

Morphological and genetic analysis of moss-dwelling tardigrades

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Tardigrades, or water bears, are a diverse phylum of microscopic invertebrates that have existed since the Cambrian. They are especially noted for being polyextremophilic existing both in hot springs as well as in solid ice. They are among the few organisms capable of suspending metabolism and entering a state of cryptobiosis. To date there are approximately 1000 described species although it is widely recognized that the group has attracted little research attention and their diversity is thus vastly underestimated. The armored tardigrades, or Heterotardigrades, have morphological characteristics such as cephalic appendages, cuticular extensions, claws and the pattern of dorsal cuticular plates, whereas the main characteristics of the naked tardigrades (Eutardigrade) are claws, the buccopharyngeal apparatus and a cuticle structure, which can be smooth, granulated or bearing tubercles. The phrase "naked" refers to the absence of cuticular dorsal plates, which are present in Heterotardigrada. We investigated a total of 192 moss samples from both man-made (botanical gardens) and natural habitats (Graz vicinity) for the presence of tardigrades. From a total of 88 moss samples with tardigrades, 104 individual slide preparations were made for morphological evaluation. Nearly all preparations ($N = 103$) could be assigned to the genus level, whereby 59 individuals were assigned to one of six species. An additional four species were determined with the aid of an mtDNA sequence (COI gene). Eutardigrades dominated the collected moss samples with *Macrobotus* as the most frequent genus and *Macrobotus cf. hufelandi* ($N = 47$) as the most common species. *Macrobotus cf. hufelandi* was found at different altitudinal levels in Styria, whereas other species were only found in specific locations. There was no apparent species-specific relationship between moss and tardigrade taxa and there was no apparent relationship between the colonization of tardigrades and various man-made substrates within the botanical gardens. Further molecular genetic analysis was hindered by the extreme divergence of the group, and thus lack of universal primers for species-level identification. Based on our own phylogenetic reconstruction of available GenBank sequences it is clear that the current systematic and taxonomic assignment of tardigrades requires substantial revision.

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