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Letter to the Editor

A rebuttal to the letter to the editor concerning 'Defining species boundaries in the genus *Phytophthora*: the case of *Phytophthora andina*'

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Dear Editor,

In their letter to the Editor in this issue of Plant Pathology, Cárdenas *et al.* (2012) used the paper of Oliva *et al.* (2010) on the species description of *Phytophthora andina* as an example to discuss the criteria used for species designation in the genus *Phytophthora*, and suggest that the current species description of *P. andina* 'cannot yet be considered as accurate'. Cárdenas *et al.* (2012) contended that Oliva *et al.* (2010) did not provide sufficient phylogenetic evidence to support designation of *P. andina* as a new species, and that the data indicate that *P. andina* is not monophyletic. We agree that, in addition to morphological data, robust phylogenetic and genealogical analyses are important when a new species is described. However, we also believe that Oliva *et al.* (2010) and several other reports have provided sufficient data to establish *P. andina* as a new species. Here, we assess the evidence for species designation of *P. andina*, comment on the polyphyletic nature of *P. andina*, and suggest areas where future research is needed.

Evidence for species designation of *P. andina*

Phytophthora andina comprises at least three clonal lineages that have been defined by multilocus genotyping and are designated EC-2(1a), EC-2(1c) and EC-3(1a), where '1a' and '1c' refer to mitochondrial haplotypes using the nomenclature of Griffith & Shaw (1998). At the time of publication of Oliva *et al.* (2010), at least five previous studies had published phylogenies based on mitochondrial loci, nuclear loci or both (Table 1) that included one or more of the lineages of *P. andina* together with other related *Phytophthora* spp.

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One of the earliest studies involving a single isolate of EC-2(1c) clearly distinguished *P. andina* from *P. infestans* and other related species based on two nuclear and four mitochondrial loci (Kroon *et al.*, 2004). Subsequently, two studies (Gómez-Alpizar *et al.*, 2007, 2008) also examined the phylogeny of the EC-2(1c) clonal lineage of *P. andina* and used both nuclear and mitochondrial gene genealogies to clearly document the evolutionary history of EC-2(1c) from *P. infestans* and other *Phytophthora* species. EC-2(1c) shares a common mitochondrial ancestor with *P. infestans* and it was also suggested by Gómez-Alpizar *et al.* (2008), Oliva *et al.* (2010) and Kroon *et al.* (2004) that *P. andina* may be of hybrid origin.

The EC-3 lineage of *P. andina* was discovered later and was not examined in all the previous studies. Nonetheless, EC-3 was clearly distinguished from *P. infestans* with nuclear *ras* loci but could not be distinguished from *P. infestans* with mitochondrial loci, since it shares the Ia mitochondrial haplotype with *P. infestans* (Gómez-Alpizar *et al.*, 2008; Oliva *et al.*, 2010). The status of this lineage was left unresolved in Gómez-Alpizar *et al.* (2008) until additional isolates could be examined.

In summary, all the studies in Table 1 were consistent in distinguishing *P. andina* from other similar *Phytophthora* species when nuclear loci were examined. With mitochondrial loci, isolates with the 1c haplotype were distinguished, while those with the 1a haplotype were similar or identical to *P. infestans*. Thus, it can be concluded that there was sufficient sequence-based phylogenetic evidence from nuclear genes for designation of the species *P. andina* at the time of publication of Oliva *et al.* (2010).

Polyphyletic nature of *P. andina*

In Oliva *et al.* (2010) it was argued that the EC-2(1a), EC-2(1c) and EC-3 lineages should all be considered

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Table 1 Summary of sequence-based studies involving both *Phytophthora infestans* and mitochondrial groups of *Phytophthora andina* at the time of publication of Oliva *et al.* (2010)

Gene	References	*P. infestans*	Number of isolates in each mitochondrial group of *Phytophthora andina*[a] 1a	1c
Mitochondrial loci				
mtDNA IGS	Wattier *et al.*, 2003		ND	
Mt cox1	Kroon *et al.*, 2004		ND	
	Gómez-Alpizar *et al.*, 2007		ND	
	Gómez-Alpizar *et al.*, 2008		8(EC-3)	
cox 11	Oliva *et al.*, 2010		7	
Mt nadh1	Kroon *et al.*, 2004		ND	
Mt P3, P4	Gómez-Alpizar *et al.*, 2007		ND	
Nuclear loci				
ITS	Oliva *et al.*, 2010			5
	Gómez-Alpizar *et al.*, 2008		8(EC-3)	10
β-tubulin	Kroon *et al.*, 2004		ND	1[c]
Intron 1 of *ras*	Oliva *et al.*, 2010		5(EC-3) 2(EC-2)	5
	Gómez-Alpizar *et al.*, 2008		8(EC-3)	10
	Gómez-Alpizar *et al.*, 2007		ND	4
N EF-1a	Kroon *et al.*, 2004		ND	1[c]
Seven nuclear genes	Blair *et al.*, 2008		ND	1

ND, not determined.

[a]Darker shading indicates similarity to *P. infestans*; lighter indicates separation based on the analysis; 1a and 1c refer to mitochondrial haplotypes as per Griffith & Shaw (1998) and defined by Adler *et al.* (2004); there are two RFLP groups, published by Adler *et al.* (2004) as EC-2 and EC-3, that have the 1a haplotype, while the 1c haplotype is only found in the EC-2(1c) lineage.

[b]Assumed to be EC-2 primarily based on timing as the other lineages were discovered later.

[c]Isolate 3421.

P. andina for two reasons. First, based on sequencing of nuclear genes (Gómez-Alpizar *et al.*, 2008; Lassiter *et al.*, 2010; Oliva *et al.*, 2010) the three lineages showed high levels of heterozygosity (unlike *P. infestans*), and shared at least one allele at many loci with *P. infestans*, which again is in agreement with the hypothesis of a hybrid origin of *P. andina*. The hybrid nature of *P. andina* was again suggested in a recent paper by Goss *et al.* (2011). Secondly, the three lineages of *P. andina* are genetically similar based on AFLP, RFLP and SSR markers (Adler *et al.*, 2004; Erselius *et al.*, 1999; Oliva *et al.*, 2007, 2010). Cárdenas *et al.* (2012) noted these studies but appear to consider them of minimal importance in species identification. We believe that random DNA variation observed with these markers can provide a close look at microevolutionary processes, particularly when supported by sequence data.

Although the EC-3 lineage was 'lumped' with the other lineages of *P. andina* in Oliva *et al.* (2010), others may choose to split this lineage into its own species as further collections and data are published. In fact, a recent paper by Goss *et al.* (2011) suggested that differences in several other nuclear loci not examined previously, including *trp*1 and PIT11126, can be used to differentiate the EC-2(1c) and EC-3 lineages of *P. andina*. The EC-3 lineage infects *Solanum betaceum*, so host isolation may be leading to speciation in this case. Goss *et al.* (2011) repeatedly used the species designation *P. andina* for isolates of both EC-2 and EC-3 lineages, which would appear inconsistent with the objection proposed in their letter to the Editor by some of the same authors.

Finally, we would like to critique the analyses done by Cárdenas *et al.* (2012) in their response. They showed that *P. andina* could not be separated from *P. infestans* using *Cox1* sequences and maximum parsimony, maximum likelihood and Bayesian analysis (Fig. 1a of Cárdenas *et al.*, 2012). If the *P. andina* isolates they examined all had the 1a mitochondrial haplotype, then one would expect them to share a clade with *P. infestans* in a *Cox1* phylogeny. However, in all previous publications, the EC-2(1c) lineage was distinct from *P. infestans* for *Cox1* (Table 1). Cárdenas *et al.* (2012) used only four isolates of *P. andina* and over 70 isolates of *P. infestans* in their study (see their Table 1) and gave no multilocus genotype information in their paper. Neither the lineage, nor the mtDNA haplotype of the isolates were given to the reader and, therefore, it is difficult to interpret their *Cox1* analysis.

Several authors (Kroon *et al.*, 2004; Blair *et al.*, 2008; Gómez-Alpizar *et al.*, 2008; Goss *et al.*, 2011) previously documented that ITS sequencing cannot resolve any of these closely related *Phytophthora* species. *Phytophthora andina*, *P. mirabilis, P. phaseoli* and *P. infestans* share 99% sequence identity in this region. Therefore, the data presented by Cárdenas *et al.* (2012) in Fig. 2a are not surprising and have been extensively

discussed by others. Finally, the lack of resolution between *P. infestans* and *P. andina* for β-tubulin in Fig. 1b of Cárdenas *et al.* (2012) is clearly inconsistent with previous studies (Table 1), regardless of the lineage of *P. andina* involved.

We suggest the continued collection of more isolates from this clade of *Phytophthora* species in the Andean region. Apparently, the Andean region is a genetic hotspot for evolution of new species in this clade and/or the presence of hybrids. Host specialization is occurring and there are bridging hosts such as pear melon (*Solanum muricatum*) that can be infected by both *P. infestans* and *P. andina* (Alder *et al.*, 2004). Multiple authors have suggested that *P. andina* is a hybrid species with either *P. mirabilis* or an unknown *Phytophthora* as a parent (Kroon *et al.*, 2004; Gómez-Alpizar *et al.*, 2008; Goss *et al.*, 2011). The fact that *P. andina* and *P. mirabilis* share closely related haplotypes at multiple nuclear loci (Gómez-Alpizar *et al.*, 2008; Lassiter *et al.*, 2010; Goss *et al.*, 2011) but do not occur sympatrically outside of Mexico is a conundrum that needs to be further explored before the true evolutionary history of the clade can be delineated.

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