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Morphological and molecular characterisation of Italian *Leptoconops* species with new recorded populations.

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The genus *Leptoconops* is one of the 4 blood-feeding genera of midges within the family Ceratopogonidae (Diptera). Although studies have been conducted on this genus over the past century, uncertainties and ambiguities persist regarding the identification and classification of many species. Molecular data remain scarce, and nothing is currently known about the potential vectorial role of these midges, despite their well-documented nuisance and painful bites, which can cause allergic reactions. These considerations are particularly relevant to the study of *Leptoconops* in Italy, where 6 species are currently known. Research has so far been limited to the Maremma area (Tuscany), characterized by coastal marshes and sandy shores, habitats that support all life stages of *Leptoconops*. Our study aimed to provide new morphological and molecular data to resolve identification issues, explore the presence of *Leptoconops* in previously unstudied areas, and implement a suitable methodology for pathogen detection in this genus. During the summer of 2024, we collected over 4,000 adult midges using traps and aspirators across two districts: the coastal area of Grosseto (Maremma) and the inland hilly district of Siena. Following morphological analyses, DNA was extracted from representative individuals for DNA barcoding, and genome sequencing was performed on two species: *L. noei* and *L. irritans*. A new qPCR protocol for pathogen detection was developed using a high-throughput microfluidic platform. Morphological identification enabled the assignment of specimens to 4 species, each showing distinct geographical distribution. Notably, the collection of *L. noei* specimens from 12 sites in the Siena district represents the first recorded presence of *Leptoconops* in an Italian inland area. SEM analyses revealed previously undescribed morphological traits and variability in sensory organs. Detailed morphological observations, combined with new DNA barcoding analyses, clarified longstanding ambiguities in the taxonomy of *Leptoconops* species. Genome sequencing is ongoing, and we have successfully annotated the mitochondrial genomes of both *L. noei* and *L. irritans*. The pathogen detection protocol has been optimized, and screening is currently underway. Our research has significantly expanded the knowledge of the distribution, morphology, and genetics of the genus *Leptoconops*, while also introducing novel tools for species identification and pathogen detection.