

## Usefulness of morphological data for the study of nematode biodiversity\*

Michel LUC<sup>1</sup>, Marcelo E. DOUCET<sup>2</sup>, Renaud FORTUNER<sup>3,\*\*</sup>, Pablo CASTILLO<sup>4</sup>,  
Wilfrieda DECRAEMER<sup>5</sup> and Paola LAX<sup>2</sup>

<sup>1</sup> 6 rue Boutard, 92200 Neuilly sur Seine, France

<sup>2</sup> Centro de Zoología Aplicada, Universidad Nacional de Córdoba, C.C. 122, 5000 Córdoba, Argentina

<sup>3</sup> La Cure, 86420 Verrue, France

<sup>4</sup> Instituto de Agricultura Sostenible (IAS), Consejo Superior de Investigaciones Científicas (CSIC), Apdo. 4084,  
14080 Córdoba, Spain

<sup>5</sup> Royal Belgian Institute of Natural Sciences, Brussels and Ghent University, Ledeganckstraat 35, 9000 Ghent, Belgium

Received: 1 March 2010; revised: 22 April 2010

Accepted for publication: 23 April 2010

**Summary** – Public interest in biodiversity has never been so high, but the necessary inventory of existing species is hindered by the scarcity of taxonomists able to characterise and describe new species ( $\alpha$ -taxonomy). This situation is particularly worrisome in nematology where only a small fraction of the estimated  $10^5$  to  $10^8$  existing species has been described and where experienced taxonomists are fast disappearing while the few that are still active find it increasingly difficult to publish morphological descriptions and to get financial support for biodiversity studies. The present disregard for  $\alpha$ -taxonomy is said to originate from the excessive reliance by funding agencies and academic authorities on the Impact Factors attributed to scientific journals by ISI (Institute for Scientific Information). Molecular studies gave back some support and prestige to taxonomy, but that approach suffers from some limitations and it cannot be used alone for reaching taxonomic conclusions. In addition, any described DNA sequence should always be linked to a named species whose morphology has been correctly described by a trained taxonomist. The authors call for a renewed interest in  $\alpha$ -taxonomy. Electronic publication according to the Code of Zoological Nomenclature would be one solution, but journals and authors seem reluctant to accept it. A variant would be to publish on paper only the diagnosis of the new species. The complete descriptions could be deposited in an Internet database but the setting-up of such a database would be a costly enterprise that would require several years of work by a team composed of morphological and molecular taxonomists and computer scientists.

**Keywords** –  $\alpha$ -taxonomy, electronic publication, Internet databases, molecular biology, morphological descriptions.

Man has always shown an interest in the various life forms that share his planet with him and, according to the Bible, taxonomy is the true ‘oldest trade on Earth’: “*And out of the ground the Lord God formed every beast of the field, and every fowl of the air; and brought them unto Adam to see what he would call them: and whatsoever Adam called every living creature, that was the name thereof.*” (King James Bible, Genesis 2:19).

In real life, men did not stop at naming every living creature; they also arranged them into categories such as

felines, bovines, snakes, and so on. These categories made identification easier and they had also some practical purposes; a bovine was good to eat while felines and snakes were dangerous. Much later, after the discovery of the fact that any species derives from another one, these categories had to reflect phylogenies and group only species that evolved from a common ancestor.

More scientifically, this old human curiosity translates into an interest in biodiversity, *i.e.*, *the diversity within species, between species and of ecosystems* (Conven-

\* This article originates from discussions held on the phone between Michel Luc and Marcelo Doucet. Michel Luc died before the text was ready but the task was completed by several individuals who wanted to publish it as a last homage to Michel. As the article embodies many of Michel Luc’s concepts on where taxonomy should go, concepts that he communicated to the other authors, we thought it was only fitting that he signs here his last article.

\*\* Corresponding author, e-mail: fortunier@wanadoo.fr

tion on Biological Diversity, Rio de Janeiro, 1992, in <http://www.cbd.int/convention>, section Text of the Convention, Article 2: Use of terms). In relation to species, diversity incorporates the number of species present in a given environment and their relative abundance, which means that the knowledge of biodiversity requires, first and foremost, an inventory of the plant and animal life in a particular habitat or in the world as a whole. Of course, it will be interesting to know the ancestry of the species that live on Earth, but this comes only after these species have been discovered, described and named, which is what is called  $\alpha$ -taxonomy.

The quest for the knowledge of biodiversity has recently taken a new urgency with the realisation that we are at the beginning of what could prove to be the sixth major extinction event where many species could disappear and hence should be discovered and described before they are gone.

The basis of any study of biodiversity is the morphological description of the existing species as they appear to us. Such descriptions must make it possible to identify other specimens of the described species. Once this is done, other aspects of the well characterised species can be studied, including its genome, its ecology (relationships with its environment), its biology (feeding, reproduction, host-parasite relationships), its geographical distribution, its interest as a biological marker, and more. But, except for some unicellular organisms such as viruses, certain bacteria, *etc.*, the basis of all of these studies will always be the morphological description of the species.

Molecular data can be very useful in providing hints at identity and relationships among species, but biodiversity is the diversity of whole organisms that can be seen living and interacting with their environment, it cannot be reduced to the diversity of DNA sequences. Taxonomy is sometimes differentiated as: *i*) descriptive taxonomy, *i.e.*, species description based on morphological features; and *ii*) molecular taxonomy, *i.e.*, use of DNA sequences to characterise species and analyse phylogenetic relationships (Godfray, 2002). This is confusing the nature – morphological *vs* molecular – of the data used in a study with the goal – species description *vs* phylogenetic analysis – of that study. It is quite conceivable to describe a species based on molecular data and conversely to use morphological data to define its phylogeny. The latter was the approach used, *e.g.*, in the reappraisal of *Tylenchina* (Luc *et al.*, 1987) or the phylogenetic study of *Xiphinema* (Coomans *et al.*, 2001).

In nematology, our knowledge of global biodiversity is far from being complete. While the number of nematode species on Earth is at least several hundred thousands, and may reach one million or more (Lambshhead, 1993, 2004), only about 28 000 of them have been described (Coomans, 2000; Hugot *et al.*, 2001; Hugot, 2002). Much work remains to be done but, at the same time, many review papers bemoan the decrease in the number of nematode taxonomists and the fact that the few remaining taxonomists find it difficult to fund taxonomic studies and to publish their work in scientific journals (Coomans, 2000).

Boero (2010) described the process that led to this unfortunate situation in nematology, as in all of biology. It started with the creation of ISI (Institute for Scientific Information) in 1960 by an American scientist, Eugene Garfield, for providing abstracts of as many scientific articles as economically possible. To cover all of the scientific journals would have been too expensive, so ISI invented the Impact Factor (IF) to rank each journal according to the number of times the papers it publishes are cited. A journal with a low IF was considered to have no scientific impact. In the next step, the IF became increasingly used by scientific hierarchy and funding agencies for evaluating the worth of a scientist. A scientist publishing papers in journal with high IF was ranked higher than one publishing in low IF journals, despite recommendations to compare only journals within the same field of research. This means that an index set up for a purely economical reason came to be used for judging the quality of scientific publications and of scientists themselves. What would have been the IF of Mendel's original paper? According to Piquemal (1992), Mendel's presentations on February 8 and March 8, 1865, at Naturforschenden Verein in Brünn, elicited no comments or discussions and the corresponding article (Mendel, 1866) was cited only twice over the next 35 years. The only way to judge the quality of an article is to actually read it.

Ranked by their 2008 IF, as found in [http://abhayjere.com/Documents/Impact factor 2008\\_PDF.pdf](http://abhayjere.com/Documents/Impact%20factor%202008_PDF.pdf), the top nematology journals are *Journal of Nematology* (IF: 1.2), *Nematology* (IF: 0.9), *Russian Journal of Nematology* (IF: 0.4) and *Nematropica* (IF: 0.2). The other nematology journals are not listed at all, including *International Journal of Nematology*, *Journal of Nematode Morphology and Systematics*, *Nematologia Brasileira*, *Egyptian Journal of Agronematology*, *Nematologia Mediterranea*, *Japanese Journal of Nematology*, *Indian Journal of Nematology*

and *Pakistan Journal of Nematology*. Among journals dedicated to taxonomy in general, one can cite *Zootaxa* (IF: 0.7) and *Zoosystema* (IF: 0.6), the latter being the successor of *Bulletin du Muséum national d'histoire naturelle*. These impact factors are very low compared with those of journals in the medical field such as *CA: a Cancer Journal for Clinicians* (IF: 74.6, best IF) or the *New England Journal of Medicine* (IF: 50.0) or of general scientific journals such as *Nature* (IF: 31.4) or *Science* (IF: 28.1), and they are also on the low side when compared with the IFs of other zoological journals, such as *Zoologica Scripta* (IF: 2.5) or *Zoological Journal of the Linnean Society of London* (IF: 2.1), and with the global average IF of 2.44 as estimated from a sample of 140 IFs randomly drawn from the 6600 IFs listed. Clearly, all journals, including nematology journals, will try to raise their IF, and all scientists will try to have their articles published in high IF journals.

Now, an article that describes a new species will not be cited in later publications, even if that species proves to be a devastating parasite. *Heterodera schachtii* Schmidt is probably one of the most damaging plant-parasitic nematodes, but the hundred of studies published on the biology and control of *H. schachtii* do not cite the article of Schmidt (1871). Even taxonomic articles are required to include full citations of all works relevant to nematode descriptions, but not for the species that are not the principal subject of the paper (*Nematology*, Notice to contributors). This certainly does not help to boost the IF of the journals that publish descriptions of new species.

Boero (2010) explains how taxonomists were not even aware of the situation created by the use of the ISI impact factor but “when scientific careers became boosted by researchers’ IF, traditional taxonomy resulted in a scientific suicide”. Journals interested in boosting their IF became more and more reluctant to publish morphological descriptions of new species and favoured other types of articles, for example, papers describing zoological diversity in an evolutionary context as in *Zoologica Scripta*, hereby giving preference to studies in phylogeny, biogeography, molecular biology and palaeontology. Such a shift in scope had some positive aspects but prevented pure morphology-based articles from being published. *Zootaxa* and *Zoosystema* do accept morphological descriptions but with very strict restrictions, and both journals specifically discourage articles describing only one species, as if diversity was not built one species at a time. Decision makers came to consider taxonomy as a science of the past, one that belongs to museums or that may even not be a science at all. Consequently, the word ‘taxonomy’ gradu-

**Table 1.** Place of taxonomy articles in the journal successively named *Revue de Nématologie* (1978-1991), *Fundamental and Applied Nematology* (1992-1998) and *Nematology* (since 1999).

Year	Total number of articles	Taxonomical articles			
		Description of n. spp.	Molecular studies	Others	Total
1978	26	7 (27%)	0	1	8 (31%)
1980	26	9 (35%)	0	3	12 (46%)
1990	56	11 (20%)	2	16	29 (52%)
2000	67	7 (10%)	10	8	25 (37%)
2009	76	21* (27%)	12	8	41 (54%)

\*Of the 21 articles with descriptions of new species, 13 include molecular data, while the other eight (10% of the articles) give morphological descriptions only.

ally became a ‘dirty word’, to the extent that any research work including it in its heading would most probably not be funded. It is true that, as indicated above, the number of described species is very low compared to the estimated number of existing species, but discouraging young scientists from entering the field of taxonomy and cutting the funds allocated to the discovery and description of new species is not going to improve this situation.

In the field of nematology, it is interesting to see the place occupied by taxonomy in the journal founded by Michel Luc, a taxonomist, under the successive names of *Revue de Nématologie* (1978-1991), *Fundamental and Applied Nematology* (1992-1998) and *Nematology* (since 1999), its current name. Table 1 concerns only the papers listed as ‘Articles’ and excludes research notes, ‘tribunes’, symposiums and other non-characteristic publications (e.g., obituaries). Table 1 shows that the percentage of articles dealing with the various aspects of taxonomy is generally constant (around 40%), the 2009 percentage (54%) being actually higher than the 1978 one (31%). However, when we consider only those articles with descriptions of new species, while their absolute number and their percentage relative to the total number of articles in the journal are holding-up (27% in both 1978 and 2009), the percentage of morphological descriptions unsupported by molecular data shows a sharp decline, from 31% in 1978 to only 10% in 2009. This may be due to the fact that recent descriptions include data types such as molecular data that were not available in 1978, which must be viewed as an enhancement of descriptions due to additional data sources, but it may also be due to the fact that descriptions not including

molecular data are rejected on sight due to current journal policies. The present authors recognise that a species description that encompasses all of the existing types of data is obviously better than one with just one type of data. However, they resent the fact that a study that includes only molecular data will be accepted (if the data and the analyses are valid), whereas a study with only morphological characters will have to fight against a systematic prejudice before it is accepted.

When the whole scientific literature is considered, the decrease in the number of new nematode species published per year becomes obvious. A recent search in *CABI Nematology Abstracts* found 172 new species descriptions in 2003, 135 in 2004 and 83 in 2005 (Eyuaem-Abebe *et al.*, 2006), with 125 new nematode species being described in 2009. While the coverage of that journal has changed over time, these numbers can still be compared to the average annual number of 364 new nematode species listed in *Zoological Records* between 1979 and 1988 (Hugot, 2002). Some might argue that this situation arose because the species descriptions became more comprehensive (with SEM and LM photos, sequence data and the like) than in the past, thus taking more time to complete. Nobody can complain about better descriptions, but, if more stringent requirements from journals caused a reduction in the number of new species described, our knowledge of diversity (*i.e.*, of the number of existing species) suffered. At a time when public interest in biodiversity has never been so high, the basis of any biodiversity study – the description of new species – is being driven out of scientific literature.

We do not agree with Paul De Ley (2000) when he wrote that “*we can no longer afford to describe new species purely for the sake of describing new species*”, nor with the report on ‘*Taxonomy in Europe in the 21<sup>st</sup> century*’ of the European Distributed Institute of Taxonomy (EDIT) recommending that ‘*formal description will only be used in taxa or instances where a formal name is essential*’ (<http://www.e-taxonomy.eu/node/22>). It is true that, from a purely taxonomical point of view, “*description of a new species per se without any new element apart from, e.g., differences in morphometrics has very little scientific value*”, and that “*we could continue for ages in this way without really furthering substantially our knowledge on the group*” (Coomans *et al.*, 2001), but these authors did add that this would give a better estimation of diversity, which is what we are talking about here. In the name of human interest in the beauty and wonder of the living world, we must describe and name any species we find

because it is there, and not just when it presents a specific taxonomical, ecological, economical or medical interest. To do otherwise would be to abandon the search for biodiversity and betray the trust of the public.

Partly because of the difficulty of publishing purely descriptive works and partly because of the interest caused by a new and powerful tool, many taxonomists turned to molecular biology. Table 1 shows that the fact that the percentage of taxonomy articles among the papers published in *Nematology* remained level, in spite of the decline of morphological publications, was due to the rise of molecular studies that went from zero in 1978 to almost half of the taxonomical articles published in that journal in 2009.

This reflects in the acceptance policies of the nematological journals with the best IF in the field. *Journal of Nematology* does not have any specific policy requiring molecular data (DNA sequence is optional), but reviewers might ask for these data if they think it is important (Kris N. Lambert, Editor-in-Chief of *Journal of Nematology, in litt.*). *Nematology* accepts single species descriptions, subject to the usual peer review, but from certain groups molecular data are required and also encouraged as support for all new taxa (David Hunt, co-Editor-in-Chief of *Nematology, in litt.*).

Molecular data are an important part of species descriptions, but so are other types of data: ecological data, physiological data, behavioural data and others, including morphological data. It is obvious that the best descriptions are those that include all of these types of data – the so-called integrated approach – but we see no reason to favour one over the others and specifically to require that, to be published, a description must include one particular type of data such as molecular data.

Going one step further, we would like to point out that molecular biologists themselves are not comfortable with the idea of describing new species based on molecular data alone. When a species is described from morphological data only it is called a new species without a qualm, whereas a population said to be different based on molecular data is called a ‘cryptic species’, cryptic meaning “of an obscure nature”, according to the Webster online dictionary (<http://www.websters-online-dictionary.org/definition/cryptic>). This may be due to old habits or it may reflect a deeply felt difference between the two types of data. In any case, concepts are evolving. When molecular techniques were developed to the point where they became available to any scientist with proper tools, back in the 1980s, the molecule became paramount.

Now, we are seeing the emergence of 'systems biology' that focuses on the way these molecules interact within complex systems. The next step might be the rediscovery that these systems operate within organs, and from organs to whole organisms would be a rather simple step. The holistic approach of systems biology is doomed to lead to a renewed interest in the morphology of whole organism, which is what  $\alpha$ -taxonomy is about.

The above-mentioned policies mean that a scientist who discovers a new species may find it very difficult, if not impossible, to describe it on morphological grounds alone. One can sympathise with the editors of journals who had to find a way out of the quandary and choose between allowing morphological descriptions of single species and accepting a drop in their IF, but it is a fact that such policies go against the goal of the study of biodiversity, which is the inventory of the diversity of all the living beings on Earth, even when some of the existing types of data are unavailable and even when the species present no special interest for humans.

As a related issue, biodiversity is particularly rich in tropical regions, but these areas include many developing countries lacking the funds needed for buying the very expensive equipment and reagents that are required for molecular research. It is very difficult to obtain the resources necessary to equip such a laboratory. Of course, science must not proceed at the pace of the slowest, but at the same time the slowest should not be kicked out of the race and they should be allowed to make their own contribution to our general knowledge of biodiversity, as humble as it might be.

Molecular studies gave back some respectability to taxonomy and it is obvious that adding molecular data can only add to the value of a description, but some taxonomists went so far as to abandon completely the morphological approach and go all-molecular. While an integrated approach is to be commended, the scientific freedom of authors should not be limited by imposing a particular type of data. Also, it must be underlined that the study of molecular data alone can only suggest the presence of potential new species whose real existence must be corroborated by integrated approaches using other types of data.

The term 'barcoding' describes the situation where DNA sequences are used as a barcode for identifying species. However, in order to discover a barcode that uniquely identifies a particular species, the molecular biologist must know what species he is working on. Molecular analyses are conducted on specimens whose species

identity was previously defined by a taxonomist on the basis of morphological criteria. The alternative would be to abandon completely the concept of morphological species and reduce the study of the diversity of living beings to the study of the diversity of DNA sequences. This would satisfy neither human curiosity nor the practical aspects of classifications.

Barcoding presents other problems in the description of new species. Supposing that the barcodes of all known species in a particular genus were known, should a specimen found with a different barcode be described as a new species? The answer is no, because the various species concepts that have been proposed require that the individual members of a species differ clearly (clear gap) by one character, differ by a number of morphological and biological characters, and/or originate from a common ancestor. Barcoding alone may show the presence of a clear gap in one character (the barcode) in exceptional circumstances (all known species of the genus are already barcoded); it cannot answer the other criteria. Barcoding cannot provide a straightforward relationship between the degree of genetic divergence and reproductively isolated entities. The tool allows a rapid comparison of new and known sequences and determines the amount of similarity or divergence between specimens or populations/species but barcoding does not determine 'what is a species?'

Originally a part of a mitochondrial gene coding for the enzyme CO subunit I was chosen as a barcode species identifier. A choice recently supported by the hypothesis that mitochondrial sequences might actually act as a drive in the speciation process (Lane, 2009). For nematodes, ribosomal genes proved promising for barcoding, mainly 18S (SSU rDNA) (Bhadury *et al.*, 2006) but also 5.8 and 28S (LSU rDNA). Non-coding regions such as internal transcribed spacers ITS1, ITS2 and D2-D3 expansion region were also used to analyse relationships. Many articles have discussed the problems raised by the molecular approach (Seberg *et al.*, 2003; Quicke, 2004). Most difficulties are related to the choice of the data (genes) to be used and the choice of the method of phylogenetic inference – MP (Maximum Parsimony), ML (Maximum Likelihood), NJ (Neighbour Joining), BA (Bayesian method) – used to analyse these data. Rubinoff *et al.* (2006) highlight, for example, the shortcomings of mtDNA for barcoding identification and emphasise the lack of a barcode-based species concept.

When molecular taxonomists rely on sequence data alone, they may describe as 'cryptic species' populations of an existing species that are morphologically identical

and are differentiated only by molecular criteria, without attempting to resolve the conflict between morphological and molecular data. Molecular data can be very useful in the case of species exhibiting large morphological variability under different environmental conditions because DNA sequences are not affected by the environment. However, barcoding should never be accepted as the ultimate answer in all cases. For example, geographically separated populations of a particular species may have distinct sequences while remaining mutually fecund. Such populations must not be described as separate species in spite of the DNA evidence (Tan *et al.*, 2010). These authors were able to resolve the status of a cryptic species (*Sepsis pyrrhosoma*, a dipteran) by using morphological and behavioural data, as well as tests for reproductive isolation. Such studies can only be conducted by a team that includes at least one 'classical' taxonomist, *i.e.*, one who is familiar with the morphology of nematodes. For the reasons mentioned above, the number of classical taxonomists is rapidly decreasing (Hugot, 2002). Far from solving the problem of the huge difference between the number of described species and the number of existing species, the increasing number of cryptic species based on molecular data is, in fact, adding to the burden of classical taxonomists who are the only scientists formed to study such species from different points of view, decide on their validity and describe their morphology. The development of molecular biology should not be used as a reason for diminishing the number of classical taxonomists as it actually requires that more classical taxonomists be hired and funded.

Regardless of the type of data used, the ultimate answer to the question 'what is a species?' can only be given by a test for reproductive isolation, which is the basic 'species concept' underlying all of the others. Of course, reproductive isolation cannot be proven in the case of the parthenogenetic nematode species, but this very mode of reproduction makes it possible to create clones from a single female. Such clones can then be used to establish the limits of intra-specific variability of morphological characters, in other words to establish species boundaries for parthenogenetic species (Fortuner & Quénehervé, 1980).

On the practical side, about 28 000 nematode species have been morphologically described over the years. Relying on barcodes alone would require the definition of 28 000 barcodes for each molecular region considered (such as *cox1*, D2-D3, and so on). In addition, the value and accuracy of some of the known sequences are

questionable. For example, GenBank accession numbers FJ661086.1, FJ661085.1, FJ661082.1 and FJ661075.1 identify the species involved only as '*Tylenchida* sp.', which is not very informative or useful.

Of course, a sequence such as, *e.g.*, FJ661086 is linked in the GenBank database to voucher specimens and it can be used in a Blast search to identify these specimens, *Deladenus* sp. in this case (anonymous reviewer, *in litt.*). However, it would be logically flawed to use this fact to confirm the validity of the original *Deladenus* sequences. Let us suppose that the sequences originally said to characterise *Deladenus* came, in fact, from *Gymnotylenchus*, a related genus in Allantonematoidea, and let us suppose that the voucher specimens of sequence FJ661086 also belong to *Gymnotylenchus*. The Blast search would still attribute them to *Deladenus*, but this would not make the original misidentification any more valid. It is essential that the specimens studied are correctly identified from morphological characters and that voucher specimens (or video images such as Digital Multifocal Images (DMI)) be available before a new nematode sequence is deposited in international databases.

Another problem is that molecular phylogenetic studies are based on a very small number of sequences. For example, Mundo-Ocampo *et al.* (2008) synonymise *Afenestrata* with *Heterodera* based largely on the study of five genes, including three rRNA genes and two nuclear genes, one coding for actin and the other for the heat shock protein (HSP) 90. This is more than most molecular studies but very limited compared to the total size of nematode genomes. For example, the genome of *Caenorhabditis elegans*, the first multicellular organism whose genome has been completely sequenced, includes 100 million base pairs representing 20 100 coding genes. It also includes at least 16 000 RNA genes. Also, we can question the relevance of the two nuclear genes selected by Mundo-Ocampo *et al.* (2008) on the morphology and physiology of the nematodes concerned. At best, actin has some action on movement and HSP 90 on resistance to heat, but there is much more than that in the physiology of nematodes. In other words, molecular markers must not be used alone but in addition to morphological and physiological characters. This is the only way to insure that the classification obtained will not only reflect common ancestry, but also have a practical interest.

Coomans *et al.* (2001) discuss other problems raised by molecular data – including randomness of the changes in DNA sequence, neutrality of molecular evolution, different rates of change of characters, absence of a universal

molecular clock – and they conclude that both morphological and molecular approaches have their own interests and limitations and that it is important to combine them “to obtain a comprehensive view of evolutionary relationships”.

To conclude, molecular biology is a wonderful tool but it suffers from limitations and cannot exist in a vacuum. Also, to be relevant for the study of diversity, molecular studies must be realised on known species, *i.e.*, on taxa whose morphology and biology have been described using the classical approach. This requires that  $\alpha$ -taxonomy regains its lost prestige.

How can this be achieved?

The most important thing is to allow taxonomists to continue publishing morphological descriptions of new species.

It has been shown above that the current trend of journals to limit the space devoted to morphological descriptions is illogical in the face of the current public interest in biodiversity and one can hope that this trend will be reversed some time in the future, but this is not happening at this moment. We must find a way to continue publishing morphological descriptions of new species. Even when a molecular study hints at the possible existence of a new species (what is called a cryptic species), the putative new taxon should be accepted only from morphological and other non-molecular criteria. Tan *et al.* (2009) show how this can be done.

It remains that taxonomists find it more and more difficult to publish morphological descriptions of new species. Calls to journals for a change of policy have been made (Eyuaem-Abebe *et al.*, 2006) but have not been answered. It is therefore necessary to find immediately applicable practical solutions.

One such solution could be found in the electronic publication of nematode taxonomic manuscripts, as discussed by Eyuaem-Abebe *et al.* (2006). The 1999 revision of the International Code of Zoological Nomenclature (ICZN) still states (Article 8.1.3.) that the work describing and naming a new species “must have been produced in an edition containing simultaneously obtainable copies by a method that assures numerous identical and durable copies” but it now accepts (Article 8.6.) “works produced after 1999 by a method that does not employ printing on paper”, as long as it “contains a statement that copies (in the form in which it is published) have been deposited in at least five major publicly accessible libraries which are identified by name in the work itself”. Article 9.8. indicates that “text or illustrations distributed by means

of electronic signals (*e.g.*, by means of the World Wide Web)” do not constitute published works and a proposed amendment of the ICZN would disallow physical works that are not paper-based, *e.g.*, CD-ROMs, DVDs (see [http://www.iczn.org/electronic\\_publication.html](http://www.iczn.org/electronic_publication.html)).

In contributions to the discussion on the proposed amendment, various authors advocate freely-downloadable PDF files (M.P. Taylor, 2009), others prefer XML (Garrity, 2009), or RTF (B. Taylor, 2009) files. Most of the persons contributing to that discussion expressed concern about the longevity of electronic data storage media, the maintenance of access to historic data in spite of technology evolution, the integrity of transferred or “refreshed” data, the special skills needed for managing the computer science aspects, and the cost of such an approach.

For answering the primary goal of the study of biodiversity, *i.e.*, the inventory of as many species as possible, in spite of the limitations placed by many journals on the publication of taxonomic works and in view of the requirements of the ICZN, a solution would be to publish on paper the minimum needed for the name to be valid. Article 13.1.1. of the ICZN states that, to be valid, a new name must “be accompanied by a description or definition that states in words characters that are purported to differentiate the taxon”. This means that the diagnosis and relationships of a new species, plus information on type host, type locality and type specimens, would be enough to establish its validity. These could be added in the “Short communications” section or in a new separate section where several diagnoses could be placed on a single page. This solution would not require any major changes in the existing publication policy, but some journals may have higher standards and insist on publishing a full description.

Obviously, such a minimal description without any illustration would be useless for more elaborate taxonomical analyses and for species identification. Still, once the ICZN requirements are met, the corresponding detailed descriptions – including complete morphological data, any other available data (ecological, physiological, molecular, *etc.*), keys or tables for identification or detailed relationships, and illustrations (drawings, photos or DMI) – could be distributed by the authors themselves as they see fit and without any involvement of the journal. These additional data and their electronic support could be modified over the years depending on future developments of information technology. The validity of the names would be maintained due to the original publication on paper.

It is interesting to note that the various formats mentioned above (PDF, XML, RTF) are well adapted for storing text and images similar to what has been traditionally, and still is, published on paper. We believe this is missing taking advantage of the infinite possibilities afforded today by information technology. After the requirements of the ICZN are met, electronic publication and data storage should be seen as an opportunity to go beyond the mere textual description of *ad hoc* characters. If a universal data format were established for the description and illustrations/photos of new species and used in a database freely accessible through the Internet together with properly designed software, such a database could allow any scientists with minimal training in nematode morphology (including molecular biologists) to identify the specimens they are working on. Development of such software would be a difficult task but not an impossible one (Diederich *et al.*, 2000a). The same database could be extended to host molecular data (sequences) and, hopefully, to link a particular gene to the protein it codes for and that protein to particular morphological or physiological characteristics. We are far from being able to define such relationships but, if and when they are defined, the structure would be ready to host them.

Concerns about the immutability and safety of the data could be alleviated by the use of distributed databases with regular automatic refreshment of the data. For example, the database could be duplicated and stored on half a dozen master servers, permanently checking each other for inconsistencies and updates. These master databases would be updated as the technology advances. With the multiplication of smart phones with increasingly gigantic memory capacities, we can even imagine that every nematologist could have his or her own copy of the database, to be updated every time they log on to the web.

However, the definition of a database schema is not a straightforward issue and any attempt made by biologists alone is doomed to fail or at least to result in a far from optimal database. For example, the Generic Biological Information System NeMys (<http://nemys.ugent.be/index.asp>) includes keys to genera in some families, but the identification characters used are *ad hoc* characters, each one being defined and valid only for the family considered, which means that the database is far from being generic. In addition, the identification process in NeMys seems to be a straightforward elimination of a number of genera every time the user chooses one of the proposed states of one of the characters. Such a system does not degrade gracefully when the user makes a single mis-

take. It would be necessary that the team gathered for setting up a taxonomic database includes computer scientists, knowledge engineers, computer analysts and programmers, in addition to various taxonomists specialising in morphological descriptions, ultrastructure, molecular data, plus nematologists familiar with ecological, parasitological, and other biological data. Such a team must be prepared to work on the project for several years, which is how long it took the Nemisys (Nematode Identification System, later Genisys, General Identification System) team, composed of one biologist and two computer scientists, to propose a database schema for morphological characters alone (Diederich *et al.*, 2000b; see also [http://genisys.prd.fr/genisys\\_home.html](http://genisys.prd.fr/genisys_home.html)).

The various electronic solutions proposed above are not well received by journals and scientists and they will require heavy funding. It remains that the classical approach to morphological description of new species has fallen into disregard, although a reversal might be in the making with the PEET (Partnerships for Enhancing Expertise in Taxonomy) program established in the USA by the National Science Foundation to enhance taxonomic research and help prepare future generations of experts (<http://www.nsf.gov/about/history/nsf0050/environment/wanted.html>). It is hoped that electronic publication and similar initiatives will give back to morphological taxonomy the prestige it should never have lost and pull back the taxonomist from the list of endangered species.

We finally ask molecular biologists to realise that there is more to biodiversity and taxonomy than molecular sequences alone. No new grouping should be proposed for molecular reasons alone. Taxonomic decisions, be it the definition of a new taxon or the synonymisation of an existing one, must consider morphological and physiological data.

The molecular approach includes two pitfalls. The first one is to use molecular biology only to give an air of respectability and objectivity to a subjective taxonomical decision. In many studies, molecular data is used to 'prove' that some taxa must be synonymised but the emended diagnosis of the valid taxon includes only morphological characters. If the sequences used in the main analysis are not mentioned in the diagnosis, why use them to demonstrate a supposed relationship between the taxa?

The second one is to base phylogeny on molecular data alone, regardless of any existing morphological or physiological differences in the taxa involved. This results in a classification of sequences, not of living beings.



The current molecular approach consists in finding a barcode unique to a nematode population and concluding from this that this population is a (cryptic) species, or concluding from the result of the analysis of a few sequences that a supra-specific grouping is or is not valid. We call on molecular biologists to turn this approach around and use molecular data only as a pointer to possible relationships and to propose new species or new grouping only after the nematodes involved have been considered as a whole.

It is difficult to imagine, at least for the time being, an alternative to the work of a taxonomist provided with the necessary literature, time and adequate optical equipment, who describes species within a group familiar to him/her and who publishes the results of this work in specialised journals. There are several activities that cannot be done without the hands and eyes of a human being. Direct contact with the organism under study is essential. A thorough observation of the specimen, its structures, shapes and measurements leads to personal interpretation of its identity. The description process involves a deep knowledge of real and tangible parameters, how they are related and the relative importance of each parameter with respect to the others.

Nematodes are worms, with specific internal and external shapes and specific physiological, ecological and parasitical behaviours, depending on the genera, species and even populations, to which they belong. In the soil of cultivated or non-cultivated fields, in rivers, lakes, and oceans, nematodes appear as living animals, not as a sequence of nucleotides. The direct contact with the organism under study is essential for proposing a meaningful classification. A good connection with the real should lead us equally to encourage the use of all the study tools to advance in the knowledge of biodiversity of this fascinating animal group.

## References

- BHADURY, P., AUSTEN, M.C., BILTON, D.T., LAMBSHEAD, P.J.D., ROGERS, A. & SMERDON, G.R. (2006). Development and evaluation of a DNA-barcoding approach for a rapid identification of nematodes. *Marine Ecology Progress Series* 320, 1-9.
- BOERO, F. (2010). The study of species in the era of biodiversity: a tale of stupidity. *Diversity* 2, 115-126.
- COOMANS, A. (2000). Nematode systematics: past, present and future. *Nematology* 2, 3-7.
- COOMANS, A., HUYS, R., HEYNS, J. & LUC, M. (2001). Character analysis, phylogeny, and biogeography of the genus *Xiphinema* Cobb, 1913 (Nematoda: Longidoridae). *Annales du Musée Royal de l'Afrique Centrale (Zoologie), Tervuren, Belgique* 287, 1-239.
- DE LEY, P. (2000). Lost in worm space: phylogeny and morphology as road maps to nematode diversity. *Nematology* 2, 9-16.
- DIEDERICH, J., FORTUNER, R. & MILTON, J. (2000a). Genisys and computer-assisted identification of nematodes. *Nematology* 2, 17-30.
- DIEDERICH, J., FORTUNER, R. & MILTON, J. (2000b). A uniform representation for the plan of organization of nematodes of the order Tylenchida. *Nematology* 2, 805-822.
- EYUALEM-ABEBE, BALDWIN, J., ADAMS, B., HOPE, D., GARDNER, S., HUETTEL, R., MULLIN, P., POWERS, T., SHARMA, J., YE, W. & THOMAS, W.K. (2006). A position paper in the electronic publication of nematode taxonomic manuscripts. *Journal of Nematology* 38, 305-311.
- FORTUNER, R. & QUÉNÉHERVÉ, P. (1980). Morphometrical variability in *Helicotylenchus* Steiner, 1945. 2. Influence of the host on *H. dihystra* (Cobb, 1893) Sher, 1961. *Revue de Nématologie* 3, 291-296.
- GARRITY, G.M. (2009). Comments on the ICZN proposed amendment on electronic-only publications (1). *Bulletin of Zoological Nomenclature* 66, 302-305.
- GODFRAY, H.C.J. (2002). Challenges for taxonomy. *Nature* 417, 17-19.
- HUGOT, J.-P. (2002). Changes in numbers of publications on the main groups of Nematoda and Helminthes between 1971 and 1995. *Nematology* 4, 567-571.
- HUGOT, J.-P., BAUJARD, P. & MORAND, S. (2001). Biodiversity in helminths and nematodes as a field of study: an overview. *Nematology* 3, 199-208.
- LAMBSHEAD, P.J.D. (1993). Recent developments in marine benthic biodiversity research. *Oceanis* 19, 5-24.
- LAMBSHEAD, P.J.D. (2004). Marine nematode biodiversity. In: Chen, Z.X., Chen, S.Y. & Dickson, D.W. (Eds). *Nematode morphology, physiology and ecology*, vol. 1. Tsinghua, P.R. China, Tsinghua University Press, pp. 438-492.
- LANE, N. (2009). On the origin of bar codes. *Nature* 462, 272-274.
- LUC, M., MAGGENTI, A.R., FORTUNER, R., RASKI, D.J. & GERAERT, E. (1987). A reappraisal of Tylenchina (Nemata). 1. For a new approach to the taxonomy of Tylenchina. *Revue de Nématologie* 10, 127-134.
- MENDEL, J.G. (1866). Versuche über Pflanzenhybriden. *Verhandlungen des naturforschenden Vereines in Brünn, Bd. IV für das Jahr 1865, Abhandlungen*, 3-47.
- MUNDO-OCAMPO, M., TROCCOLI, A., SUBBOTIN, S.A., DEL CID, J., BALDWIN, J.G. & INSERRA, R.N. (2008). Synonymy of *Afenestrata* with *Heterodera* supported by phylogenetics with molecular and morphological characterization of *H. koreana* comb. n. and *H. orientalis* comb. n. (Tylenchida: Heteroderidae). *Nematology* 10, 611-632.

- PIQUEMAL, J. (1992). Mendel (Gregor Johann) 1822-1884. In: *Encyclopedia Universalis, Corpus*. Encyclopedia Universalis France, vol. 14, pp. 959-960.
- QUICKE, D.L.J. (2004). The world of DNA barcoding and morphology. Collision or synergism and what of the future? *Systematist* 23, 8-12.
- RUBINOFF, D., CAMERON, S. & WILL, K. (2006). A genomic perspective on the shortcomings of mitochondrial DNA for "barcoding" identification. *Journal of Heredity* 97, 581-594.
- SCHMIDT, A. (1871). Über den Rüben-Nematoden (*Heterodera schachtii* A. S.). *Zeitschrift der Vereinten Rübenzuckerindustriellen Zoologieverein*, v. 21 n. F. 8, 1-19.
- SEBERG, O., HUMPHRIES, C., KNAPP, S., STEVENSON, D., PETERSEN, G., SCHARFF, N. & ANDERSEN, N. (2003). Shortcuts in systematics? A commentary on DNA-based taxonomy. *Trends in Ecology and Evolution* 18, 63-65.
- TAN, D.S.H., ANG, Y., LIM, G.S., BIN ISMAIL, M.R. & MEIER, R. (2010). From 'cryptic species' to integrative taxonomy: an iterative process involving DNA sequences, morphology, and behaviour leads to the resurrection on *Sepsis pyrrhosoma* (Sepsidae: Diptera). *Zoologica Scripta* 39, 51-61.
- TAYLOR, B. (2009). Comments on the ICZN proposed amendment on electronic-only publications (3). *Bulletin of Zoological Nomenclature* 66, 306-307.
- TAYLOR, M.P. (2009). Electronic publication of nomenclatural acts is inevitable, and will be accepted by the taxonomic community with or without the endorsement of the Code. *Bulletin of Zoological Nomenclature* 66, 205-214.