Utility of Classical α-Taxonomy for Biodiversity of Aquatic Nematodes

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Abstract: "Classical α-taxonomy" has different interpretations. Therefore, within the framework of an integrated taxonomic approach it is not relevant to divide taxonomy in different components, each being allocated a different weight of importance. Preferably, taxonomy should be seen in a holistic way, including the act of delimiting and describing taxa, based on different features and available methods, and taxonomy can not be interpreted without looking at evolutionary relationships. The concept of diversity itself is quite diverse as is the measure of diversity. Taxonomic descriptions of free-living aquatic nematodes are very valuable as they provide basic phenotypic information that is necessary for the functional ecological, behavioral, and evolutionary interpretation of data gathered from molecular analyses and of the organism as a whole. In general, molecular taxonomic analyses have the advantage of being much faster and of being able to deal with a larger number of specimens but also possess the important advantage of dealing with a huge amount of features compared to the morphology-based approach. However, just as morphological studies, molecular analyses deal only with partial of an organism.

Key words: morphology, molecular analyses, taxonomic descriptions.

The authors, having expertise in taxonomy based on morphology as well as on molecular data, were invited to discuss the future role, if any, of α-taxonomy for biodiversity assessment of aquatic nematodes in the current era of molecular/genetic data-based research and metadata.

At the beginning of the 21st century, many discussions on the taxonomic impediment and the advantage of molecular identification techniques emphasized the shortcomings and pitfalls of α-taxonomy, questioning its utility and future (Tautz et al., 2002). Remedies have been proposed and new technologies have been developed to counter the negative perspective of taxonomy (Will et al., 2005) and create fast-track taxonomy by combining digital imaging, molecular techniques, and highly accurate diagnoses in online wiki databases (Riedel et al., 2013). To have a sound discussion, one must be clear on the terminology used. Therefore, we will first look at the different interpretations of the terminology used through time, more particular how do we define taxonomy and systematics? Why differentiate taxonomy into subdisciplines/sublevels as, e.g., α-taxonomy, descriptive taxonomy, molecular taxonomy, and integrated taxonomy? Despite the nature, definition and delimitation of species remain controversial issues, one can nevertheless provide guidelines for a good nematode species level taxa descriptions,

informative illustrations, and nomenclatural rules to follow. The second part of this paper then deals with the current/future role of α-taxonomy in nematode biodiversity assessments in which species distributions, community structures, and ecosystem functioning are analysed. Nematodes are an ubiquitous and very abundant group of metazoans, which play an important role in the nutrient mineralization process and cycling. Hence, to understand aquatic ecosystem functioning, one must first address nematode diversity (Ristau et al., 2013). However, the concept of diversity itself is quite diverse, as is the measure of diversity (Hodda et al., 2009). We will discuss the role of α-taxonomy in nematode biodiversity assessments in which species distributions, community structures, and ecosystem functioning are analysed.

α-TAXONOMY

The study of aquatic nematode biodiversity starts with an inventory of the nematodes in the environment. The first approach is a phenotypic one. Although each specimen is unique, in reality specimens are sorted and grouped into taxonomic units and ordered (classified) on the basis of phenotypic, usually morphological characteristics, similarity, and contiguity, a methodology indicated as α-taxonomy by Simpson (1960). Names of the taxa thus defined are given by applying the Linnean nomenclature.

The term α-taxonomy was first coined by Turrill (1935) who distinguished α-taxonomy or "traditional taxonomy" based on morphology and Ω-taxonomy or "perfected taxonomy" which is more inclusive and builds upon a broader base of information from morphology, physiology, ecology, genetics, and including relationships. Later on, different interpretations of

taxonomy/α-taxonomy were given, thereby dividing taxonomy into two or three main components (Fig. 1). Mayr (1969) recognized three levels of taxonomy: α-taxonomy involves the characterization and naming of species, β-taxonomy deals with the arrangement of species into a natural system, and γ-taxonomy refers to studies of various biological and evolutionary aspects. More recently, taxonomy is seen as consisting of basic taxonomy *s.s.*, also referred to as descriptive taxonomy, complemented by taxonomy *s.l.* or phylogenetic taxonomy that deals with the study of diversity, classification, and relationships (Godfray, 2002). The latter part was named biosystematics by Tillier et al. (2000), which together with taxonomy (read α-taxonomy) constitutes the discipline systematics. Dayrat (2005) introduced the term integrative taxonomy for a holistic approach based on multiple and complementary perspectives; he also provided seven guidelines for naming a species, e.g., taking into account the necessary number of specimens to obtain information on possible intraspecific and interspecific variations of characters; the need for support based on broad evidence provided by morphology, ecology, etc; or the preservation of type specimens in museum collections and their availability for molecular studies. In general, α-taxonomy stands for the process of species discovery, description, and naming (Luc et al., 2010). Martens and Segers (2005) defined α-taxonomy as the basic and classical description of new taxa and considered it a basic methodology in which the description of taxa is comparable with obtaining DNA sequences, an opinion we do not share. α-Taxonomic data, similar to DNA sequences, provide the tools for future analyses of, for example, phylogenetic relationships or the development of integrated databases which can be used to analyse distribution patterns or in monitoring aquatic ecosystem health. In their recommendations, they confirm that α-taxonomy *sensu* basic and classical description remains a vital part of inventory biodiversity but unlike sequence data stored in a web-based database, α-taxonomy should result in publications and be seen as a direct means to be able to conduct further analyses such as phylogenetic reconstructions. Yeates et al. (2011) referred integrative taxonomy to as iterative taxonomy because it lacked statistical evidence. Currently, integrative taxonomy is widely used for a combined morphology- and molecular data-based approach when delimitating and describing taxa. The term "integrative" highlights what any science, including taxonomy, should be, i.e. not just descriptive but hypothesis driven and based on a holistic approach to test the hypothesis of for example, a new taxon. Taxonomy should combine different types of features, at different levels of biological organization (from morphological features to genes) as well as search for new advanced characters. Defining a new species for example, goes beyond the act of describing, but also involves the interpretation of the features used, look for homology of
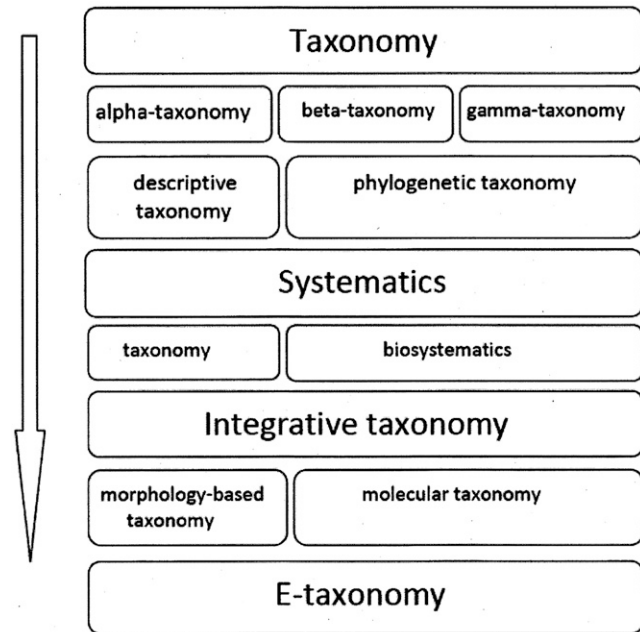

FIG. 1.   Chart on evolution in interpretation of taxonomy.

diagnostic characters, analyse relationships and establish a classification. E-taxonomy or web-based taxonomy refers to the advantages of the Internet as a medium to connect taxonomists and facilitate access to larger amount of different type of data.

Morphology is the basis for understanding animal structures and "basic" or "classical" description is the term for a morphology-based description. Considering α-taxonomy *sensu* "basic" species description as a basic methodology is somewhat misleading since especially with small animals, such as free-living aquatic nematodes, light microscopy (LM) observations have to be interpreted and made visible via drawings or pictures, and depending on the observer's precision, are more or less subjective. Anyway, species descriptions are not similar to obtaining DNA sequences since DNA sequences are objective strings of nucleotides, independent from how or by whom they were determined. Species descriptions, in contrast, depend on the person who produces them and on the methodology by which morphological features were assessed.

The Species as a Taxonomic Unit

The majority of definitions of α-taxonomy include as last step the naming of species. Naming a species implies the knowledge on how to determine species boundaries. Species descriptions rarely indicate explicitly how species boundaries were determined (i.e., under which species concept the new species is to be interpreted), although it was one of the recommendations for taxonomic submissions to the journal *Hydrobiologia* (Dodson and Lee, 2006). The morphological

species concept is the most widely used and morphological species are defined on the basis of particular diagnostic characteristics, whereby morphological distinctiveness is considered as an indicator of "lineages independence" (Decraemer et al., 2008). Yet, there are many more, different species concepts without reaching a generally accepted consensus (Coomans, 2002; Hey, 2006). More recently, de Queiroz (2007) proposed a unified species concept that separates (i) the generally accepted theoretical concept of the species as separately evolving metapopulation of lineages or segments of such lineages as a necessary property of species from (ii) the different operational criteria (properties) relevant for species delimitation such as intrinsic reproductive isolation, ecologically divergent, monophyletic, or phenetic distinction. Under the unified species concept, any "property" (read operation) that provides evidence of lineage separation is relevant to inferring species boundaries (de Quieroz, 2007).

Typification

α-Taxonomy deals with species descriptions; they form the main basic part of a taxonomic paper. However, taxonomic papers are governed by the nomenclatural rules imposed by the International Code of Zoological Nomenclature (ICZN) (http://iczn.org/) and the Principle of priority (Minelli, 2005). A single specimen, the holotype, is the name bearer or type of a species that is fixed in the original publication by the original author(s) (ICZN: art. 73) and serves as the universal reference for that name. Due to the Principle of priority for a taxon name, all papers, including old and poor papers, remain important for nomenclatural purposes and must be checked when introducing a name for a new taxon. The ICZN does not provide guidelines for species descriptions, but defines the rules according to which the task can be undertaken and gives recommendations on the relevant data on the holotype to be included in species descriptions, e.g., morphometric data, developmental stage, parasite hosts, and information on locality, sampling depth, collection, and number and name of collector. Type material should be deposited preferably in museums. The availability of well-preserved and accessible type material is important for accurate differentiation of a new taxon through comparison. Unfortunately, there is a general tendency in papers on free-living aquatic nematodes and nematodes in general, to abandon comparison with type material or voucher specimens. This is largely because of the numerous problems encountered, such as bad conditions of slides (drying out and flattening of specimens) especially microscopic slides with glycerine mounts sealed by glycol (Abebe et al., 2014); also more recent paraffin slides may be subject to invisible cracking and drying out. Other problems are the refused access to or lack of information on type material; many slides of type material were not deposited and lost (e.g., the marine species described by Wieser, 1953a). To limit risks, the loss or deterioration of type material, most museums do no longer send type material upon demand. Abebe et al. (2014) argued for e-typing of nematodes as a solution to improve access and comparison with type specimens. With the cooperation of museum curators, e-types such as high-quality digital multi-focal video images (De Ley & Bert, 2002) could supplement type material and online open access of the images would ameliorate and speed up accurate species identification.

Species Descriptions of Free-Living Aquatic Nematodes

Free-living aquatic nematodes are usually small, about 1 mm, although longer species such as leptonematids, exist. They have to be fixed and processed for mounting on microscopic slides and observation at high magnification (×1000). Consequently, a good fixation is very important for adequate detailed observations (Coomans, 2000). Correct use of the microscope, including optimal regulation of the light source, is essential for observation of small structures and complex systems in fixed specimens. Making drawings with the use of a camera lucida is needed for a better understanding of nematode morphology/functioning than observation of a micro-photograph or video capture of a fixed specimen; living specimens, however, provide the most accurate information (Fig. 2).

The holotype fixes the name and the species diagnosis provides the "definition" of the named species, commonly based on morphology alone. A description or definition is one of the requirements for a new name published after 1930 (ICZN art. 13.1.1.). A description is not complete without a species diagnosis. It describes the most important diagnostic features and is complemented with information on differentiation from similar species and information on how the species boundaries were determined. The description also provides information on type locality and habitat, on other localities when available and includes information on sampling and abiotic and biotic features if not presented under "Material and Methods" in the publication. An explanation on the etymology of the species name is also included.

A good species description should be concise, presented in a telegraphic style and does not include the general characteristics at genus level. It deals first with the gender of the holotype which for most free-living aquatic species is the male and describes the holotype as well as possible variation observed in the paratype specimens. The description starts with the general appearance (often indicated as "habitus"), the external structures such as body cuticle and ornamentations,
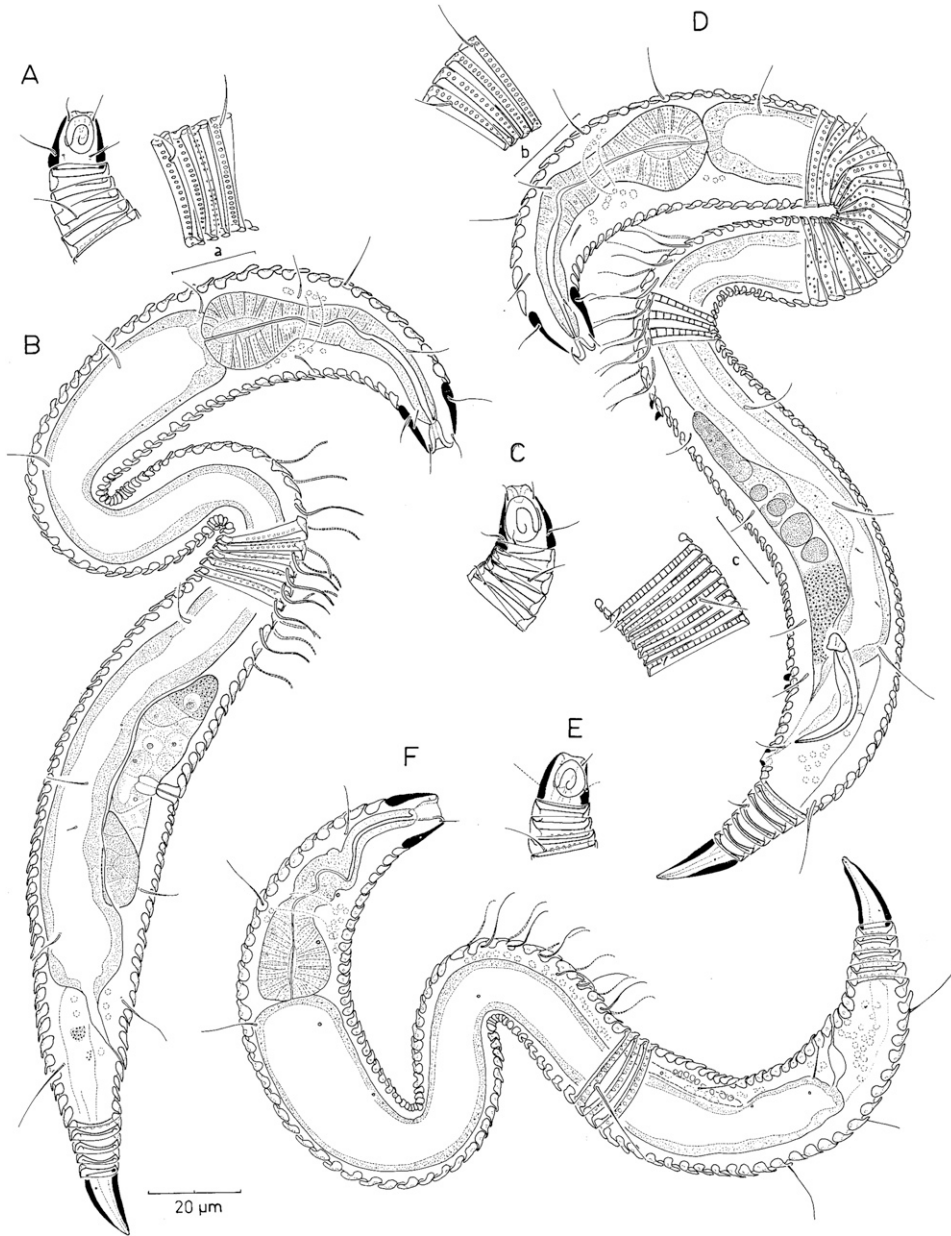
FIG. 2. Example of an informative illustration of a species description. *Epsilonema oodamphids*, (A) Surface view of head region (paratype ♀). (B) Whole mount of paratype ♀ with indication of the level at which the body cuticle is shown in surface view. (C) Surface view of head region (holotype ♂). (D) Whole mount of holotype ♂ with indications b, c of the levels at which the body cuticle is represented in surface view. (E) Surface view of the head region of a third-stage juvenile (paratype). (F) Whole mount of a third-stage juvenile with parts of body cuticle in surface view (paratype). From Decraemer and Gourbault (1987), courtesy Bulletin van het Koninklijk Belgisch Instituut voor Natuurwetenschappen.

arrangement of the somatic setae, head shape, and arrangement of anterior sensilla; the order is from anterior to posterior, from LM to scanning electron microscopy (SEM) observations. It continues with the internal structures and groups information per system, starting with the digestive system, followed by structures/systems in the pseudocoel such as the nerve ring, presence of pseudocoelomocytes, the secretory–excretory system, the reproductive system and copulatory apparatus, and secondary sexual features in male, and ends with the tail region. This is followed by the description of the other gender and the different juvenile stages (when available) in similar order.

Illustrations should, if possible, include different approaches and preferably include line drawings as well as microphotographs. Line drawings should provide information on all diagnostic features of the holotype and the opposite sex as well as on juvenile stages when available. For aquatic nematodes, this includes the general appearance, details of body cuticle and ornamentation, the head region in surface view, the stomodeum (details of stoma including feeding apparatus

if present, pharynx, and cardia), position of nerve ring and secretory–excretory system when present, the reproductive systems with details of the copulatory apparatus in male or the vaginal region in female.

The basic illustrations can be completed with SEM microphotographs. All figures must include a scale. Video captures (De Ley and Bert, 2000) are not included in the publication but are preferably available upon demand or by access to an open database (Abebe et al., 2014).

The description must include information on the type specimens (holotype, paratypes), with indication of slide numbers of the nematode collections where deposited and name and address of the institute hosting the nematode collection. For free-living aquatic nematodes, an allotype is often indicated as the specimen of opposite sex to the holotype, but the term allotype is not recognized by the ICZN. A relative larger number of specimens, e.g., 10 to 20 per sex and per developmental stage will provide insight into intraspecific variation of specific characteristics and allow to choose the holotype as the most informative specimen in optimum condition. Whenever, possible morphometric data should be treated statistically enabling a more objective approach for species discrimination based on multivariate analysis such as canonical discriminant analysis. Morphometric data of juveniles should not be compiled and information on J1, J2, J3, and J4 must be presented separately.

Shortcomings and Pitfalls of α-Taxonomy

Time-consuming species descriptions: One of the main stumbling blocks in taxonomy, is the many inadequate species descriptions and poor illustrations, based on a single or a few specimens, often representing only one of the sexes in amphimictic species (as is the case for the majority of free-living aquatic nematode species), and in the worst case only juveniles. Old species descriptions of aquatic nematodes, but also some recent ones, are often superficial and lack information on diagnostic features or intraspecific variability/gender dimorphism, e.g., due to the small number of specimens observed. The interpretations of structures are not correct, not presented in a logical order and infringe correct terminology. For example, the symmetries of the cephalic setae and position of the buccal armature are often misinterpreted. Cephalic setae are derived from somatic setae and follow the bilateral symmetry (Coomans, 1979), whereas outer and inner labial sensilla follow the radial (hexaradial) symmetry. However, the outer labial sensilla are often described as anterior cephalic sensilla (English school see Platt and Warwick, 1983). Stomatal teeth are mainly positioned in the stegostom (De Ley et al., 1995), the anteriormost part of the pharynx with a triradial symmetry. Therefore, the teeth in the ventrosublateral sectors of the triradial symmetry should be indicated as ventrosublateral teeth and not as subventral teeth (Coomans, 1979). Another common error is the indication of a genital branch as gonad, while the gonad refers only to the ovary in female or testis in male (Southey, 1973).

The poor or incomplete descriptions and illustrations, as well as the lack of access to type specimens hamper the matching of molecular sequences with existing species descriptions (De Ley et al., 2005). The traditional morphology-based approach is artisanal, time consuming, and diagnostic morphological features are few in number at first sight, and they are very limited in number compared to the large amount of features (each nucleotide is a feature) in molecular analyses. Consequently, correct identification is hampered and poor interpretations will contribute to nomenclatural instability (De Ley et al., 2005). Nematode descriptions and identifications have to be improved using additional approaches including ultrastructure information and polytomous illustrated identification keys. A very useful attempt was made by NEMYS (Deprez et al., 2004). A response to these problems in α-taxonomy could be found in the creation of an E-taxonomy or web-based taxonomy that is not only based on morphological α-taxonomy as interpreted in general but should also include molecular information of species (Mayo et al., 2008, Abebe et al., 2011, 2014). However, as De Ley (2000) mentioned *we can no longer afford to describe species purely for the sake of describing new species* (sic) or extending the diversity catalogue. We have to focus on species in terms of ecological, economic, or medical relevance and use them as models in other types of biological research or as added taxonomic value in those groups with few species, e.g., possessing so far unknown structures (De Ley, 2000).

Cryptic species (hidden species under the same name): Nematode species recognition and description is not always easy. Nematode morphology is highly conserved. Morphometric data often overlap so that species differentiation is not straightforward. Looking only at morphological features of an animal is looking at a partial picture of the animal's phenotype. Many features involved in species recognition and behaviour (e.g., attraction, mating), involve chemicals (e.g., hormonal cues detected by the nematode's well-developed sensory system), remain unnoticed by light microscopy. However, good morphological observation, especially when features are small and differences subtle, needs first of all a good knowledge of nematode morphology, and requires time investment and training. However, intraspecific morphological variation, especially morphometric variation, cannot always be distinguished from interspecific variation. Statistical analysis can help in differentiating taxa, but sometimes, morphology needs a hint from molecular analysis (Palomares-Rius et al., 2014).

BIODIVERSITY ASSESSMENT OF FREE-LIVING AQUATIC
NEMATODES: IS THERE A ROLE FOR SPECIES
DELIMITATION AND DESCRIPTION (α-TAXONOMY)?

According to article 2 of the Convention on Biological Diversity, Rio de Janeiro, 1992, (http://www.cbd.int/convention) biodiversity concerns the diversity within species, between species and of ecosystems, and can be of different levels: genetic diversity, species diversity, and ecological diversity. Nematodes are the dominant group in the meiofauna of most lakes, rivers, and marine environments so knowledge of their biodiversity is important to understand aquatic ecosystem functioning. Since nematodes show a high diversity and abundance, they are interstitial dwellers and burrowers that are present at all levels of the food chain, have a low mobility, a wide range of tolerance, different life strategies showing short to long generation times, and react fast to disturbance (Brinke et al., 2011), and they can provide essential information for sustainable management of these ecosystems and the protection of freshwater resources (Barbuto and Zullini, 2005; Wu et al., 2010). Nematodes are very useful bio-indicators and possess several traits advantageous for ecotoxicological studies (Brinke et al., 2011).

However, there is neither general agreement on how diversity should be measured, nor on the geographic scale and time span over which it should be assessed (Hodda et al., 2009). In its simplest form nematode diversity is measured by the number of taxa, mostly family or genus level and to a lesser extent species or putative species level (Ferrero et al., 2008; Heininger et al., 2008; Moreno et al., 2011). Because of the taxonomical complexity of the group and time-consuming aspects of species identification, field studies mostly use nematode genera or families as unit to measure and describe patterns of diversity next to other units such as ecological and trophic categories (Wieser, 1953b; Bongers, 1990), and groupings by biomass (Losi et al., 2013b) or body size and shape (Schratzberger et al., 2007). King (2009) described the need for including phylogeny in biodiversity measurements. However, trophic categories derived from stoma morphology may be misleading since conflicts exist between stoma morphology and feeding habit and feeding selectivity; such flexibility may cause intraguild resource partitioning (Moens et al., 2004). More recently, information on global biodiversity is obtained by environmental DNA sequencing based on a metagenomic approach (Bik et al., 2012), whereby distribution patterns are based on operationally clustered taxonomic units mostly molecular, which in general have no formal correlation with published species descriptions (Floyd et al., 2002; Blaxter et al., 2005; Creer et al., 2010). In a metagenomic approach, collective gene information (e.g., two regions of about 400 bp regions of 18S rRNA or D2-D3 of 28S rRNA) of a habitat's mixed community of organisms is obtained (Creer et al., 2010; Bik et al., 2012).

α-Taxonomy-based biodiversity: α-Taxonomy in biodiversity studies on aquatic nematodes is biased toward marine nematodes. As such, there are about three times more marine nematode species described than freshwater species. Due to an increased interest for the conservation and protection of freshwater ecosystems and freshwater resources, freshwater nematode diversity received slightly more attention during the last two decades resulting in several taxonomic papers (e.g., Abebe and Coomans, 1995; Abebe and Coomans, 1996a, 1996b; Abebe, 2000; Eyualem, 2002; Gagarin, 2000; Tsalolikhin, 2001; Zullini et al., 2002; Gagarin et al., 2003; Esquivel and Arias, 2004), checklists (Heyns, 2002) and books on freshwater nematodes (Smith, 2001; Thorps & Covich, 2001; Abebe et al., 2006). Nearly 2000 species of freshwater nematodes have been described (Abebe et al., 2006) but studies show extreme regional bias with those from the southern hemisphere remaining underrepresented also in current studies. Several species show an apparent cosmopolitan distribution though their identifications need to be checked for possible species complexes. For the freshwater habitats, Dorylaimida is the most species-rich order with about one-third of known freshwater species (Abebe et al., 2008). The order Mermithida with juvenile stages J2 and J3 being parasites of aquatic insects are the second species-rich group with more than 400 species. The number of new taxa published per year remains extremely low. Abebe et al. (2014) found that on a total of 301 taxonomic nematode papers published in the period 2006 to 2009, only 15.6% dealt with free-living marine species and hardly 3.9% were on free-living freshwater species. A recent update for 2012 and 2013 based on the Web of Science, showed that only 5 and 6 new free-living freshwater nematode species have been described, respectively versus 25 and 26 for new free-living marine species.

Reverse taxonomy for cryptic species: In free-living marine nematodes, many cryptic species have been discovered recently during population genetic studies (Derycke et al., 2010) and were fully characterized in an integrative approach (Apolônio Silva de Oliveira et al., 2012). Instead of looking first at nematode morphology, a reverse molecular approach (reverse taxonomy in Markmann and Tautz, 2005) may be necessary to detect cryptic species, to interpret morphological variation and to validate a new species. The utility of α-taxonomy or morphology-based taxonomy in the process of clarifying the taxonomic status of molecular lineages in two free-living nematode species, viz. *Rhabditis* (*Pellioditis*) *marina* and *Halomonhystera disjuncta* is described in Fonseca et al. (2008). The authors first analysed the phylogenetic relationships among nucleotide sequences of the mitochondrial COI gene and two nuclear gene fragments (ITS1, ITS2, and D2D3 of the 28S

rRNA) of several populations of the morphologically suspected species complexes, subsequently looked if concordant lineages were also supported morphologically, based on multivariate morphometric analysis and when the latter were confirmed, performed α-taxonomy (indicated as typological taxonomy) to identify fixed and nonoverlapping features between lineages. The example illustrates that molecular studies using different genes provide a more reliable support for species differentiation and avoid inconsistencies between conspecific taxa (De Ley et al., 2005), but are also dependent on morphology and α-taxonomy to identify species and determine species diversity.

As for free-living aquatic nematodes, it is often assumed (based on morphology) that many freshwater nematode species have a cosmopolitan distribution accompanied by a high degree of gene flow between populations (Abebe and Coomans, 1995; Michiels and Traunspurger, 2005; Abebe et al., 2008; Schabetsberger et al., 2013). Molecular analyses using different nuclear and mitochondrial genes revealed the existence of three distinct genetic lineages within *Tobrilus gracilis*, suggesting that the morphospecies represents a species complex (Ristau et al., 2013). In a paper entitled "Without morphology, cryptic species stay in taxonomic crisis following discovery," Schlick-Steiner et al. (2007) discussed the importance of morphology-based- α-taxonomy in linking cryptic species to Linnean nomenclature and to propose evolutionary hypotheses, biogeographic scenarios, and conservation planning. Biological knowledge from nematode cultures could help to elucidate patterns and origin, as well as consequences of cryptic diversification.

Sequence technologies have led to DNA-based identification, such as DNA barcoding. These new approaches massively increase speed of recognizing and defining known and new species; however, they are not perfect either (Bhadury and Austen, 2010) and a combined approach is needed (De Ley et al., 2005; Abebe et al., 2011).

Species as a Unit for Aquatic Nematode Biodiversity

In a recent synthesis on marine biodiversity in European seas, Narayanaswamy et al. (2013) emphasized among others, the lack of advanced understanding of species diversity and the requirement for more complete biodiversity surveys. Knowledge on free-living aquatic nematode diversity is often related to the presence of a nematologist or interest of a nematode taxonomist in a certain geographic region (Decraemer et al., 2001).

Studies on nematode biodiversity provide important contributions in determining water quality and impact of pollutants. To assess the impact of pollutants such as cadmium, one of the priority heavy metal substances listed in Annex 10 of the European Union Water framework Directive (Brinke et al., 2011), on meiofauna in freshwater sediments, the use of small-scale microcosms with natural nematode communities was investigated. The structure of the nematode community was identified to species level and the maturity index based on the colonizer–persister (cp) classification was determined. The results showed that even within a family, genera/species can differ in their sensitivity to heavy metals. Cd-induced changes in nematode species composition also indicated that a new cp-classification might be needed to assess pollution in freshwater sediments with nematodes (Brinke et al., 2011). Ristau and Traunspurger (2011) analysed littoral nematode communities to study trophic state and eutrophication effects in southern Swedish lakes. They found that trophic level was strongly influenced by species richness, with oligotrophic and mesotrophic lakes showing the greatest species numbers and a shift in species composition along the threshold from mesotrophic to eutrophic conditions.

Discussion and Conclusion on the Utility of α-Taxonomy

In view of the generally recognized need for an integrative taxonomic approach (Pereira et al., 2010), in our opinion, it is not relevant to divide taxonomy in different components based, for example, on the type of information obtained or measure used such as α-taxonomy versus systematics or phylogenetic taxonomy, or based on the technique used such as classical (= morphology-based) taxonomy versus molecular taxonomy, each being allocated a different weight of importance as can be deduced from the journals in which results are published or the criteria by which research projects are selected (Luc et al., 2010). A free-living aquatic nematode species description should strive to include, as far as possible, information from a holistic approach, i.e. data from different fields (morphology, ecology, behaviour, phylogeny, etc.), at different levels of biological organization (morphological features, molecular data) and obtained by different techniques. Both, the "α-taxonomy" mainly based on morphological features and the molecular approach (from barcoding to metagenomics; nuclear or mitochondrial), survey only a (small) part of the whole animal. Each of the approaches/techniques has its pitfalls and hence the results they produce can be improved, refined or extended by combining different approaches.

Taxonomic descriptions and illustrations can be improved and made more accessible by a better training of students, digitized type material and voucher specimens and, video captures can bridge the gap of vouchers when the whole animal is used for sequencing, forming a link between DNA analyses and morphology/α-taxonomy/phylogeny, functional and molecular ecology (Creer

et al., 2010). Accessible databases of species descriptions (NeMys) or digital pictures of organisms and sequences and storage of organism's DNA would allow taxonomist–morphologists to check and improve species information in GenBank. The use of scratchpads (http://scratchpads.eu) as for example, the *Pristionchus* scratchpad (Herman and Sommer, 2014) can stimulate collaboration between taxonomists and widen access, e.g., to literature (Abebe et al., 2011).

DNA barcoding provides a much faster method for biodiversity identification, and is easier to implement than morphology-based discrimination; it has helped to identify new species and to recognize cryptic species. However, there is no standard gene for DNA barcoding of marine nematodes and the limited number of sequences of known species (based on morphology-based taxonomy and mostly from European waters) hampers identification of marine nematodes. The results obtained from sequencing of 18S and 28S rRNA for DNA barcoding of free-living marine nematodes appeared very useful (Meldal et al., 2007), whereas the mitochondrial COI gene is more difficult to amplify.

Cryptic species are often detected by molecular analyses (Fonseca et al., 2008). A posteriori morphological studies of these cryptic species represent a reversed taxonomic approach. Hence, molecular analyses stimulate morphology/taxonomy and show the need for this discipline (Derycke et al., 2013). Many free-living aquatic nematodes have been considered cosmopolitan generalists, but population genetic analyses reveal them to represent species complexes of cryptic species. A recent investigation of cosmopolitan taxa in free-living deep-sea nematodes of the order Enoplida suggested the existence of closely related, globally distributed species complexes whereby phylogenetic analysis showed that within genera, deep-sea and shallow-water species were sister taxa (Bik et al., 2010). Additional data are needed to add further insight to these patterns (De Mesel et al., 2006; Bik et al., 2010).

Ultrasequencing of aquatic meiofauna may be a promising solution in nematode diversity assessment compared to the time-consuming and complex task of aquatic nematode identification. Also the metagenomic approach through which information is obtained from a mixed community of organisms, is not complete since it screens only a small part of the genome. Interpretation of the results on nematode diversity, generally constituting about 50% of operational taxonomic units (OTU) found, needs to be done cautiously because of the risk of putative recombinant DNA molecules (chimeras) and thus of nonexisting species in the data set. For interpretation of the OTU-based phylogeny and linking with known taxa, morphology/taxonomy is needed. Molecular technology and mass collection of information, put morphology/taxonomy back in the picture (Luc et al., 2010). Morphology is hence a crucial component of species delimitation and underpins molecular species identification (DeSalle, 2006). The creation of a web-based E-taxonomy *s.l.* including morphology-based taxonomy and sequence-based taxonomy (Abebe et al., 2011), so far not functional for free-living aquatic nematodes, would be very beneficial for taxonomist and ecologists.

Aquatic nematode biodiversity studies use different units to measure biodiversity, among which rarely the species, so does this mean that α-taxonomy is no longer needed (Losi et al., 2013a)? In contrast, it is a very exciting period for the free-living nematode morphologist/taxonomist, for example, to check if the outcomes of molecular analyses agree with those based on morphology, the challenge to search for features that can differentiate cryptic species found by sequencing and check species so far been considered as cosmopolitan species. However, it is unlikely that morphology-based taxonomy will ever match the speed at which molecular analyses gather information. As such, the purpose of taxonomy per se is not to have all free-living nematode species described and/or named, but more importantly to use morphology/taxonomy to explain the functionality of the data gathered from metagenomics or other diversity measures used, e.g., to understand the role of nematodes in aquatic ecosystems, ecosystem functioning, and evolution, and focus on those species which appear more important/informative in terms of ecological, biogeographic, and other relevance.

New techniques, the novel type of data and the need for a holistic approach illustrate the utility or role of morphology and "α-taxonomy" still can play.

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