

AGATA CESARETTI¹, JAN-NIKLAS MACHER^{2,3}, M.
ANTONIO TODARO^{1,4}

1 Dipartimento di Scienze della Vita, University of Modena and Reggio Emilia,
Via G. Campi 213/D, 41125 Modena, Italy.

2 Naturalis Biodiversity Center, Leiden, The Netherlands.

3 Department of Environmental Biology, Institute of Environmental Sciences
(CML), Leiden University, Leiden, The Netherlands.

4 NBFC, Piazza Marina 61, 90133 Palermo, Italy.

Hidden diversity in marine Gastrotricha: Mediterranean lineages are genetically distinct from North Sea species

Marine meiofaunal organisms present a long-standing biogeographic paradox: despite their limited dispersal ability, many nominal species have been reported to exhibit remarkably broad, even cosmopolitan, distributions. This pattern is particularly evident in Gastrotricha, where cryptic diversity and persistent taxonomic impediments have long obscured species boundaries. Here, we investigated whether four marine gastrotrich species shared between the North Sea and the Mediterranean Sea represent single widespread species or geographically structured evolutionary lineages. Populations of *Xenotrichula intermedia*, *Halichaetonotus aculifer*, *H. atlanticus*, and *Turbanella bocqueti* were analysed using Oxford Nanopore long-read sequencing, from which the nuclear (18S, 28S) and mitochondrial (COI) markers were recovered for multilocus phylogenetic reconstruction. Species boundaries were subsequently evaluated using six complementary population genetic, distance-based, coalescent-based, and tree-based species-delimitation approaches (4× rule, ABGD, ASAP, GMYC, PTP and bPTP). All four nominal species consistently recovered Mediterranean and North Sea populations as distinct evolutionary lineages, although the strength of genetic divergence varied among taxa. *X. intermedia* exhibited the strongest pattern, with Mediterranean specimens further subdivided into distinct Ligurian, Adriatic and Tunisian lineages, revealing substantial cryptic diversity within the Mediterranean basin. Additional internal structuring was detected within the northern populations of *H. aculifer* and *T. bocqueti*, whereas *H. atlanticus* exhibited a shallower, although still consistent, north–south differentiation. Despite relying on conceptually different analytical frameworks, all species-delimitation methods converged on the same general pattern of lineage diversification, supporting the long-term evolutionary independence of the identified lineages. Overall, our results reveal a pervasive north–south phylogeographic structure and extensive hidden diversity across the investigated species. The recurrent recovery of geographically structured lineages in taxonomically and biologically diverse gastrotrich species suggests that repeated north–south phylogeographic divergence is a major driver of diversification in European marine Gastrotricha. This, in turn, indicates that hidden diversity is a widespread feature of marine Gastrotricha rather than the result of isolated cases of cryptic speciation.