algeriensis* COI sequences from Spain, Germany and Sweden and a dataset of ITS2 sequences, available in GenBank only from Spain specimens, were used for sequence alignments and to generate the respective phylogenetic trees (NJ method-Tajima-Nei distance).

Figure 1. Map of the Gargano area (Foggia Province). Lesina Lake (yellow dot) and Salso Lake (red dot) collection sites.

Figure 2. Head and thorax of *Anopheles algeriensis*

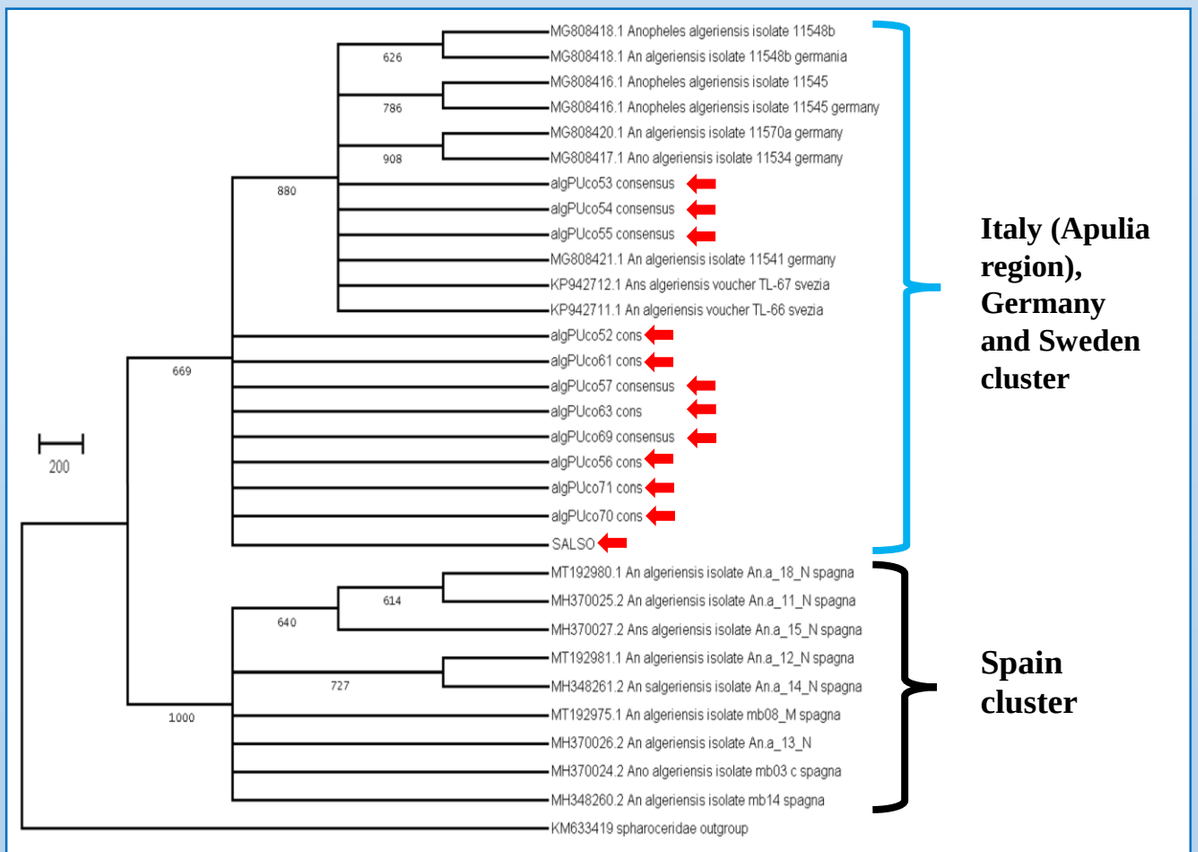

Figure 3. Phylogenetic tree generated for COI sequences. Red arrows indicate the 12 representative sequences obtained from 25 Apulian specimens; the other sequences were obtained from the article Delgado-Serra *et al.* 2021.

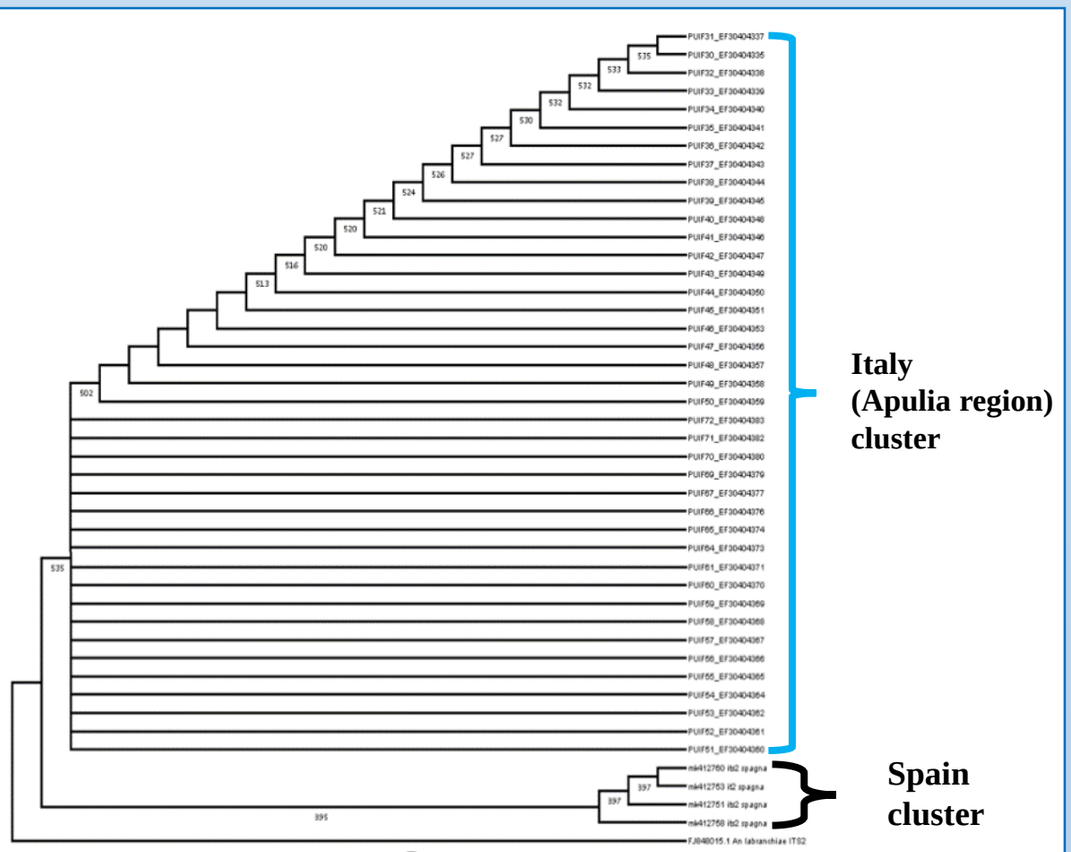

Figure 4. Phylogenetic tree generated for ITS2 sequences. This phylogenetic tree includes 40 representative sequences of 63 obtained from Apulian specimens and 4 representative sequences of 10 available from Spain in GenBank.

RESULTS AND CONCLUSIONS. In total, 494 specimens were identified, showing the typical morphological characteristics of *An. algeriensis*. In addition, 25 specimens and 63 specimens were successfully characterized for COI and ITS2 markers, respectively. Phylogenetic tree generated for COI sequences (Fig. 3) showed that Apulian population forms a cluster with German and Swedish specimens, whereas specimens from Spain form a distinct cluster. Alike, ITS2 phylogenetic tree (Fig. 4) showed two-separated clusters, one including Apulian *An. algeriensis* sequences and another one formed by Spanish sequences. As already observed in a previous study on *An. algeriensis* COI characterization (Delgado-Serra et al., 2021 J Med Entomol 58:608-15), our analyses on both COI and ITS2 sequences point out the presence of two separate molecular species within the single morphospecies *An. algeriensis*. Further investigation should be carried out to better investigate molecular diversity of *An. algeriensis* populations from different countries.

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