Wallemia sebi is a xerophilic food- and air-borne fungus. The name has been used for strains that prevail in cold, temperate and tropical climates. In this study, multi-locus phylogenetic analyses, using the internal transcribed spacer (ITS) regions, DNA replication licensing factor (MCM7), pre-rRNA processing protein (TSR1), RNA polymerase II largest subunit (RPB1), RNA polymerase II second largest subunit (RPB2) and a new marker 3´-phosphoadenosine-5´-phosphate (HAL2), confirmed the previous hypothesis that W. sebi presents a complex of at least four species. Here, we confirm and apply the phylogenetic analyses based species hypotheses from a companion study to guide phenotypic assessment of W. sebi like strains from a wide range of substrates, climates and continents allowed the recognition of W. sebi sensu stricto and three new species described as W. mellicola, W. Canadensis, and W. tropicalis. The species differ in their conidial size, xerotolerance, halotolerance, chaotolerance, growth temperature regimes, extracellular enzyme activity profiles, and secondary metabolite patterns. A key to all currently accepted Wallemia species is provided that allow their identification on the basis of physiological, micromorphological and culture characters.