

“Application of micromorphological, morphometric and molecular methods in the identification of selected species of *Alchemilla* L. (Rosaceae)”

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The subject of this doctoral thesis is the application of micromorphological, morphometric and molecular methods in the identification of selected species of the genus *Alchemilla* L. (Rosaceae).

The genus *Alchemilla* comprises a taxonomically challenging group of plants. Numerous species are morphologically very similar, and the overlap of traditionally used diagnostic characters makes unambiguous species identification difficult. Additional complexity results from biological and evolutionary processes, including apomixis, polyploidy and potential hybridisation. Despite previous research, there is still a need to assess which sources of data can support species differentiation and what limitations affect their diagnostic value.

The main objective of the study was to assess morphometric, micromorphological and molecular variation among selected species of *Alchemilla* L. (Rosaceae) and to evaluate their potential taxonomic utility in species identification. The research comprised three parts based on partly different sets of species and specimens. These focused on achene morphometry and micromorphology, leaf morphometry, and leaf micromorphology combined with molecular analyses. Morphometric analyses included shape descriptors and statistical analyses, including PCA, PERMANOVA, PERMDISP and NMDS. Micromorphological characters were examined using scanning electron microscopy, whereas molecular differentiation was assessed using phylogenetic and network analyses. One part of the study was additionally supplemented by an assessment of nuclear DNA content using flow cytometry.

The analyses showed that the diagnostic value of the investigated characters varied among the different sources of data. Achene morphometry revealed considerable overlap among species, indicating limited taxonomic utility of achene shape. In contrast, achene micromorphological characters, particularly those related to surface ornamentation and epidermal cell morphology, showed greater diagnostic potential. Leaf morphometric analyses also revealed differentiation among some of the taxa studied, although considerable overlap limited their usefulness for specimen identification. Overall, leaf morphometry did not allow unambiguous separation of the morphologically identified species or reliable assignment of specimens identified as *Alchemilla* sp. Macro- and micromorphological analyses of leaves revealed variation in the relative size of the apical tooth

and in trichome abundance and distribution. However, none of the observed combinations of characters was restricted to a single morphologically identified species.

Among the molecular markers analysed, the nuclear ITS region contained the highest proportion of variable and parsimony-informative sites and provided the most informative phylogenetic reconstruction. In contrast, the plastid markers *matK*, *rpoCl* and *trnH-psbA* showed more limited discriminatory power. Neither phylogenetic nor network analyses provided consistent species-level separation of the investigated taxa. Nuclear DNA content showed relatively limited variation and considerable overlap among taxa.

Overall, none of the investigated datasets alone provided unambiguous identification of all the species studied. Selected achene micromorphological characters showed greater diagnostic potential than achene shape, although their taxonomic utility requires further evaluation. Morphometric, micromorphological, molecular and cytometric data should therefore be regarded as complementary sources of taxonomic information. Their combined interpretation provides a more comprehensive assessment of variation among the investigated taxa and supports the use of an integrative approach in taxonomic studies of the genus *Alchemilla*.