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.

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 1a 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...
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)

<table>
<thead>
<tr>
<th>Gene</th>
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<th>P. infestans</th>
<th>1a</th>
<th>1c</th>
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<tr>
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<td>mtDNA IGS</td>
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<td>Kroon et al., 2004</td>
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<td>ND</td>
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<tr>
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<td>cox1</td>
<td>Oliva et al., 2010</td>
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<td>7</td>
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<td>ß-tubulin</td>
<td>Kroon et al., 2004</td>
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ND, not determined.

*aDarker 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.

*bAssumed to be EC-2 primarily based on timing as the other lineages were discovered later.

*cIsolate 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 trp1 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 phylogeny. However, in all previous publications, the EC-2(1c) lineages of P. andina 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.

References

Adler NE, Erselius LJ, Chacon MG et al., 2004. Genetic diversity of Phytophthora infestans sensu lato in Ecuador provides new insight into the origin of this important plant pathogen. *Phytopathology* 94, 154–62.


