

INTEGRATIVE TAXONOMY OF HOPLOLAIMIDAE, HETERODERIDAE AND CRICONEMATIDAE

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The tasks of *systematics* are (i) to name, identify and catalogue of organisms (*taxonomy*), (ii) to discover the ancestral relationships among organisms (*phylogeny*) and (iii) to organize information about organism's diversity into a hierarchical system (*classification*). *Integrative taxonomy*, called also as *polyphasic taxonomy*, is defined as the science that aims to delimit the units of life's diversity from multiple and complementary perspectives, including phenotypic, genotypic and phylogenetic, ecology, development, behavior and others. The integrative taxonomy approach leading to a conclusion based on consensus of all available data and information has been successfully applied for resolving of systematics issues in bacteriology, botany, zoology and nematology. This approach follows no strict rules or guidelines, may integrate any significant information on the organisms, and results in a consensus, satisfying most but not necessarily all users of taxonomic results. Integrative analysis leads to a transition type of taxonomy in which a compromise can be formulated on the basis of result presently having at hands. Integrative taxonomy is an attempt to synthesize the real data and a step toward a synthetic taxonomy, which will be made possible through the development of new informative strategies. The first step for developing of an integrated taxonomy of the group is to identify how and where the different approaches are in conflict.

Molecular tools represent powerful instruments for taxonomy, however, a researcher can come to incorrect conclusion without understanding of species concepts, evolution process and principles and problems of sequencing and phylogenetic analysis and knowledge of structure of molecular markers. There are many problems in working with molecular datasets. Two of them are referred to correctness of reference materials and sequences (incorrect identification and sequence reading mistakes) and reliability of taxon or sequence positions in inferred phylogenetic trees (incorrect applied model and analysis, anomalous evolution of gene marker). Some examples of the resolutions of these problems are provided.

Hemicriconemoides strictathecatus is one of the oldest among 50 of sheathoid nematodes. In the beginning of our project several sheathoid populations collected in South Africa, China, Taiwan and Venezuela were characterized morphologically and molecularly and proposed to be considered *H. strictathecatus*, although no DNA sequences of topotype specimens of *H. strictathecatus* were available at the time to validate this morphological identification. However, further analysis of topotype species from Florida revealed that that the original description of *H. strictathecatus* was based only on the features of the morphotype with a rounded tail terminus. The morphotype with a pointed tail terminus was missed at the time of the original description because of the limited number of specimens collected and examined. This study allowed to re-consider previously published identification and underlined the necessity of molecular characterization of the type materials including topotype specimens.

Among the spiral nematodes, *H. pseudorobustus* is one of the most commonly reported species distributed in both temperate and tropical regions. Identification of *H. pseudorobustus* and related species is a difficult, if not impossible, process, because most characters used for their diagnosis appear to vary within species. In our study we provided morphological and molecular characterisations of several spiral nematodes, *H. broadbalkiensis*, *H. digonicus*, *H. dihystra*, *H. microlobus*, *H. paxilli* and *H. pseudorobustus*, collected in different geographical areas of USA, Switzerland, Italy, New Zealand, Spain, UK, South Korea and Russia. We used a tree-based method and sequence analysis for species delimiting of *Helicotylenchus* and

suggested that *H. microlobus* and *H. pseudorobustus* are valid species separated from each other morphologically and molecularly. The delimiting of these species made in our study does not contradict with phenetic analysis published by some authors.

Understanding of evolution of molecules and genes, especially, ribosomal RNA gene and mitochondrial *COI* gene is principal for correct application of these markers in phylogenetic reconstruction. Knowledge of rRNA structure is increasingly important to assist phylogenetic analysis through reconstructing optimal alignment, utilizing molecule features as an additional source of data and selection of appropriate models of evolution of the molecule. In several studies using the doublet model with sixteen states of nucleotide doublets for the stem region and the standard model of DNA substitution with four nucleotide states for loops and bulges, we demonstrated that using structural information for phylogenetic analyses led to trees with lower resolved relationships between clades and likely eliminated some artefactual support for misinterpreted relationships, such as paraphyly of *Helicotylenchus*, *Rotylenchus*, *Mesocriconema* and *Hemicriconemoides* as consequence, generated a more realistic picture of the relationships.

The reniform nematodes of the genus *Rotylenchulus* with 11 valid species are semi-endoparasites of numerous herbaceous and woody plant roots and are mainly distributed in tropical and subtropical regions. *Rotylenchulus reniformis* is the most important species in the genus and is considered as a major pest of cotton and other in several other countries. In the results of analysis, we revealed two rRNA gene types in four species *Rotylenchulus* species. *R. reniformis*, *R. macrosoma*, *R. macrosomoides*, *R. macrodoratus*. The rRNA gene sequence trees showed paraphylies of these species, whereas they were monophyletic in the *hsp90* and *COI* trees. The presence of two distinct classes of rRNA genes in a single genome has been reported in organisms belonging to all three domains of life and this phenomenon should be seriously considered in interpretation of phylogenetic results.

Species delimiting within a group is more successful if we know what caused their origin and determined their evolutionary history. Our studies with the *Avenae* group of the genus *Heterodera* could be a good example of an integrated taxonomy. This group contains a total of 12 species. Ten of them, *H. arenaria*, *H. aucklandica*, *H. australis*, *H. avenae*, *H. filipjevi*, *H. mani*, *H. pratensis*, *H. riparia*, *H. sturhani* and *H. ustinovii*, are morphologically closely related and represent the *H. avenae* species complex, and the other two, *H. hordecalis* and *H. latipons*, are morphologically more distinct from this complex. In series of studies we provided analyses of morphological and morphometrical characters, isoelectrophocusing of proteins, PCR-RAPD, PCR-ITS-RFLP. We also made phylogenetic and phylogeographic analyses using Bayesian inference, maximum likelihood and statistical parsimony of several hundred sequences of the ITS rRNA and *COI* genes from more than 150 populations collected in 26 countries sequence and. *COI* gene sequences allowed to confirm species status and distinguished *H. arenaria*, *H. australis* and *H. sturhani* from each other and other species. A putatively new cyst nematode species related to *H. latipons* was revealed from the analysis of *COI* and ITS rRNA gene datasets. Based on the results of phylogeographical analysis and age estimation of clades with a molecular clock approach, it was hypothesised that several species of the *Avenae* group primarily originated and diversified in the Irano-Anatolian hotspot during the Pleistocene and Holocene periods and then dispersed from this region across the world. Different geographic barriers, centres and times of origin might explain current known distribution patterns for species of the *Avenae* group. Possible pathways, including a long distance trans-Atlantic dispersal, and secondary centres of diversification were proposed.

Despite of the fact that morphological studies will retain an outstanding role in nematode taxonomy, the use of sequence information of from single to multi genes and whole genomes, from trees to coalescent patterns of gene genealogies will enhance our understanding of nematode biodiversity. Collaborations between taxonomists, evolutionary biologists and bioinformaticists become critical for application of principles of integrative taxonomy in Nematology.