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Species delimiting of the genus *Pratylenchus* (Nematoda: Pratylenchidae) plant-parasitic nematodes

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ABSTRACT

Root-lesion nematodes of the Pratylenchus are an important pest parasitizing a wide range of vascular plants, including several economically important crops. However, morphological diagnosis of more than 100 species is problematic due to the low number of diagnostic features, high morphological plasticity, and incomplete taxonomic descriptions. In order to employ bar-coding-based diagnostics, we analyzed the most important species of *Pratylenchus* based on COI sequences. In this study, we reconstructed the species delimitation of the *Penetrans* group using mitochondrial gene sequences. A combination of different methods for species delimitation allowed us to establish the species boundaries within the *Pratylenchus* group and, as such, clarify long-standing controversies about the taxonomic status of *Pratylenchus*. Therefore, in 2024, a study was undertaken at the University of Limpopo to compare species delimitation analyses within the *Pratylenchus* genus based on the cytochrome c oxidase I (COI) region. In this study, Assemble Species by Automatic Partitioning (ASAP) and Automatic Barcode Gap Discovery (ABGD) species delimitation approaches were employed. A total of 73 sequences GenBank were utilized, representing 13 species (*Pratylenchus* sp.*, P. zeae, P. parazeae, P. vulnus, P. scribneri, P. brachyurus, P. speijeri, P. coffeae, P. loosi, P. horti, P. hippeastri, P. penetrans, P. neglectus,* and *P. thornei*). The phylogenetic analyses grouped all the species into 14 clades, which separated all species clearly. The ASAP and ABGD analysis reveal a remarkable amount of cryptic biodiversity within the genus *Pratylenchus*, confirming that identification based on COI marker alone can be inconclusive in this taxonomically confusing genus. The phylogenetic tree supported these analyses. In conclusion, our analyses indicate COI to be a good barcode gene, as all Operational Taxonomic Units (OTUs) do not only form monophyletic groupings in the COI phylogeny.

Key words: COI mtDNA, *Pratylenchus*, phylogeny, ASAP, ABGD, species delimitation

INTRODUCTION

Root-lesion nematodes of the genus *Pratylenchus* are migratory endoparasites belonging to the family Pratylenchidae (Nematoda, Tylenchida). Root-lesion nematodes are ranked as the third most important group of plant-parasitic nematodes in terms of economic loss in agriculture and horticulture (Castillo and Vovlas, 2007). In some cases, yield loss can extend up to 85% of the expected production (Nicol *et al.*, 2011). Several species of *Pratylenchus*, such as *P. penetrans*, *P. brachyurus, P. coffeae*, and *P. vulnus* have a wide geographical distribution and can parasitize a wide range of host plants (Janssen *et al.*, 2017). Within the genus *Pratylenchus*, 98 species were recognized by Geraert (2013), after which three additional species have been described: *Pratylenchus oleae* (Palomares-Rius *et al.*, 2014), *Pratylenchus quasitereoides* (Hodda *et al.*, 2014), and *Pratylenchus parazeae* (Wang *et al.*, 2015), bringing the total species number to 101. However, morphological diagnosis of root-lesion nematodes is problematic due to the low number of diagnostic features and high intraspecific variability.

Despite the existence of a wide spectrum of identification methods, no specific approach has been widely accepted, yet in fact, most species identification methods have been tested for only a limited number of Pratylenchus taxa (Troccoli *et al.*, 2016). In recent years, several species of Pratylenchus have been matched to molecular sequences,

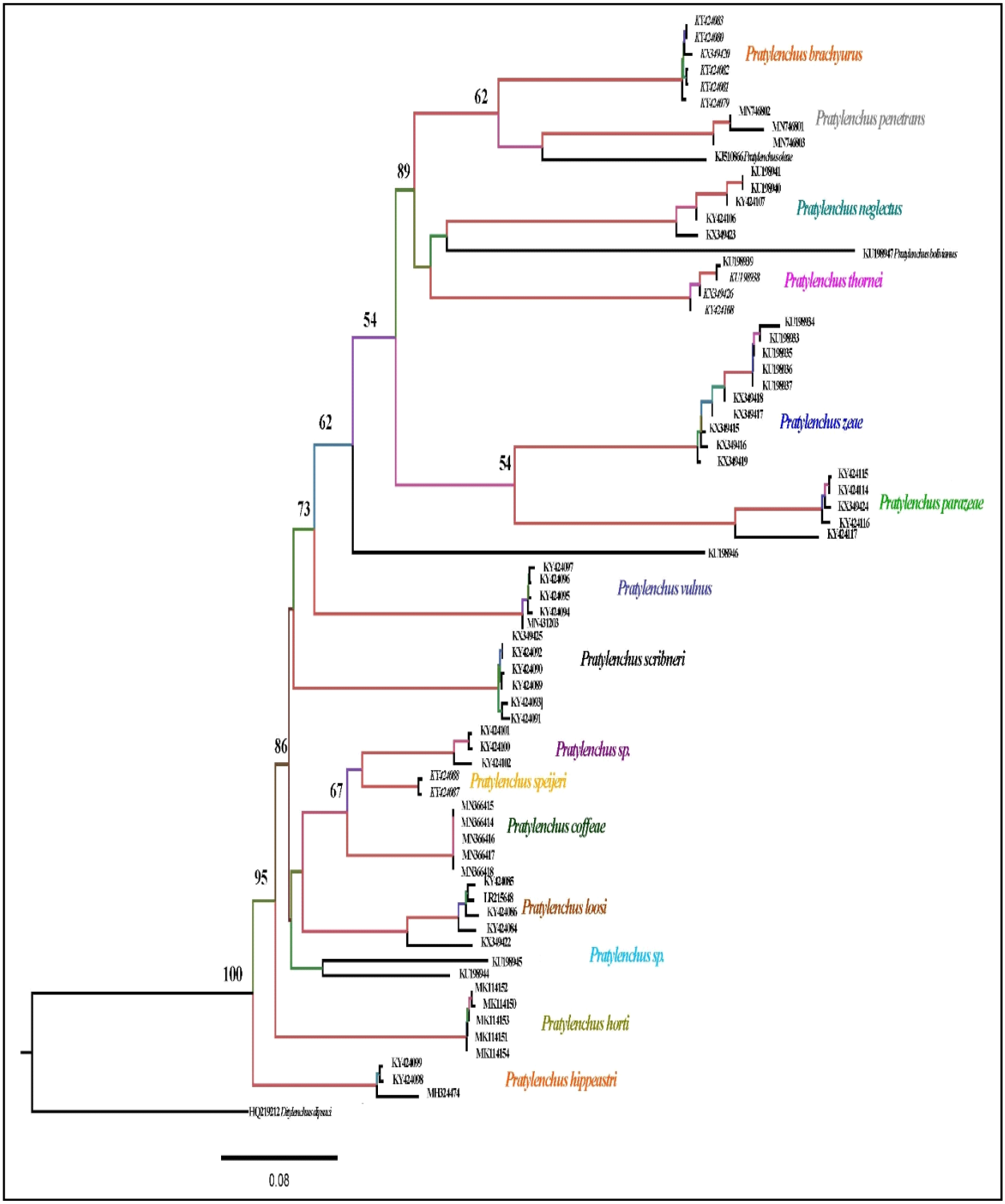

Fig. 1. Maximum likelihood tree of *Pratylenchus* based on the sequence of the COI mtDNA region.

revealing the existence of cryptic species complexes (De Luca *et al.*, 2012). Collecting topotype material has often proved to be the only way to confidently connect DNA sequences to formerly described morphospecies (Troccoli *et al.*, 2016). However, despite these efforts, the vast majority of morphospecies remain unlinked to DNA sequences (Geraert, 2013). Therefore, identifying the genus *Pratylenchus* is important. The study aimed to delimit

species of the *Pratylenchus* genus based on COI markers for some of its species. It is important to identify the species correctly because this affects the trade of plants and plant products, adding even more importance to clarifying the taxonomic status of this species (Handoo *et al.*, 2001).

MATERIALS AND METHODS

The present investigation was conducted in 2024 at the University of Limpopo. A total of 73 sequences were utilized from GenBank, representing 13 species of *Pratylenchus* sp., *(P. zeae, P. parazeae, P. vulnus, P. scribneri, P. brachyurus, P. speijeri, P. coffeae, P. loosi, P. horti, P. hippeastri, P. penetrans, P. neglectus,* and *P. thornei*).

Phylogenetics Analysis

Sequences were analyzed in BioEdit (Hall, 1999), confirming the variable sites' presence by manually inspecting the chromatograms. Sequence alignment was performed by MEGA X (Kumar *et al.*, 2018). The phylogenetic tree was elaborated using the Neighbour-joining (NJ) with 1000 bootstrap values implemented in MEGA X (Kumar *et al.*, 2018). The NJ tree files in Newick format were visualized using FigureTree (v 1.4.4) (Rambaut, 2017).

Species Delimitation Analysis

Species boundaries within each species were explored using Assemble Species by Automatic Partitioning (ASAP) (Puillandre *et al.*, 2021) and the Automatic Barcode Gap Discovery (ABGD) species delimitation methods. The ASAP analyses were based solely on the mtDNA sequence dataset. The ASAP analysis was run on the webserver (https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html) using the default parameters. In this method, p distance parameter was preferred simple distance (p-distances). The ABGD analysis was used to cluster the sequences of each conspecific into putative species based on the barcoding gap using the K2P nucleotide substitution model. The analysis was run on the webserver (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html) with default settings.

RESULTS AND DISCUSSION

The COI alignment contained 73 sequences of the Penetrans group and was 639 base pairs long. Based on our phylogenetic analysis, the *Pratylenchus* genus consists of 14 distinct clades with highly supported (95%) bootstrap values (Fig. 1).

Molecular species delimitation using ASAP and ABGD revealed 23–22 Operational Taxonomic Units (OTU's) (Fig. 2), and differential operational taxonomic units were estimated within *P. hippeastri* according to different models and variable prior intraspecific divergence. This variable number of estimated OTU's is not surprising given that molecular species delimitation analyses are known to generate a variety of different species hypotheses (Kekkonen and Hebert, 2014). In this study, we opted to follow the most stringent hypothesis, where only the OTU's that are supported by all ABGD and ASAP species delineation methods were retained. The minimal number of OTU's, as estimated by the ABGD analysis yielded 22 OTU's. Even following the most conservative estimate, *P. loosi, P. neglectus,* and *P. parazeae* are all delimitated as separate taxonomic entities, confirming signatures of independent evolution as predicted by population genetic theory (Fujisawa and Barraclough, 2013). Consequently, our species delimitation analyses confirmed the species hypotheses made by previous taxonomic studies (Palomares-Rius *et al.*, 2010). Our analyses indicate COI to be a good barcode gene, as all OTU's in the COI phylogeny (Figs. 1 and 2).

CONCLUSION

In conclusion, the mtDNA partial sequences are applicable for species delimitation analyses and phylogenetic relationships at low-level taxa (genus and species). Therefore, mitochondrial DNA consists of stop-coding regions with a slow evolutionary rate that can separate all *Pratylenchus* species. Although COI is a good marker for the identity of the *Pratylenchus* genus, more investigation with different markers is recommended.

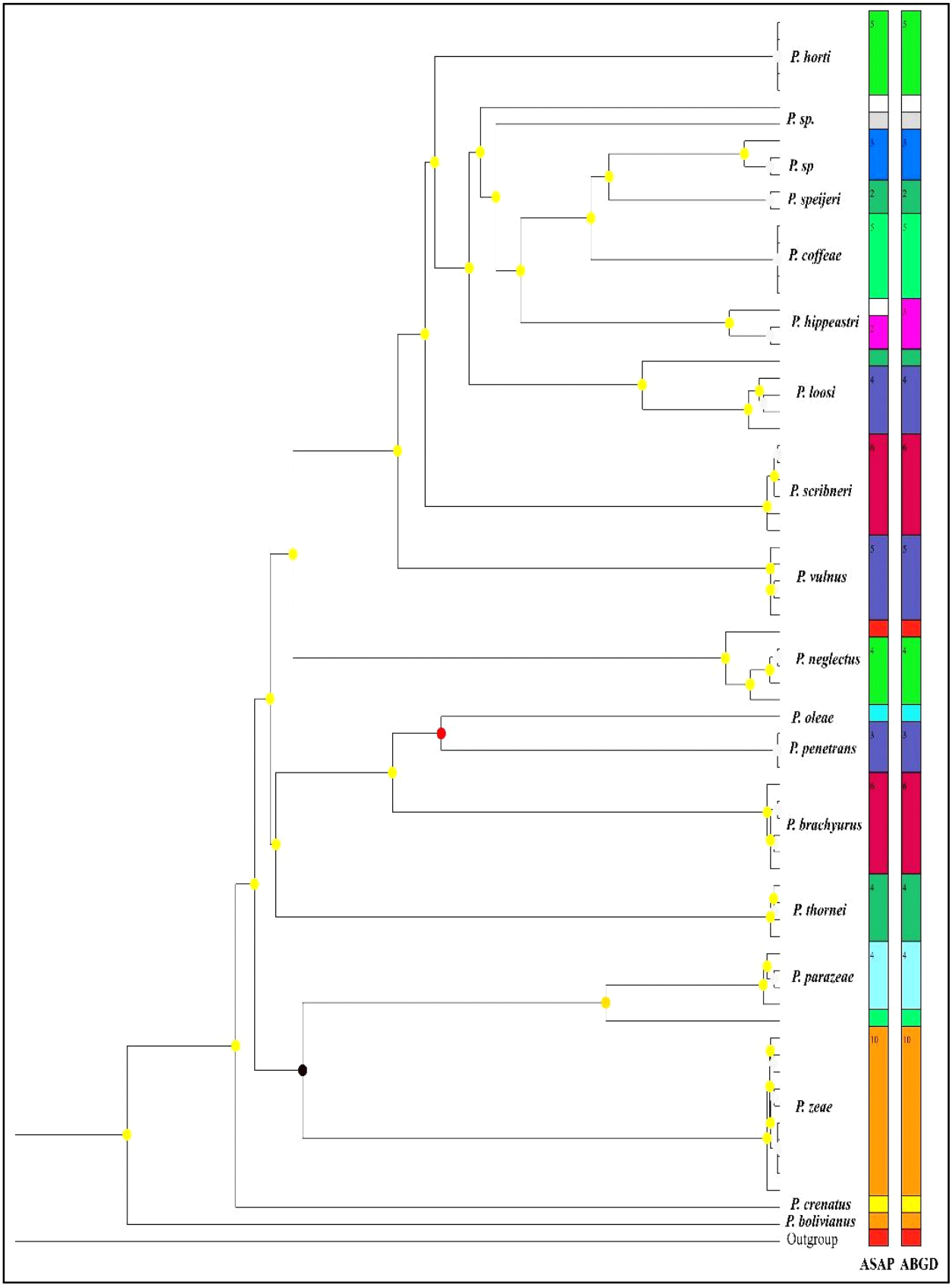

Fig. 2. Comparative analyses of species delimitation based on COI region.

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