SUPPLEMENTAL MATERIAL

DNA extraction. Fungal biomass (0.3 g wet weight) was placed in a 2-mL screw cap plastic tube containing approximately 200 mg of 0.5 mm silica and zirconium beads (Fisher Scientific, USA). Then, 1 mL of Carlson lysis buffer (1) and 1.5 µL β-mercaptoethanol (Fisher Scientific, USA) were added to each tube, followed by two rounds of bead-beating at room temperature for 5 minutes in an Omni bead ruptor 24 (Omni International, Kennesaw, GA) at 5.65 m/s. After sample incubation at 74 °C for 30 minutes, an initial chloroform:isoamyl alcohol (24:1, [v/v]) wash step was followed by two additional wash steps with 750 µL phenol:chloroform:isoamyl alcohol (25:24:1 [v/v]) before 750 µL chloroform:isoamyl alcohol (24:1, [v/v]) was added. The upper aqueous phase was transferred to a fresh tube, to which 0.6 volumes of ice-cold isopropyl alcohol was added, followed by cooling at -20 °C for at least 2 hours. Samples were then centrifuged at 4 °C for 15 minutes at 10,000 x g, the supernatants discarded, and the pellet washed with ice-cold 70 % ([v/v]) molecular grade ethanol. Following a final centrifugation step (10,000 x g, 5 minutes), the supernatants were discarded, and the pellets air-dried at room temperature. The pellets were suspended in 100 µL DNase-free H_2O and stored at -20 °C until analysis. DNA extraction from soil samples was performed using the FastDNA spin kit for soil (MP Biomedicals, Santa Ana, CA) following the manufacturer's protocol. DNA concentration and purity were analyzed with a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA) and a Qubit 2.0 fluorometer (Life Technologies, Carlsbad, CA) using the dsDNA BR Assay Kit following manufacturer's recommendations (Life Technologies, Carlsbad, CA).

Primer design. To design primers targeting *p450nor* gene sequences, primary literature resources were queried to identify available *p450nor* gene and protein sequences. These

reference P450nor sequences were assessed using multiple sequence alignment (2) to identify conserved residues, followed by selection of additional sequences by querying public sequence databases (GenBank, UniProt) using BLASTx (3). Overall, 38 *p450nor* reference gene sequences were obtained and aligned using the program T-Coffee (2) followed by manual inspection. PAL2NAL (4) was used to generate a codon-aware nucleotide alignment to assist in selecting sites for primer design. In order to design primers specific to fungal *p450nor* sequences and prevent binding to other genes present within the diverse cytochrome P450 protein family, features unique to the 38 P450nor reference sequences were identified (5–7). Specifically, mutations in a secondary structural element of the P450nor protein, referred to as the B helix, abolished the reducing capacity of the enzyme and implicated specific amino acid residues in NADH or NADPH co-substrate binding (5). Additionally, the B helix has been proposed as a unique and variable structural element of the CYP55 family of P450 cytochromes (8); therefore, an adjacent, conserved site was selected for forward primer binding (*p450nor*394F, Table 1). Further inspection of the 38 reference protein sequences revealed conserved secondary structural elements 650-750 nucleotides downstream of the forward priming site suitable for reverse primer design (Table 1).

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SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Stereomicroscopic images of representative members of isolated fungal groups (A) and singletons (B) identified by sequencing of the cloned ITS region from each fungal isolate. The group identifier and putative taxonomic designation are listed in the white boxes to the left of the images. Scale bars and fungal isolate ID number are located in the upper left and lower left corner of each image, respectively.

Figure S2. Alignment of P450nor sequences from select organisms displaying primer binding sites (pink), and key residues involved in cofactor (NAD(P)H) binding and P450nor activity. The N-terminal region of the alignment has been removed for clarity. Stars below the alignment (blue, red, black) indicate positive, negative and charged amino acids and key residues involved in NAD(P)H binding, respectively. Sites outlined in black with all bold amino acid characters indicate 100% amino acid identity. Columns highlighted yellow and outlined in black indicate ≥ 80% amino acid similarity. Stars with a white "S" and "H" indicate involvement in a salt bridge and proton channel, respectively. PP stands for a residue believed to be involved in binding pyrophosphate on the NAD(P)H molecule. The B' helix (helix 3α above) is a hypervariable region of P450 proteins and represents an area of NAD(P)H recognition by P450nor. P450nor secondary structure is provided by the PDB accession 1JFB and was added using the ESPript 3.0 web server. F_oxysorum = *Fusarium oxysporum*, F_lichenicola = *Fusarium lichenicola*, C_globosum = *Chaetomium globosum*, T_virens = *Trichoderma virens*, S_macrospora = *Sordaria macrospora*, G_graminis = *Gaeumannomyces graminis*, A_terreus = *Aspergillus terreus* NRRL 255, A_flavus = *Aspergilus flavus* NRRL 3357, U_reesii = *Uncinocarpus reesii*, C_apollinis = *Coniosporium apollinis*, M_gypseum = *Microsporum gypseum*, M_brunnea = *Marssonina brunnea*, M_phaseolina = *Macrophomina phaseolina*, T_cutaneum = *Trichosporon cutaneum*, C_reinhardtii = *Chlamydomonas rheinhardtii*, C_variabilis = *Chlorella variabilis*.

Figure S3. N_2O production in soil microcosms amended with chloramphenicol and streptomycin to inhibit bacterial activity. NO_3^- (2 mM) or NO_2^- (1 mM) were added as the sole nitrogen source. Acetate (●), formate (□), pyruvate (▲), or plant residue (◊, dashed line), were added as carbon source and electron donor for NO_3^- or NO_2^- reduction, respectively, in Havana (left

column) or Urbana (right column) microcosms. Note the use of different scales on the y-axes. Carbon sources were not monitored during the enrichment.

Figure S4. NO_3^- (▲), NO_2^- (■), and N_2O (○) dynamics in transfer cultures from Havana and Urbana microcosms amended with ampicillin and kanamycin to prevent bacterial activity. NO_3^- (2 mM) or NO_2^- (1 mM) were provided as the sole nitrogen source. Acetate (A, B, C, D), formate (E, F, G, H), plant residue (I, J, K, L), or pyruvate (M, N, O, P) were added as carbon source and electron donor. Note the differences in scale on the y-axes.

Figure S5. Intron structure of the region amplified by *p450nor* primer set *p450nor*394F/*p450nor*809R in 47 *p450nor* sequences. Only one to four introns are detected in the amplified region. Thick black lines indicate the gene, and black rectangles above this line indicate the coding sequences (CDS). Thin lines between CDS rectangles indicate an intron. Green triangles indicate *p450nor*394F/*p450nor*809R binding sites. Some genes have been reversed for clarity (indicated by red REV next to sequence name).

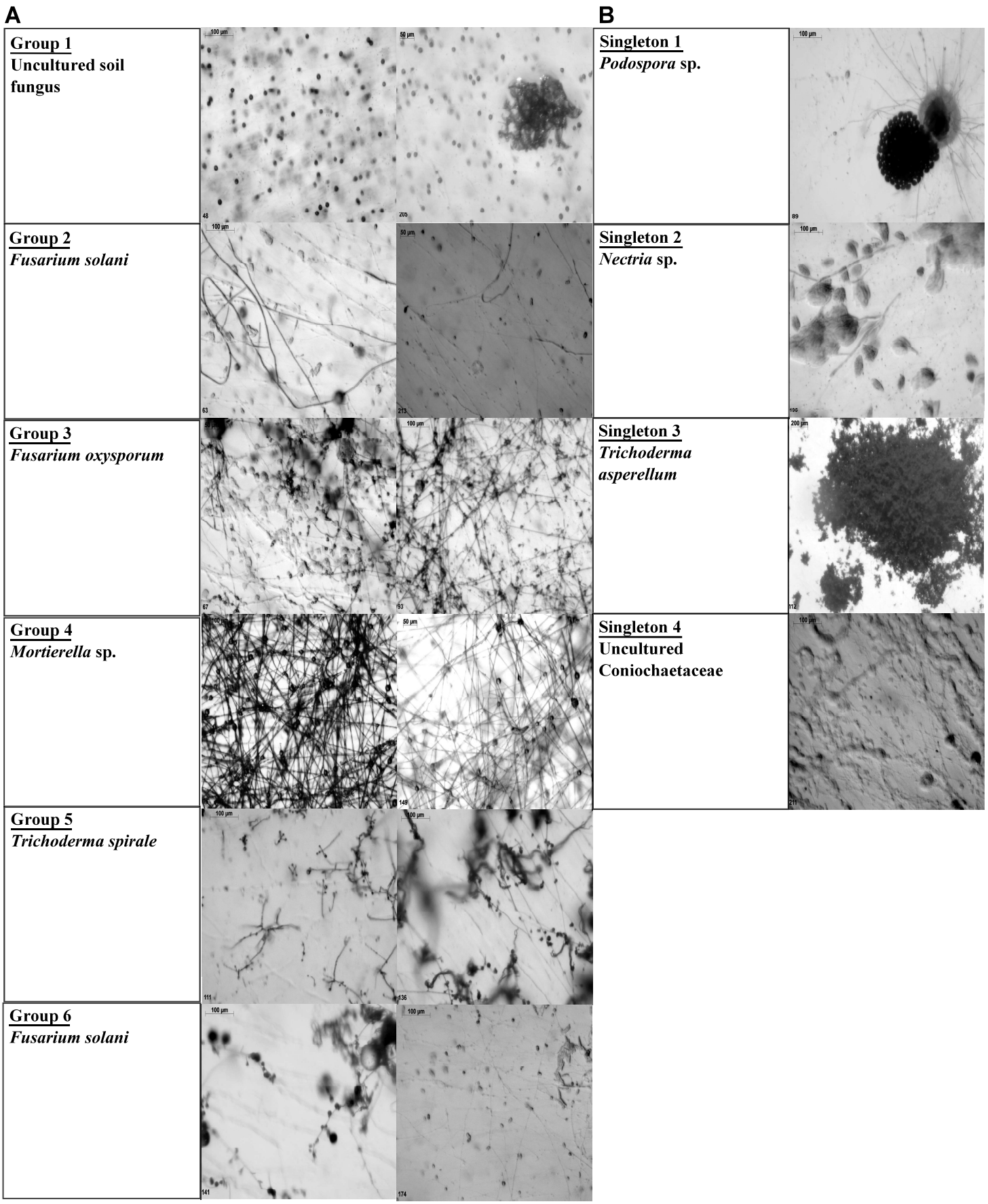
SUPPLEMENTAL DATASET LEGENDS

Dataset S1. NCBI protein GI and Accession numbers of P450nor sequences used for primer design.

Dataset S2. Accessory metadata for the fungal isolates from Havana and Urbana soils.

Dataset S3. The SILVA 18S rRNA gene classification of 37 fungal isolates in Figure 1.

Dataset S4. *p450nor* amplicon lengths and number of introns in amplified region using primer set *p450nor*394F/*p450nor*809R.



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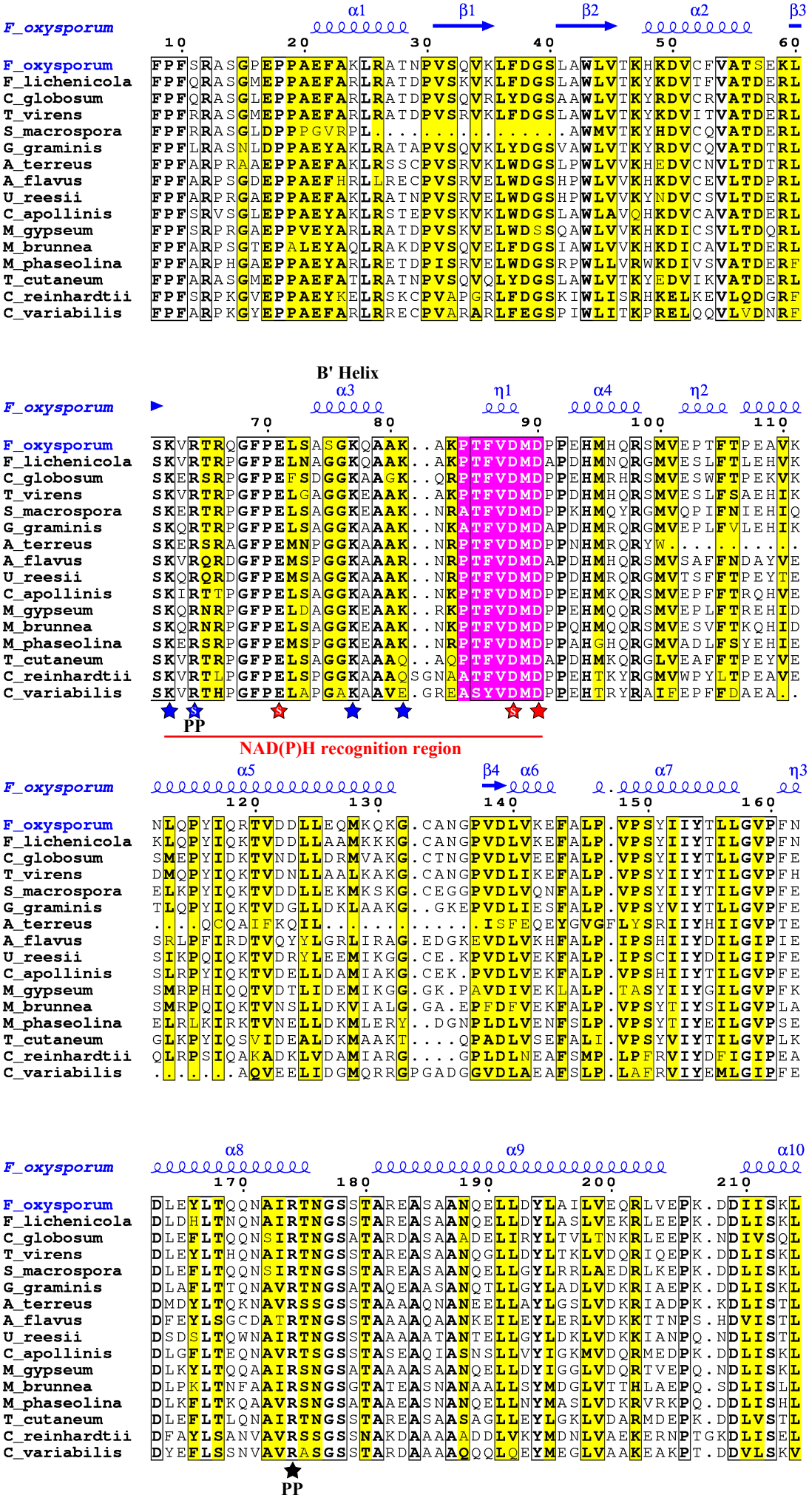
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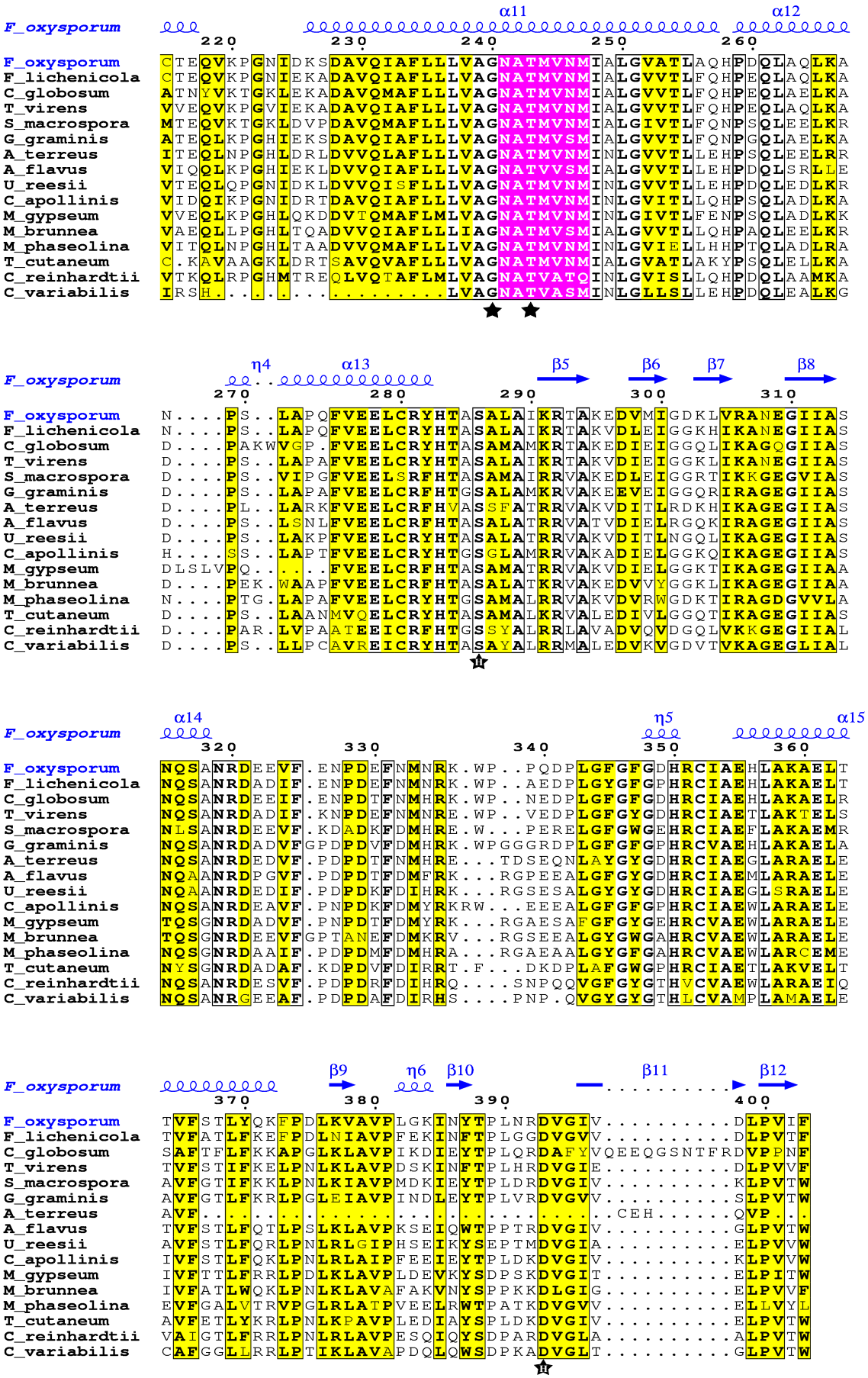
PP
NAD(P)H recognition region
PP



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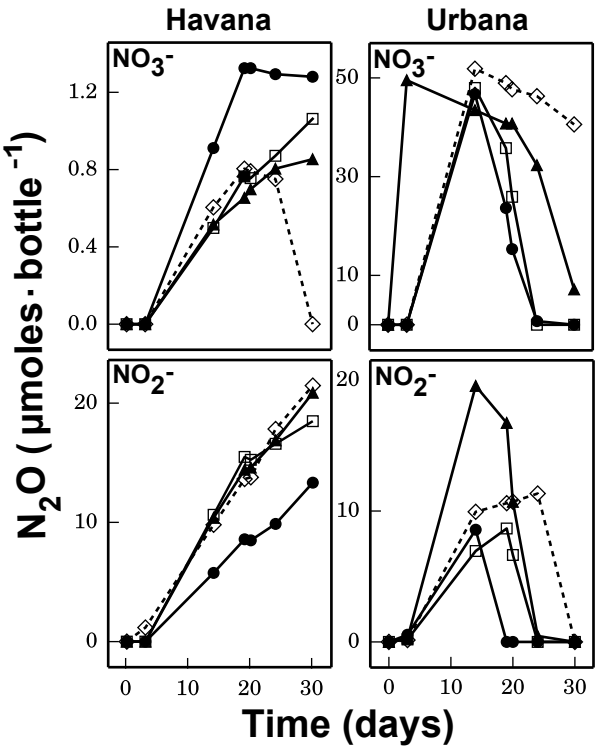

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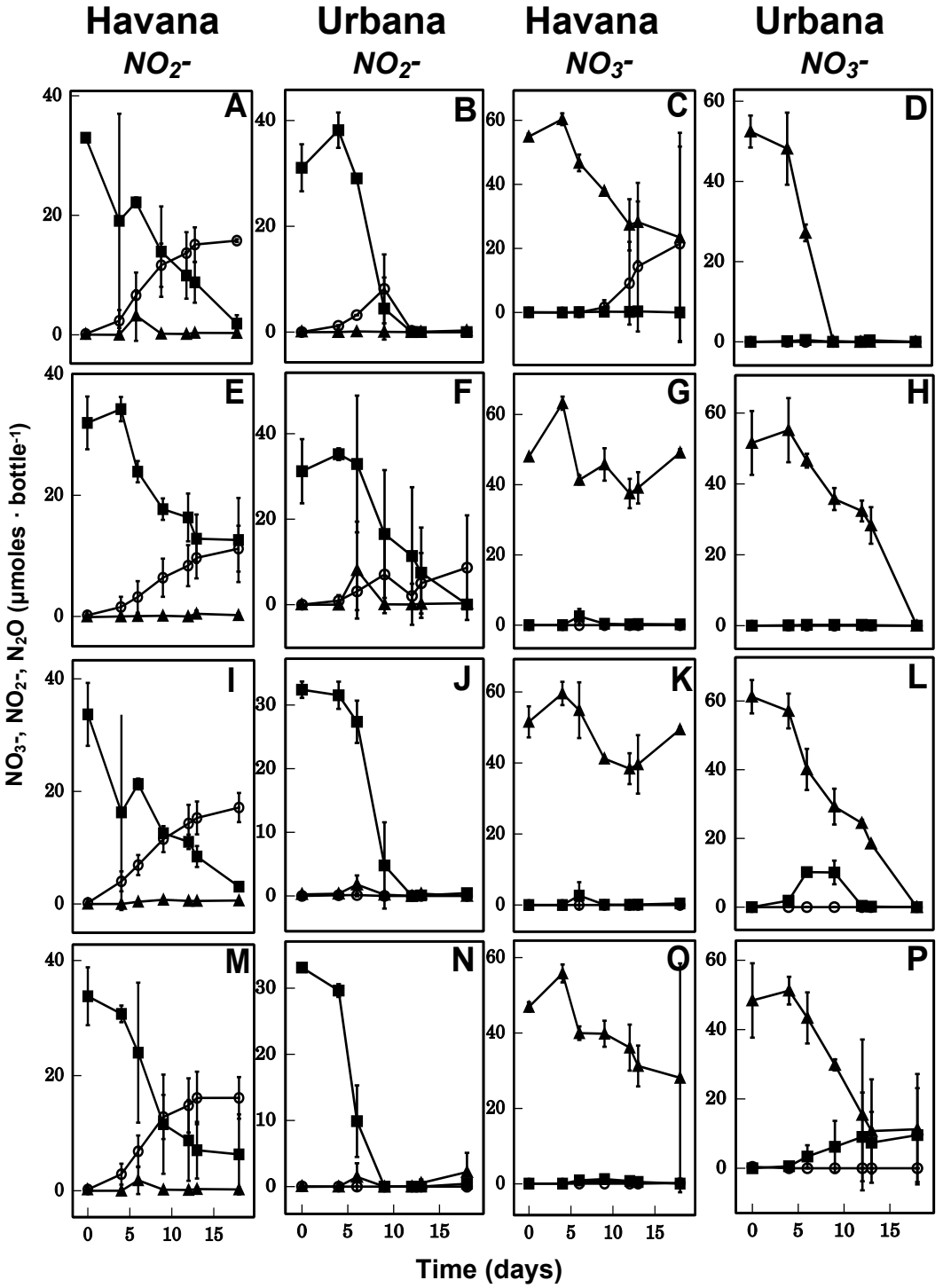

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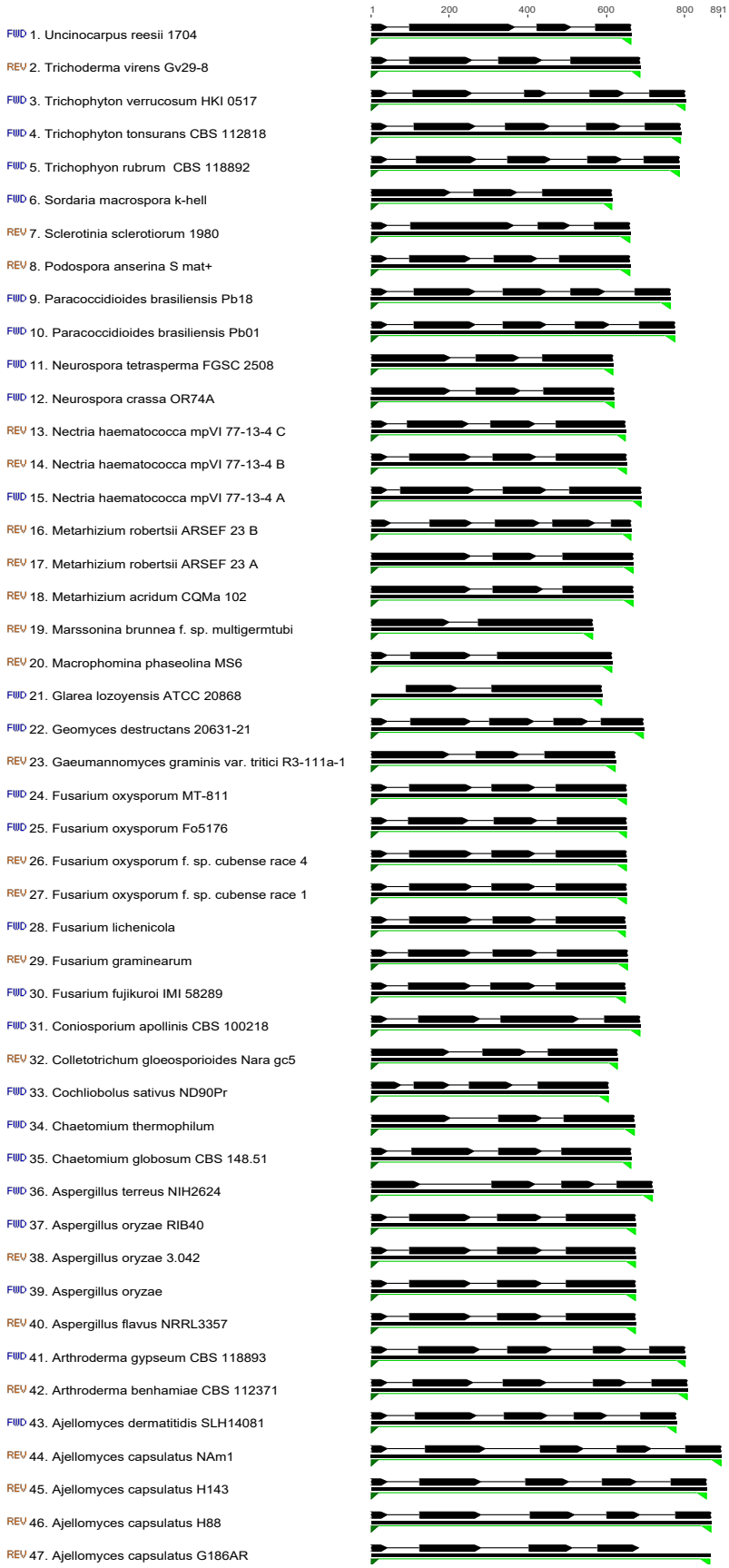

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