Validation of ITS from GenBank database in diagnosing medically important black yeasts and relatives species of Herpotrichiellaceae

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Objectives

To investigate the use of ITS from GenBank database and NCBI blast searching in diagnosing black yeast and relatives species of Herpotrichiellaceae.

Materials and Methods

- 356 ITS-sequences (ITS1-5.8S-ITS2) of 115 species of medical and saprophytic black yeasts and relatives in family Herpotrichiellaceae viz. *Cladophialophora* (33 sp.), *Exophiala* (40 sp.), *Fonsecaea* (9 sp.), *Phialophora* (23 sp.), *Rhinocladiella* (10 sp.), were collected from GenBank database by using the criteria of legitimate names in Mycobank deposition and type or the reference strains.
- To investigate the species cluster produced from the ITS dataset, the Neighbor joining (NJ) analysis with K2+G model was performed in MEGA6.
- To observe precision and accuracy of ITS in identification, each of type or reference sequence was tested by NCBI BLAST® megablast searching for the identity values (%).

Results (Fig 1)

- NJ analysis;
 - Species boundaries of 104 species (90.43%) were well defined with high supports.
 - Species boundaries of 11 species were ambiguous viz.
 - 6 species, the members mixed with another species in the same cluster viz. *C. devriesii* & *F. brasiliensis*, *F. compacta* & *F. pedrosoi*, *P. chinensis* & *P. verrucosa*.
 - the members of 3 species, *P. mustea*, *P. calyciformis* and *P. brunnescens* were clustered together.
 - the taxonomic positions of *P. intermedia* and *P. phaeophora* could not be indicated because each species member showed two different positions in the analysis.
- NCBI megablast searching;
 - 109 species (94.78%) were 94-100% identical with maximum scores and specific to their own types or reference species.
 - ITSs of six species were cross-specific to another type species viz. *C. devriesii* & *F. brasiliensis*, *F. compacta* & *F. pedrosoi*, *P. chinensis* & *P. verrucosa*, concordant to the results from NJ.
 - P. mustea*, *P. calyciformis* and *P. brunnescens* were 99-100% identical to *Pleurostoma richardsiae*, the ex-member of *Phialophora* which is now classified into Calosphaeriaceae.

Conclusion

- ITS sequence (ITS1-5.8-ITS2) could delimit 104 species-boundaries of black yeasts and relatives in Herpotrichiellaceae.
- The ITS could be used as a tool to identify 109 species and highly specific to type and reference strains without cross-specific to other species (94-100% identity).
- Cross-specific identity might reflect ambiguous classification of some taxa, revision of them might have to be considered.
- The hazardous causative agents of CNS infection viz. *C. bantiana*, *E. dermatitidis* and *R. mackenziei*, could be specifically identified by ITS with megablast searching (96-100% identity).
- GenBank database and NCBI blast searching are reliable and standardized enough to use for correct identification of black yeasts and relatives in Herpotrichiellaceae.
- Validation of ITS for identifying black yeasts and relatives in other families in the GenBank database should be further investigated.

Fig 1 Neighbor joining tree from 356 ITS-sequences of 115 species of black yeasts and relatives in Herpotrichiellaceae. Numbers on the branches are percents of 1,000 bootstrap replications.