ISBN: 978-85-66836-20-2

INTEGRATIVE TAXONOMY OF THE NEMATODE GENERA *Pratylenchus* AND *Meloidogyne*

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Only a small fraction of the estimated existing nematode species has been described. Some nematologists still have confidence in methods that date back to the 17th century, while others want to abandon species as fundamental units of diversity. Comprehensively described species are still benchmarks to characterise nematode diversity, but we also provide experimental evidence showing the serious shortcomings of traditional morphological methods. Nowadays, nematode taxonomy is confronted with the challenge to fully incorporate new theory, methods and data from disciplines that study the origin, limits and evolution of species. These recent methodological advances hold promise for species delimitation methods that reflect “true” speciation events. However, these advanced methods have their limitations in daily taxonomic practice, and therefore it is evident that forwarding one best possible taxonomical method is currently impossible. Our priority of coping with the enormous nematode diversity lies in integrative work, including morphological observations linked to informative molecular barcodes, comprehensive databases, and a phylogenetic framework along with other relevant features. Examples of this integrative work are illustrated here using three case studies: (1) species delimiting of the genus *Pratylenchus*, (2) a molecular barcode identification method for tropical *Meloidogyne* spp., and 3) an integrative taxonomy approach for *Meloidogyne africana.*

The first case study focuses on root-lesion nematodes of the genus *Pratylenchus*, an important pest parasitizing a wide range of vascular plants including several economically important crops. However, despite their tremendous importance, morphological diagnosis of the 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 barcode-based diagnostics, a link between morphology and species specific sequences has to be established. We reconstructed a multi-gene phylogeny of the *Penetrans* group using nuclear ribosomal and mitochondrial gene sequences. A combination of this phylogenetic framework with molecular species delineation analysis, population genetics, morphometric information and sequences from type location material allowed us to establish the species boundaries

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within the *Penetrans* group and as such clarify long-standing controversies about the taxonomic status of *P. penetrans*, *P. fallax* and *P. convallariae*. Our study also reveals a remarkable amount of cryptic biodiversity within the genus *Pratylenchus* confirming that identification on morphology alone can be inconclusive in this taxonomically confusing genus. Furthermore, taxonomic expertise is decreasing and sequence-based identification is growing rapidly and we demonstrate that this incorrect labelling resulted in a cascade of erroneous interpretations, as shown by the reports of '*P. goodeyi*' on banana in China and on cotton in India. This clearly illustrates the risk of mislabeled sequences in public databases and a strong link between morphology and DNA sequences will be of crucial importance in order to prevent, or at least minimize, sequence-based misidentifications.

The second case study focuses on the polyphagous parthenogenetic tropical root-knot nematodes of the genus *Meloidogyne* that are considered to be the most significant nematode pest in agriculture. Despite the crucial need for correct diagnosis, identification of these pathogens remains problematic. The traditionally used diagnostic strategies, including morphometrics, host-range tests, biochemical and molecular techniques, now appear to be unreliable due to the recently-suggested hybrid origin of root-knot nematodes. In order to determine a suitable barcode region for these pathogens nine quickly-evolving mitochondrial coding genes were screened. Resulting haplotype networks revealed closely related lineages indicating a recent speciation, an anthropogenic-aided distribution through agricultural practices, and evidence for reticulate evolution within *M. arenaria.* Nonetheless, nucleotide polymorphisms harbor enough variation to distinguish these closely-related lineages. Furthermore, completeness of lineage sorting was verified by screening 80 populations from widespread geographical origins and variable hosts. Importantly, our results indicate that mitochondrial haplotypes are strongly linked and consistent with traditional esterase isozyme patterns, suggesting that different parthenogenetic lineages can be reliably identified using mitochondrial haplotypes. Especially the barcode region Nad5 can reliably identify the major lineages of tropical root-knot nematodes.

Finally, the importance of an integrative approach is illustrated based on a case study for *Meloidogyne africana.* Looking to this species from multiple sides resulted in a cascade of results, from a taxonomic revision up to new insights into the cytogenetic evolution of root-knot nematodes. During sampling of several *Coffea arabica* plantations in Tanzania severe root galling, caused by a root-knot nematode was observed. From pure cultures, morphology and morphometrics of juveniles and females matched perfectly with *Meloidogyne africana*, whereas morphology of the males matched identically with those of *Meloidogyne decalineata*. Based on their *COI* sequence, however, the recovered juveniles, females and males were confirmed to belong to the same species, creating a taxonomic conundrum. Adding further to this puzzle, re-examination of *M. oteifae* type material showed insufficient morphological

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evidence to maintain its status as a separate species. Consequently, *M*. *decalineata* and *M*. *oteifae* are synonymized with *M*. *africana*, which is herewith redescribed based on results of light and scanning electron microscopy, ribosomal and mitochondrial DNA sequences, isozyme electrophoresis, along with bionomic and cytogenetic features. Multi-gene phylogenetic analysis placed *M*. *africana* in a “basal” position together with *M*. *coffeicola*, *M*. *ichinohei* and *M*. *camelliae*. This phylogenetic position was confirmed by several morphological features, including cellular structure of the spermatheca, egg mass position, perineal pattern and head shape. Moreover, *M*. *africana* was found to be a polyphagous species, demonstrating that basal *Meloidogyne* spp. are not as oligophagous as had previously been assumed. Cytogenetic information indicates *M*. *africana* (2n = 21) and *M*. *ardenensis* (2n = 51±54) to be a triploid mitotic parthenogenetic species, revealing at least four independent origins of mitotic parthenogenesis within the genus *Meloidogyne*. Furthermore, *M*. *mali* (n = 12) was found to reproduce by amphimixis, indicating that amphimictic species with a limited number of chromosomes are widespread in the genus, potentially reflecting the ancestral state of the genus. The wide variation in chromosome numbers and associated changes in reproduction modes indicate that cytogenetic evolution played a crucial role in the speciation of root knot nematodes and plant-parasitic nematodes in general.