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Usability of DNA Sequence Data: from Taxonomy over Barcoding to Field Detection. A Case Study of Oomycete Pathogens

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Oomycetes belong to the kingdom Straminipila, a remarkably diverse group which includes brown algae and planktonic diatoms, although they have previously been classified under the kingdom Fungi. These organisms have evolved both saprophytic and pathogenic lifestyles, and more than 60% of the known species are pathogens on plants, the majority of which are classified into the order Peronosporales (includes downy mildews,

Phytophthora, and *Pythium*). Recent phylogenetic investigations based on DNA sequences have revealed that the diversity of oomycetes has been largely underestimated. Although morphology is the most valuable criterion for their identification and diversity, morphological species identification is time-consuming and in some groups very difficult, especially for non-taxonomists.

DNA barcoding is a fast and reliable tool for identification of species, enabling us to unravel the diversity and distribution of oomycetes. Accurate species determination of plant pathogens is a prerequisite for their control and quarantine, and further for assessing their potential threat to crops. The mitochondrial *cox*2 gene has been widely used for identification, taxonomy and phylogeny of various oomycete groups. However, recently the *cox*1 gene was proposed as a DNA barcode marker instead, together with ITS rDNA. To determine which out of *cox*1 or *cox*2 is best suited as universal oomycete barcode, we compared these two genes in terms of (1) PCR efficiency for 31 representative genera, as well as for historic herbarium specimens, and (2) in terms of sequence polymorphism, intra- and interspecific divergence. The primer sets for *cox*2 successfully amplified all oomycete genera tested, while *cox*1 failed to amplify three genera. In addition, *cox*2 exhibited higher PCR efficiency for historic herbarium specimens, providing easier access to barcoding type material. In addition, *cox*2 yielded higher species identification success, with higher interspecific and lower intraspecific divergences than *cox*1. Therefore, *cox*2 is suggested as a partner DNA barcode along with ITS rDNA instead of *cox*1.

Including the two barcoding markers, ITS rDNA and *cox*2 mtDNA, the multi-locus phylogenetic analyses were performed to resolve two complex clades, *Bremia lactucae* (lettuce downy mildew) and *Peronospora effuse* (spinach downy mildew) at the species level and to infer evolutionary relationships within them. The approaches discriminated all currently accepted species and revealed several previously unrecognized lineages, which are specific to a host genus or species. The sequence polymorphisms were useful to develop a real-time quantitative PCR (qPCR) assay for detection of airborne inoculum of *B. lactucae* and *P. effusa*. Specificity tests revealed that the qPCR assay is specific for detection of each species. This assay is sensitive, enabling detection of very low levels of inoculum that may be present in the field. Early detection of the pathogen, coupled with knowledge of other factors that favor downy mildew outbreaks, may enable disease forecasting for judicious timing of fungicide applications.