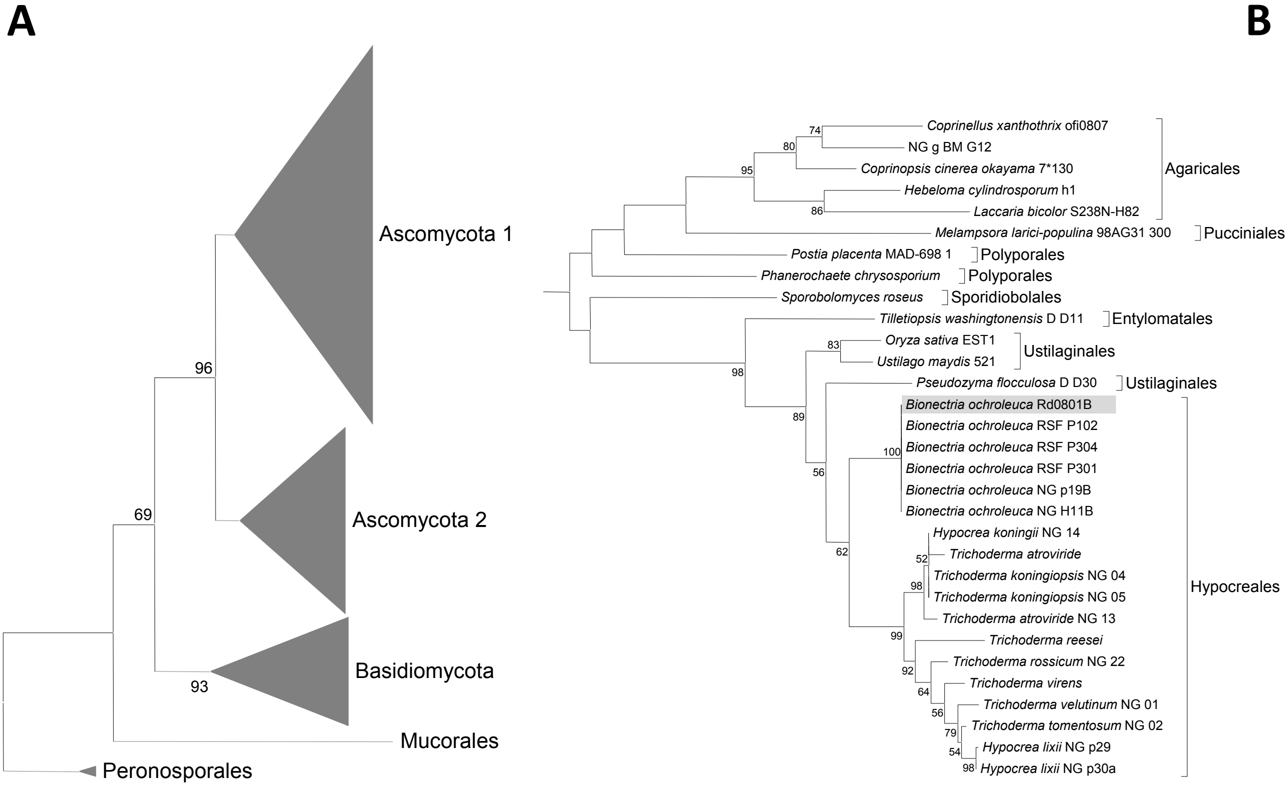
Supplementary Figure 1 Phylogenetic tree of full-length EukNR. The hypervariable N-terminal region was excluded from the analysis, the Bacillariophyta were used as outgroup. Positioning of EukNR from *Mucor circinelloides* is consistent with acquisition of the nitrate assimilation gene cluster in the fungal kingdom before the divergence of Mucoromycotina and Dikarya.

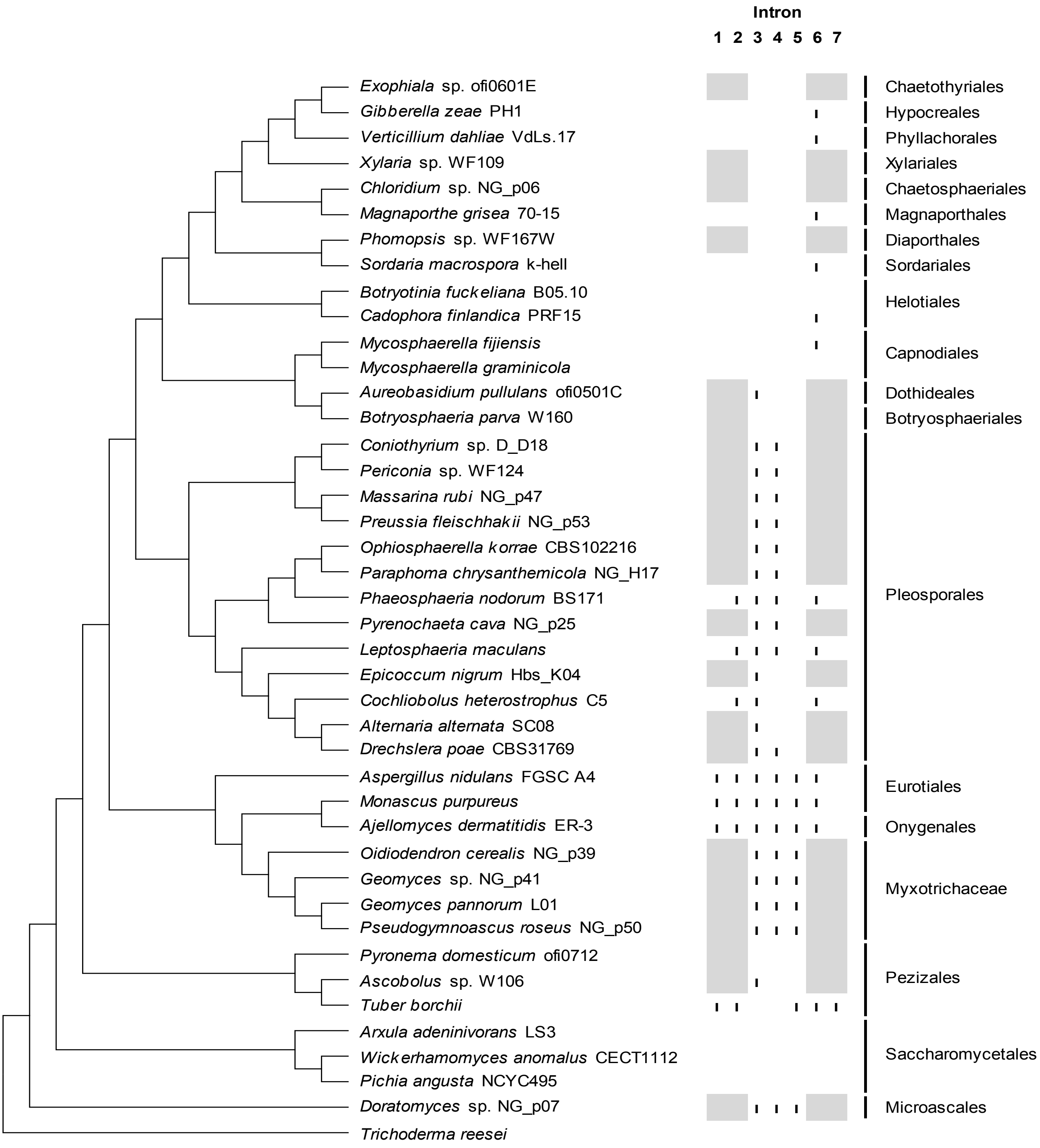


Supplementary Figure 2 Phylogenetic tree of partial EukNR. Phylogenetic analysis was based on the partial MoCo binding domain of EukNR (Region A in Figure 1). *Phytophthora* spp. (Peronosporales) were used as outgroup, bootstrap values above 50 % are indicated. In panel A a schematic overview with the major branches collapsed is given. In panel B the basidiomycetous branch including EukNR from *Trichoderma*/*Hypocrea* and *Bionectria* is uncollapsed. The two uncollapsed ascomycetous subbranches are shown on the next page. Ascomycota 1 (next page panel C) contains the Dothideomycetes, Leotiomycetes and Sordariomycetes and Ascomycota 2 (next page panel D) contains the Saccharomycetales, Pezizales and the Eurotiomycetes including EukNR from the Myxotrichaceae.

Duplicated EukNRs in *Bionectria ochroleuca* Rd0801 (panels B and C) and *Phialophora mustea* KSS12_F02 (panel C) are shaded in grey.

Saccharomycetales, Pleosporales, Dothideales, Helotiales, Phyllachorales and Microascales, which is currently represented only by the genus *Doratomyces*, appear monophyletic with high bootstrap support. Eurotiales and Botryosphaeriales, which are currently represented only by the genus *Botryosphaeria*, are monophyletic with low bootstrap support. Capnodiales and Hypocreales (excluding *Trichoderma*/*Hypocrea* and the basidiomycetous EukNR from *Bionectria*) appear monophyletic without bootstrap support; *Diaporthe eres* MB08391 (Diaporthales) and *Phialophora mustea* KSS12_F02 (Chaetothyriales) are currently nested within the Capnodiales and Hyporcreales, respectively. The remaining orders (Pezizales, Onygenales, Xylariales and Sordariales) appear thus far polyphyletic.

Supplementary Figure 2 - continued



Supplementary Figure 3 Presence/absence of introns at conserved positions in ascomycetous *euknr* genes. The phylogenetic tree is based on the partial molybdenum cofactor binding site and partial cytochrome b5 domain of EukNR (Region B in Figure 1), *Trichoderma reesei* was used as outgroup. Only representative species from each order are shown. Presence of the respective introns is indicated by a vertical bar. Were only partial sequence information is available, missing data are indicated by grey shading. In general good conservation of introns at conserved positions is found at the ordinal and even at the class level. According to the available dataset only minor variations are found within the orders Helotiales, Capnodiales, Pleosporales and Pezizales. Major deviations from the general pattern in the presence/absence of introns at conserved positions coincide with unusual positions of EukNR in the phylogenetic tree: The intron pattern in the Myxotrichaceae (Leotiomycetes) resembles the pattern found in the Eurotiales and Onygenales (Eurotiomycetes), which is in agreement with a proposed horizontal gene transfer based on EukNR phylogeny (see Figure 2). Similarly, *Doratomyces* sp. (Microascales, Sordariomycetes), does not cluster with the remaining orders from the class Sordariomycetes. More data are, however, needed to propose another horizontal gene transfer.