



*Ph.D. Thesis Defense*

*Student Presentation*

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**“Reconstructing the evolutionary history of terrestrial isopods (Isopoda: Oniscidea) at different phylogenetic levels”**

Terrestrial isopods (Oniscidea) exhibit some unique properties that make them an excellent case within global biodiversity, offering research opportunities on many biological fields, such as evolution and ecology, but also ecotoxicology, bioacoustics and ecophysiology. With more than 3,700 described species, Oniscidea is the largest and at the same time the only terrestrial isopod suborder. The sea-land transition most probably took place during the Carboniferous, ~300 Mya. Terrestrial isopods are the most successful colonizers of the terrestrial realm among Crustacea, exhibiting a series of diverse morphological, ecological, and behavioural adaptations that allowed them to conquer land. Remarkably, extant species represent almost all evolutionary steps that allowed them to occupy the whole range of terrestrial habitat types, with a few exceptions, including the very harsh desert environments, salt lakes, and subterranean freshwaters, where they have secondarily returned. Oniscidea presence extends from the sea level to very high elevations (>4800 m), excluding polar regions.

Despite the constantly accumulating genetic and morphological data, we still lack a comprehensive and robust phylogeny of Isopoda suborders, hence also of Oniscidea. Within the framework of this thesis, the evolutionary dynamics of this group was examined at different

taxonomic levels. More precisely, phylogenetic relationships were examined at the following levels: i) deep phylogeny - relationships among the five main Oniscidea clades and with closely related aquatic Isopoda suborders, ii) family/genus level – relationships among some of the major Crinocheta families and among genera of one of the species-richest families (Porcellionidae), and iii) species/population - among *Armadillo officinalis* species populations, covering a large part of its distribution but focusing mostly on intra-insular divergence within Cyprus.

Aiming to reconstruct the evolutionary history along such a multilevel approach, a series of mitochondrial (12s, 16s, COI, Cytb) and nuclear (18s, 28s, NAK, PEPCK) markers, as well as genome-wide SNPs were retrieved, employing Sanger and ddRADseq sequencing, respectively.

At the deep phylogeny level, our results undermine the widely accepted monophyly of Oniscidea. More specifically, the amphibious genus *Ligia* appears to be evolutionary more closely related to aquatic isopod taxa than the rest of the terrestrial isopods included in the analysis. Considering the evidence against the monophyly of Ligiidae presented herein, we suggest the assignment of *Ligidium* and of the closely related genera *Taurologidium* and *Typhloligidium* to a new family, Ligididae, that possesses a basal position within Oniscidea phylogeny. The produced phylogenetic pattern of the remaining terrestrial taxa reflects the complex evolutionary history of the group in view of the transition from the marine to the terrestrial realm.

Time-calibrated phylogenetic analyses at family/genus level questioned both the monophyly of Porcellionidae and *Porcellio*, one of the family's richest genera. Excluding the genera *Leptotrichus* and *Brevurus* that are not grouped with the rest of family members, the now monophyletic Porcellionidae seems to have an African origin that dates back to the Oligocene (~32 Mya).

Focusing on species/population level, in the case of *A. officinalis* we found high genetic divergence, not suggested by any of the morphological traits examined so far. The presence of five cryptic genetic lineages on Cyprus was revealed. Cladochronological dating indicates that the species arrival on the island occurred ~6 Mya, probably facilitated by the Messinian Salinity Crisis. Furthermore, results highlight the role of paleogeographic history and of human presence in shaping patterns of genetic diversity.

Our results call for a re-evaluation of morphological characters traditionally used in terrestrial isopod taxonomy, under the light of the constantly accumulating genetic data. This way, we can attain more robust and comprehensive phylogenies which will allow us to describe the complex evolutionary history of this unique group that managed to conquer land.