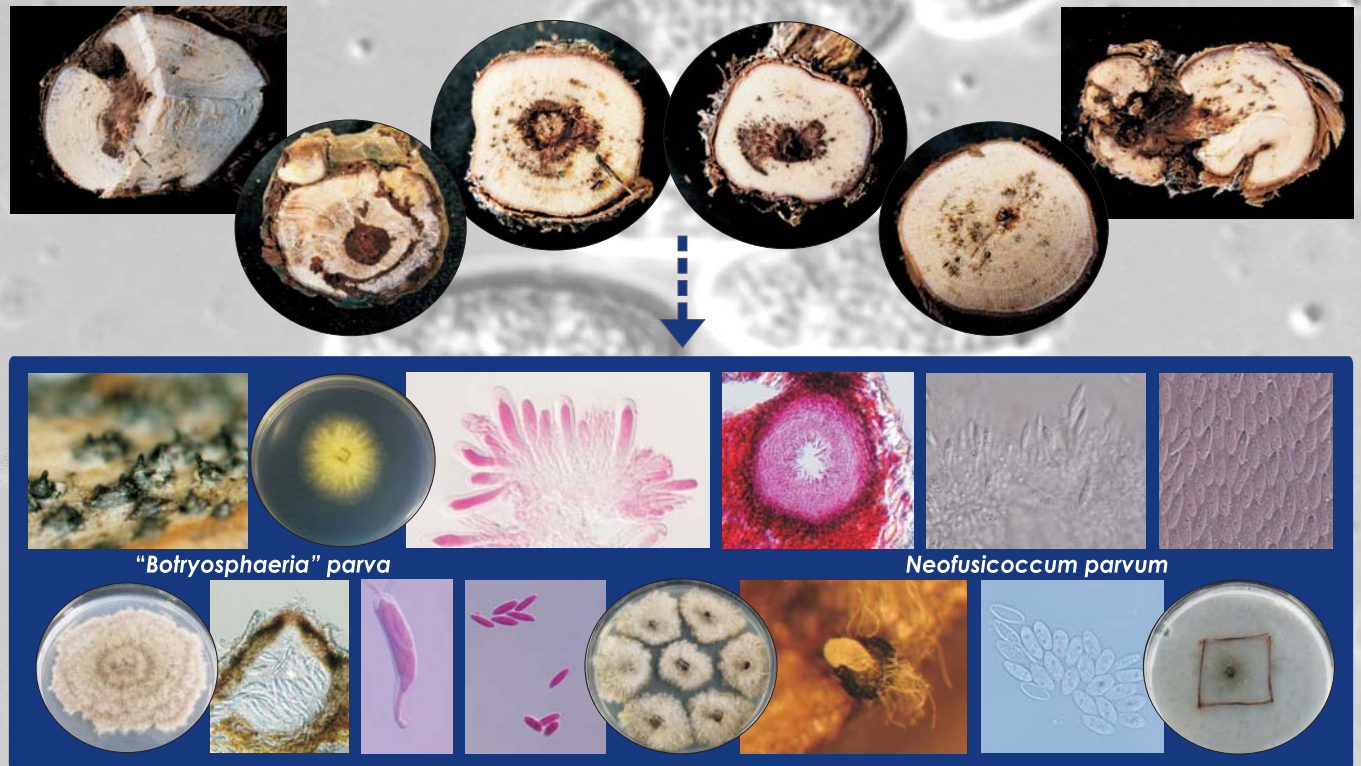


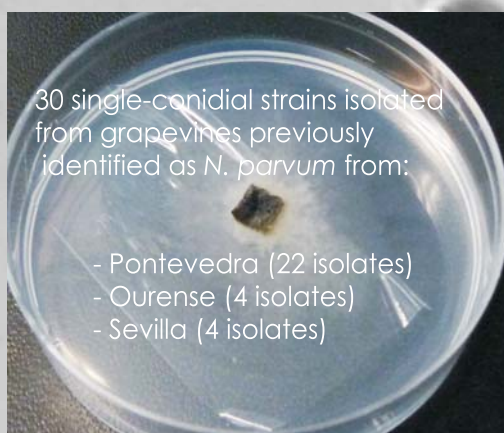
INTRODUCTION

Neofusicoccum parvum is a cosmopolitan, polyphagous fungal pathogen and one of the most virulent Botryosphaeriaceae species found on grapevine. *Neofusicoccum ribis* "sensu stricto" was detected on grapevine for the first time in 2011. *Neofusicoccum parvum* and *N. ribis* are two closely related species. In fact, the variability and overlapping in the morphological and culture characteristics of both species makes the identification of both species difficult only on the basis of the morphological criteria.

Recent molecular studies have aided to clarify boundaries between these two species by using a multiple loci approach, which revealed the existence of eight cryptic species in the complex: *N. batangarum*, *N. cordaticola*, *N. kwambonambiense*, *N. oculatum*, *N. parvum*, *N. ribis*, *N. umdonicola*, and *Neofusicoccum sp. karanda*.



MATERIALS AND METHODS



30 single-conidial strains isolated from grapevines previously identified as *N. parvum* from:

- Pontevedra (22 isolates)
- Ourense (4 isolates)
- Sevilla (4 isolates)

DNA
EXTRACTION

AMPLIFICATION AND SEQUENCING

- Internal transcribed spacer (ITS)
- Beta-tubulin (BT)
- Translation elongation factor (EF)
- RNA polymerase subunit II (RPB2)

The alignments include sequences available from TreeBase (S10644- www.treebase.org)

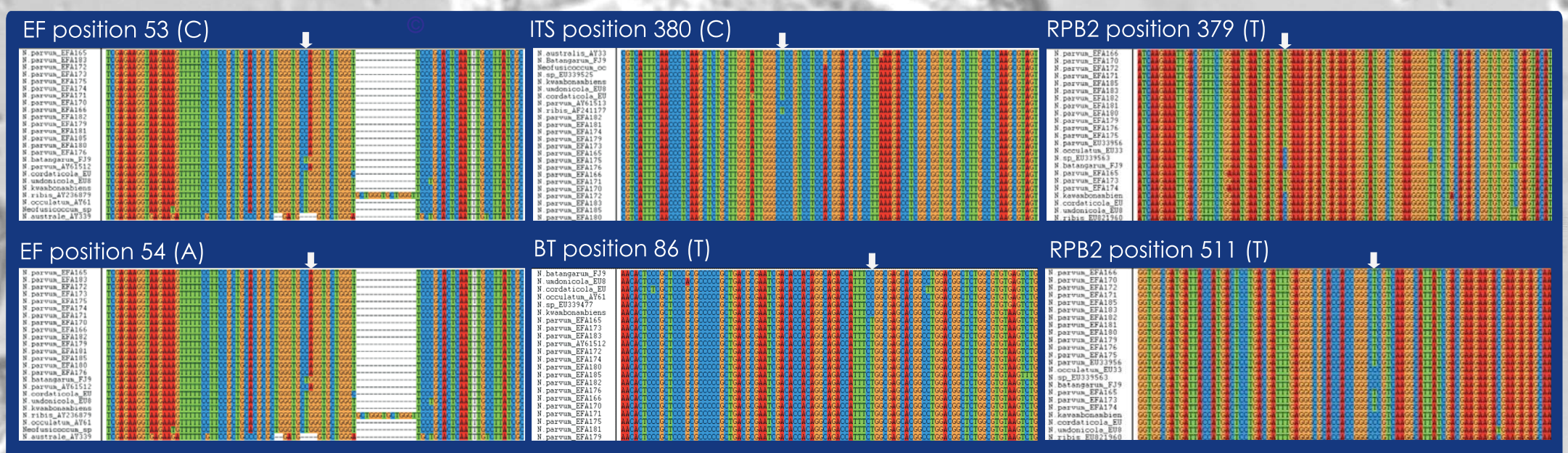
Table 1. Polymorphisms of *N. parvum/ribis* complex species from four gene regions (Sakalidis *et al.* 2011).

	ITS	BT	EF	RPB2	Total
<i>N. oculatum</i>	0	179(A)	0	0	1
<i>N. sp. karanda</i>		148(T)	21(T)		2
<i>N. batangarum</i>	390(G)				1
<i>N. cordaticola</i>	142(C)	35(T)	64(C)	136(A)	12
	373(G)	99(T)	229(C)	148(T)	
	417(C)	319(G)		301(A)	
				446(C)	
<i>N. kwambonambiense</i>	164(T)	178(T)	0	85(G)	9
	174(G)	238(A)		418(A)	
		254(A)		457(A)	
				562(C)	
<i>N. parvum</i>	380(C)	86(T)	53(C)	379(T)	6
			54(A)	511(T)	
<i>N. ribis</i>		160(T)	65-78 ^a	0	3
			213(A)		
<i>N. umdonicola</i>	169(C)	43(A)	82(T)	316(T)	4

a: *N. ribis* has an indel of 14 bp in the EF-1 α region. This is normally coded as one evolutionary event in phylogenetic analysis.

RESULTS

Six unique nucleotides across four loci that characterize *N. parvum* were observed: ITS position 380 (C), BT position 86 (T), EF positions 53 (C) and 54 (A) and RPB2 positions 379 (T) and 511 (T). All isolates analyzed in this study were identified as *N. parvum*, thus confirming the initial identification. However the analysis of other positions in the sequences indicated differences among our isolates. These differences will be the subject of future research. The alignments of the four regions of 15 isolates are presented in the following figures.



Acknowledgements

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Reference

Sakalidis M.L., Hardy G.E.St.J. and Burgess, T.I. 2011. Use of the Genealogical Sorting Index (GSI) to delineate species boundaries in the *Neofusicoccum parvum*-*Neofusicoccum ribis* species complex. *Molecular Phylogenetics and Evolution*, 60:333-344.