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MONOGRAPH

Taxonomy faces speciation: the origin of species or the fading out of the species?

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ABSTRACT Efficient field sampling and new investigation tools, including barcoding and other molecular techniques, are bringing to light an unexpected wealth of new species, including sets of morphologically quite uniform, but genetically distinct cryptic species. On the other hand, increasing appreciation of the dynamic nature of the species and a better knowledge of speciation processes and introgression phenomena challenges the taxonomists' efforts to shoehorn all diversity of life into a formal classification of which the species would be the basic unit. Unfortunately, there is probably not a single best notion of species, either in theory or in practice.

KEY WORDS barcoding; cryptic species; hybridization; speciation; species concepts.

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THE SPECIES - A SOLID PILLAR OF OUR REPRESENTATION OF LIVING NATURE?

A substantial percentage of recent books and articles in zoology, botany, palaeontology, biogeography and ecology may suggest that the species has passed undamaged through the Darwinian revolution. Although everybody, or so, in these disciplines is likely ready to accept that species are products of evolution, in practice a great many professionals describe and analyze the living world of the past and present time in terms not that different from those of Linnaeus and the other pre-Darwinian authors.

In the title-page of his magnum opus, Linnaeus (1758) announced an arrangement of his Systema naturae per regna tria naturæ, secundum classes, ordines, genera, species. Firmly placed at the bottom of the hierarchy, the species category was thus proposed as the fundamental unit of classification. In the author's creationist and largely fixist views, species have been created at the beginnings of time and the naturalist's job is to piously explore Nature with the aim of completing their inventory.

What does survive to our time, of this reassuring pre-Darwinian conception of biological diversity? Little, if anything, in theory, but quite a lot in practice. This is true both of the approach with which taxonomists continue Linnaeus' project for a global inventory of biodiversity and of the perspective from which most of their colleagues in biology, ecology, biogeography and stratigraphy look at the extant or extinct forms of life that are the object of their studies.

To be sure, there are also the 'professionals of the species problem,' that is, biologists - but also philosophers of biology - who take very seriously the Darwinian challenge and specifically focus on all those contexts where the boundaries between species are less precise or less complete, and often largely arbitrary.

The species problem has, in fact, two main aspects. One is conceptual, the other is practical. The conceptual aspect of the species problem is how the species can, or should be defined, provided that this question can be eventually answered to the general satisfaction of biologists and philosophers alike. The practical aspect is, how species are recognized by taxonomists working on the different groups of organisms and, most important, whether taxonomists can all agree on a single species concept, to be adopted as the universal currency in describing the diversity of life. A comparison of taxonomic practice as performed by leading specialists in a diversity of taxa, from mammals to fungi, from bacteria to flowering plants, has abundantly demonstrated that the entities called species in a group have little in common with the entities called species in another group (Claridge et al., 1997). Unfortunately, this heterogeneity is concealed under the (nearly) universal use of Linnaean binomens. It is thus all too easy to take taxonomic species as a set of broadly comparable units, of which we can make statistics for the most different purposes, e.g. biodiversity assessments and comparisons of extant or extinct faunas and floras. This practice should be best avoided (Minelli, 2000) but we do not have a real substitute for it; the global biodiversity estimates offered below are not exempt from this 'original sin.'

In this article I will focus on this practical aspect to the species problem, mostly taking examples from papers published in 2014: with this temporal restriction I only wish to stress the lively interest surrounding these questions. The relevant literature is enormous, and rapidly increasing with the increasing availability of morphological and especially molecular methods, and their massive application to the most diverse kinds of organisms. Enormous is also the literature about the conceptual aspects of the species problem, but I will only mention here two articles (Bernardi & Minelli, 2011; Mallett, 2013) to which I refer the interested reader and summarize in Table 1 the most important among the more than 20 different species concepts proposed to date.

HOW MANY SPECIES?

Even if we temporarily ignore the problems caused by the lack of a satisfactory species concept applicable to every kind of living things and thus simply frame the question in terms of taxonomic (named) species, it is difficult to say how many species we know at present and, still worse, how many species still await description. Estimates of 'valid' described species range between 1.5 million and 2 millions; a document issued in 2011 by the International Institute for Species Exploration gave a figure of 1,922,710 species as described throughout 2009.

Something, however, must be wrong with many of these estimates. In the last few decades, the number of new species described each year has been in the order of 17500 (International Institute for Species Exploration, 2012). This means that since 1985 about half a million new entries have been added to the list of described species. The net increase has been sensibly smaller, because of the number of nominal species that in the same time interval have been recognized to be just synonyms of other species. However, the net increase has been probably in the order of 350 000-400 000, whereas the most recent estimates of the number of described species are not correspondingly larger than the estimates produced 30 years ago.

In the last two decades of the XX century a number of papers offered estimates of the number of living species that still await description, one of the first and most often cited being May (1988). Some estimates were based on the percentage of undescribed species in small but dense samplings in areas and habitats with high diversity, e.g. Hodgkinson & Casson (1991) for tropical insects and Grassle & Maciolek (1992) for deep-sea animals. Other estimates included ecological considerations, such as the degree of hostplant specialization of phytophagous insects, as in Erwin's (1982) pioneering paper or Stork's (1988) revisitation of the same. One of the most recent papers on the subject adjusts the estimates to ~8.7 million (±1.3 million SE) eukaryotic species globally, of which ~2.2 million (±0.18 million SE) are marine (Mora et al., 2011); another, more sensible one (Scheffers et al., 2012) acknowledges the plurality of unknown or poorly known factors, as a consequence of which uncertainty remains between a global total as low as 2 million species, microbes excluded, and estimates as high as 50 millions and over.

PROLIFERATION OF NEW SPECIES

Strong catalysts favouring the description of new species are the new megajournals specifically devoted to taxonomy. In zoology, the leading role of Zootaxa and ZooKeys is by now unrivalled and undisputed. According to the journal's editor-in-chief, in 2010 Zootaxa contributed about 20% of all animal taxa described that year as new, that is, a number in the order of 4000 (Zhang, 2011).

Launched a few years after their zoological equivalents, Phytotaxa and PhytoKeys have been also rapidly growing and by now outcompete the biggest journals long established in the field. According to Zhang et al. (2014), the total number of new plant taxa described in 2011 was 6024 (of which 575 in Taxon, 473 in Phytotaxa, 183 in Novon, 169 in the Botanical Journal of the Linnean Society); in 2012, the total was 6647 (of which 632 in Phytotaxa, 465 in Systematic Botany, 340 in Phytoneuron, 301 in the Kew Bulletin, 267 in Taxon); in 2013 the number decreased to 5116 (of which 501 in Phytotaxa, 248 in PhytoKeys, 199 in Biodiversity Research and Conservation, 196 in the Botanical Journal of the Linnean Society). In discussing these numbers, it is necessary to consider that these include taxa proposed at any taxonomic rank.

Despite the large and largely unknown degree of uncertainty surrounding the estimates mentioned above, these are nevertheless important. Besides the fact that these figures help bringing the urgency of biodiversity conservation to the public attention, estimates of gaps of knowledge to be filled can stimulate targeted efforts aiming at filling them.

Some research groups are currently addressing this specific problem through well-planned field work in lesser investigated and species-rich areas, with special regard to hyperdiverse taxa such as weevils. For example, a German team, supported by local investigators in tropical areas, has recently produced a couple of excellent papers on the wingless weevils of the genus *Trigonopterus*.

Previous to the most recent researches, this genus included 91 described species ranging from Sumatra to Samoa and from the Philippines to New Caledonia. Of these, 50 species of *Trigonopterus* had been described from New Guinea, the center of the genus' diversity. But new targeted samplings in seven localities across New Guinea have resulted in the recognition of 279 *Trigonopterus* species, most of which new to science; of these, a first set of 101 species have been described by Riedel et al. (2013). Another 98 new species of *Trigonopterus* have been described in a paper (Riedel et al., 2014) devoted to materials recently collected in Indonesia (Sumatra, Java, Bali, Palawan, Lombok, Sumbawa, Flores), a large area from where only one species of *Trigonopterus* was previously known.

Perhaps less expected, there are also large numbers of undescribed species in the Lepidoptera, especially among the so-called micros. A recent study of the gelechioid genus *Ethmia* in Costa Rica revealed the presence of 22 undescribed species in addition to 23 described in the past (Phillips-Rodríguez et al., 2014).

Virtually unfathomed is, in some specialists' view, the world of Fungi, of which the number of existing species is estimated between 1.5 and 5 million, i.e. 15 to 50 times the number of currently described species. The wealth of undescribed fungal diversity is not limited to the microscopic forms: a recent study reported the identification of at least 126 species (and potentially up to 400) within a taxon of macrobasidiolichens currently regarded as one species (*Dictyonema glabratum* (Sprengel) D. Hawksw. also known as *Cora pavonia* E. Fries) (Lücking et al., 2014).

The use of new investigation tools such as barcoding (discussed below) is precious, indeed, in revealing the existence of a multiplicity of cryptic species hitherto shoehorned under one species name. I give here four examples, three of which from papers published last year.

In polychaetes, for examples, cryptic species crop up with virtually every accurate study. The detailed review published by Nygren (2014) includes several dozen examples, of which only the most conspicuous ones (those with ≥5 cryptic species inferred to be present within a taxon currently treated as a single species) are listed in Table 2. The taxonomic complexity revealed by this study is probably nothing more than the tip of a huge iceberg of species diversity in the annelids. Most of the cryptic diversity discovered to date in polychaetes is still formally undescribed, one of the few exceptions being the five species of *Archinome* listed in the Table.

Impressive are the results of some studies focusing on individual genera, where a systematic use of barcoding procedures has revealed an astonishing diversity of species, morphologically very uniform, as in some amphipods living in desert spring of the southern Great Basin of California and Nevada, USA, where 33 'provisional species' have been recognized within a clade hitherto referred to the one species, *Hyalella azteca* Saussure, 1858 (Witt et al., 2006).

A cornucopia of cryptic species, to use the words of the authors (Winterbottom et al., 2014) has been discovered in a DNA barcode analysis of the gobiid fish genus *Trimma*. Here, 473 specimens initially assigned to 52 morphological species revealed the presence of 94 genetic lineages separated by a sequence divergence usually typical of inter- rather than intraspecies differences.

To a quite smaller extent, but still worth mentioning here, new species are still being described at a sensible rate even in groups such as mammals, where a long tradition in taxonomy could be expected to have adequately accounted for extant species diversity. Taxonomic unrest is obviously larger in species-rich clades such as rodents or bats. For example, several new species of the bat genus *Miniopterus* have been recently described from Madagascar and the neighbouring Comoros archipelago, and at least seven out of the 18 species-level taxa recognized in the most recent study still require formal taxonomic treatment (Christidis et al., 2014).

TESTING THE BARCODE

"*In 2003, Paul Hebert, researcher at the University of Guelph in Ontario, Canada, proposed "DNA barcoding" as a way to identify species. Barcoding uses a very short genetic sequence from a standard part of the genome the way a supermarket scanner distinguishes products using the black stripes of the Universal Product Code (UPC). Two items may look very similar to the untrained eye, but in both cases the barcodes are distinct. [...] The gene region that is being used as the standard barcode for almost all animal groups is a 648 base-pair region in the mitochondrial cytochrome c oxidase 1 gene ("CO1"). COI is proving highly effective in identifying birds, butterflies, fish, flies and many other animal groups. COI is not an effective barcode region in plants because it evolves too slowly, but two gene regions in the chloroplast, matK and rbcL, have been approved as the barcode regions for plants*."

This is the way this technique is described, in very simple terms, in the official Barcode of Life website http://www.barcodeoflife.org/.

During the last few years, DNA barcoding has become a popular method for the identification of species. How efficient and reliable is it? The question can be reasonably asked in respect to groups and areas for which an exhaustive taxonomic treatment was already available, based on morphology, and the recent barcoding effort has covered a large percentage of the species recognized thus far.

In the case of insects, most published DNA barcoding studies focus on species of the Ephemeroptera (Ball et al., 2005; Ståhls & Savolainen, 2008), Trichoptera (Zhou et al., 2011), Lepidoptera (deWaard et al., 2009; Hausmann et al., 2011a, 2011b; Strutzenberger et al., 2011), Hymenoptera (Smith & Fisher, 2009; Zaldívar-Riverón et al., 2010) and Coleoptera (Raupach et al., 2010, 2011; Greenstone et al., 2011; Astrin et al., 2012; Woodcock et al., 2013).

Raupach et al. (2014) have recently tested the efficiency of DNA barcoding for the Heteroptera of Central Europe. Based on a conventional quantitative threshold currently accepted as a minimum molecular difference between two species, they found that species identification based on barcoding sequences is correct in a 91.5% of cases. In 21 cases, the molecular distance between two traditionally accepted species is lower (in ten cases, actually zero). To the contrary, intraspecific differences larger than the conventional species-level threshold have been found for 16 species traditionally regarded as valid. These results suggest that the barcode cannot be blindly accepted as a tool that allows quasi-automatic identification of all species, but at the same it turns to be a useful tool to discover taxa, or groups of closely related taxa, that are in need of in-depth revision. In particular, Raupach et al.'s study has provided evidence for ongoing hybridization events within various genera (e.g. *Nabis*, *Lygus*, *Phytocoris*) as well as the putative existence of cryptic species, e.g. within the aradid *Aneurus avenius* (Dufour, 1833) and the anthocorid *Orius niger* (Wolff, 1811).

Much larger success was obtained by Huemer et al. (2014) in the identification via barcode of 1004 species of Lepidoptera shared by two European countries, Austria and Finland, ca. 1600 km apart. Correct identification was possible for

98.8% of the taxa. However, deep intraspecific divergence, larger than the conventional threshold accepted as separating intra- from interspecific difference, was detected in as many as 124 taxonomic species hitherto recognized based on morphology. Authors concluded that despite the intensity of past taxonomic work on European Lepidoptera, nearly 20% of the species shared by Austria and Finland require further work to clarify their status.

The information obtained by systematically applying the barcoding method to groups for which traditional taxonomy is inadequate has different consequences. For example, this technique has been applied to the biting midges (Ceratopogonidae) of the county of Finnmark in northern Norway. Results indicated the presence of 54 species, of which 14 likely new to science, 16 new to Norway, and one new to Europe (Stur & Borkent, 2014). Another study involved a New World genus of Curculionidae (*Conotrachelus*). Two sets of specimens were compared, those emerged from some 17 500 seeds collected in six Central American rain forests and those collected in the same forests using interception traps that capture flying insects. Barcoding data suggested the presence of 17 species in the trapped samples, and 48 species among the specimens obtained from the attacked seeds. Little hope to use previous knowledge to identify them, however, as the barcoding of representatives of 24 species from museum collections provided matches for only three of the 17 species from the traps and no match at all for the putative 48 reared species (Pinzón-Navarro et al., 2010).

Overall, barcoding methods have proven much less informative for plants than the results obtained from animals would have allowed to hope. A near complete failure has been a study on willows (*Salix*) species, using two to seven plastid genome regions. Of the 71 Holarctic species in that study, only one has a unique barcode (Percy et al., 2014)!

THEORY-DRIVEN SPECIES INFLATION

This legitimate, welcome progress in the appreciation of species diversity in lesser investigated groups contrasts, to some extent, with a recent proliferation of 'new species' proposed by some authors in a revisitation of the taxonomy of popular mammal clades such as carnivores and ungulates. The theoretical background advocated by the zoologists responsible for this 'taxonomic inflation' is the phylogenetic species concept, according to which any arguably monophyletic and practically diagnosable lineage deserves to be considered (and eventually named) as a distinct species. With the increasing use in taxonomy of molecular techniques (e.g. barcoding), finding a differential trait between two populations, e.g. a single nucleotide difference, has become all too easy.

A first application to mammals of the phylogenetic species concept led Cracraft et al. (1998) to raise the Sumatran tigers to species status (*Panthera sumatrae* Pocock, 1929) based on three diagnostic sites in the mitochondrial cytochrome b gene. Shortly thereafter, Mazak & Groves (2006) added a third tiger species, the Javan tiger *P. sondaica* (Temminck, 1844), to the previously established *P. tigris* (Linnaeus, 1758) and *P. sumatrae*. Similarly, based on mtDNA and their analysis of morphological diagnosability, Groves & Grubb (2011) distinguished three species of European red deer: *Cervus elaphus* Linnaeus, 1758 (West European red deer), *C. pannoniensis* Banwell, 1997 (East European red deer) and *Cervus corsicanus* Erxleben, 1777 (Corsico-Sardinian and North-African red deer). Moreover, these are only a fraction of the total of 12 species recognized by these authors for the entire red deer/wapiti complex. Further examples of oversplitting caused by the application of the phylogenetic species concept include the 11 species of klipspringer recognized within one traditional species, *Oreotragus oreotragus* (Zimmermann, 1783), based on size differences and different sexual dimorphism, and the splitting of the mainland serow *Capricornis sumatraensis* (Bechstein, 1799) into six species (Groves & Grubb, 2011). Zachos et al. (2013), who are very critical of this trend in mammal taxonomy, acknowledge however that in other groups more than one species must be in fact recognized, as in the case of the African elephants (the forest elephant *Loxodonta cyclotis* Matschie, 1900 and the savanna elephant *Loxodonta africana* (Blumenbach, 1797); cf. Rohland et al., 2010), and the giraffe, within which six or more distinct species should be probably recognized (Groves & Grubb, 2011).

Agamospecies Concept	an operational, morphologically defined unit in organisms that reproduce asexually or by uniparental reproduction (without fertilization)	Cain (1954)
Biological Species Concept	a group of interbreeding natural populations, reproductively isolated from other similar groups	Dobzhansky (1935, 1937, 1970), Mayr (1940, 1942, 1963, 1970), Mayr & Ashlock, 1991)
Cladistic Species Concept	a group of organisms bounded by two events of speciation or by a speciation and an extinction event	Ridley (1989)
Cohesion Species Concept	the most inclusive group of organisms within which genetic and/or demographic exchange can occur	Templeton (1989)
Ecological Species Concept	a set of populations isolated through occupation of a specific ecological niche	Van Valen (1976).
Evolutionary Species Concept	an evolutionary lineage of populations in ancestor-descendant relationship, that maintains its identity vs. other lineages so defined, and with its own specific evolutionary trends and historical destiny	Simpson (1951, 1961)
Genetic Species Concept	the largest reproductive community of sexual interfertile individuals that share a common gene pool; or a field for gene recombination	Dobzhansky (1950), Carson (1957)
Hennigian Species Concept	a reproductively isolated natural population, or group of natural populations, issued from the dissolution of a stem species in a speciation event, that ceases to exist for extinction or speciation	Meier & Willmann (2000)
Least Inclusive Taxonomic Unit	a taxonomic group defined on the basis of apomorphies	Pleijel & Rouse (1999), Pleijel (2000).
Morphological Species Concept	a community or a number of related communities, whose distinctive morphological characters are, in the opinion of a competent systematist, sufficiently defined to qualify it or them with a specific name	Regan (1926)
Phylogenetic Species Concept - diagnosable version	the smallest diagnosable grouping of organisms, within which there is a pattern of ancestor-descendant relationship	Cracraft(1983)

Phylogenetic Species Concept - monophyly version	a monophyletic group of individuals characterized by one or more autapomorphies	Rosen (1978), De Queiroz & Donoghue (1988)
Phenetic Species Concept	a set of organisms that are phenotypically similar and that look different from other sets of organisms	Sneath (1976)
Recognition Species Concept	a group of organisms that share a common fertilization system, or better, a Specific Mate Recognition System	Paterson (1979, 1985)

Table 1. A selection of species concepts, with short definitions, mainly in accordance with Bernardi & Minelli (2011) and Mallett (2013), and some key references. Concepts that specifically apply to extinct organisms (the Successional Species Concept in the two versions: George's (1956) Chronospecies Concept, and Simpson's (1961) Paleospecies Concept) are not included.

Current taxon name(s)	**Inferred number of species**
Archinome jasoni Borda et al., 2013, *A. tethyana* Borda et al., 2013, *A. levinae* Borda et al., 2013, *A. rosacea* (Blake, 1985), *A. storchi* Fiege et Bock, 2009	**5**
Branchiomma spp.	**11**
Capitella capitata (Fabricius, 1780)	**12+**
Eumida sanguinea (Örsted, 1843)	**11**
Harmothoe imbricata (Linnaeus, 1767)	**6**
Leitoscoloplos pugettensis (Pettibone, 1957)	**5**
Marenzelleria viridis (Verrill, 1873), *M. bastropi* Bick, 2005, *M. neglecta* Sikorski et Bick, 2004, *M. wireni* Augener, 1913, *M. arctia* (Chamberlin, 1920)	**5**
Marphysa sanguinea (Montagu, 1815)	**5**
Ophryotrocha labronica Bacci et La Greca, 1961	**14**
Owenia fusiformis Delle Chiaje, 1844	**5**
Palola spp.	**16**
Sabellastarte spp.	**7**
Scoloplos armiger (Müller, 1776)	**5–6**
Syllis alternata Moore, 1908	**5**

Table 2. Cryptic diversity revealed in some polychaete 'species' taxa by recent molecular investigations (data compiled from Nygren, 2014, Table S1).

TRICKY SPECIES COMPLEXES

Better investigated groups reveal a complexity of interrelationship within which any formal taxonomic arrangement is likely to remain provisional, or at least arbitrary. Species complexes are particularly intractable when the reproductive behavior of some of the forms involved deviates from the typical biparental scheme. Exemplary in this respect is the complex of the European green frogs, which includes a number of hybridogenetic entities whose survival strictly depends on an uninterrupted availability of sperm from a closely related biparental species, as in the case of the Edible Frog, i.e. the hybridogenetic *Pelophylax* klepton *esculentus* (Linnaeus, 1758). This hybrid between the Pool Frog *Pelophylax lessonae* (Camerano, 1882) and the Marsh Frog *Pelophylax ridibundus* (Pallas, 1771) is fertile, but usually unable to produce balanced gametes of the two sorts, whereas it usually survives by female hybrids mating with males of one of the parental species, usually *P. lessonae* (e.g., Spolsky & Uzzell, 1986; Christiansen, 2009). Local conditions are indeed extremely diverse and are hardly amenable at a conventional taxonomic treatment. In Central and Western Europe the hybrid *P. esculentus* lives in sympatry with the parental species *P. lessonae* (LE-system), but there are also gamete-exchanging systems of *P. ridibundus/P. esculentus* (RE) and *P. ridibundus/ P. lessonae/P. esculentus* mixed populations (RLE) (reviewed by Günther, 1991; Plötner, 2005), and also rare all-hybrid populations (EE-system) reproductively independent of the parental forms (Graf & Polls Pelaz, 1989) but dependent for sperm on the presence of triploid individuals; the latter are obtained when diploid eggs produced by diploid hybrid females (LR) are fertilized by haploid sperm of diploid or triploid males (LR, LLR, LRR) (Arioli et al., 2010).

The taxonomic treatment of uniparental organisms is generally difficult and controversial. Linnaean species are quite pacifically recognized in some groups, e.g. in bdelloid rotifers, but in this group thelytokous parthenogenesis is a very old phenomenon and a number of largely fixed differences among strains have been fixed, that allow recognizing species- and genus-level taxa around which there is not much dispute. Things are different in groups where parthenogenesis, or apomyxis, is a recent phenomenon and phenotypic differences between clonal strains are much more subtle and their taxonomic evaluation much more subjective. In the case of brambles (*Rubus* spp.) and dandelions (*Taraxacum* spp.) thousands of names have been introduced to accommodate slightly divergent phenotypes at what some specialists consider the taxonomic rank of species. In many instances, however, uniparental reproduction is accompanied by variation in ploidy level and/or by morphological and molecular distances comparable to those ordinarily existing between related bisexual species, or even larger. An interesting example has been recently illustrated by Marotta et al. (2014) in the freshwater oligochaetes of the genus *Tubifex*. Despite the occurrence of different reproductive mechanisms (biparental reproduction vs. thelytoky), many populations referable to this genus have been traditionally classified as a single species *Tubifex tubifex* (Müller, 1774). Under this name, however, is concealed an unexpected diversity, as suggested by a careful karyological and molecular analysis of samples collected in just one limited area, the Lambro River near Milano. Alongside a diploid form, for which a distinct name (*T. blanchardi* Vejdovský, 1891) is available in the literature, the authors found several polyploid lines (3n, 4n, 6n), with karyological differences matching with large molecular divergence in the 16S rRNA and COI sequences. It will be no surprise if this diversity will eventually emerge as just the tip of a still unfathomed iceberg.

The identification of gene flow between related species is very important when taxa of economic and especially medical or veterinary importance are involved. Fontaine et al. (2015; see also Clark & Messer, 2015) have recently demonstrated introgression in a medically important group of sibling species of Afrotropical mosquitos (*Anopheles gambiae* Giles, 1902, *A. coluzzii* Coetzee et al., 2013 and *A. arabiensis* Patton, 1905) that differ in behaviour and thus in medical importance. Allele exchanges between these malaria vectors have been found to involve most of their autosomal genes, it is therefore possible that traits enhancing vectorial capacity may be gained through interspecific gene flow.

SPECIATION

Sooner or later, the taxonomist must confront the issue of speciation, traditionally a focal issue in evolutionary biology, thus basically approached through the tools of population genetics. Eventually, even the good practicing taxonomist who is happy applying Regan's (1926) morphological species concept (cf. Table 1) is brought by the intricacies of his/her study material to admit how right was Darwin when he acknowledged that "*No line of demarcation can be drawn between species … and varieties*" (Darwin, 1859, p. 469). It is beyond the scope of this article to present here even a short summary of current awareness, and current debates, on the issue of speciation. The interested reader is referred to Coyne & Orr's (2004) monograph, which is both a synthesis of modern understanding of speciation problems, a guide to older literature and a solid background against which to read the literature of the last decade. I will thus skip the traditional main issues, beginning with the geographic scenarios of speciation (allopatric, parapatric, sympatric). I will only glean from the very recent literature some exemplary cases that show how cautious should be the taxonomist in front of the temporal and spatial change to which natural populations are subjected. The more we know about these aspects, the more critical should be our attitude towards a taxonomic delimitation of species.

A first warning concerns the tempo of evolution. An unwarranted generalization of Darwin's depiction of evolution as proceeding through the gradual accumulation of changes happening at a very slow and essentially uniform pace led in the past to assume that a speciation event should take on the average some hundred thousand years or more. There is no reason, however, for us to expect that living nature adopts an essentially uniform pace of change. Indeed, we have now well-documented proofs of very rapid speciation events, and also of extremely conservative species pairs whose remote splitting is concealed under an amazing degree of morphological stasis. As a consequence, the taxonomist must be cautious in inferring relatedness from morphological, ecological or biogeographic evidence without the further support of molecular estimates of divergence times.

Consider, for example, that the divergence between two species of amphioxus, both currently classified in the same genus, *Branchiostoma floridae* Hubbs, 1922 and *B. lanceolatum* (Pallas, 1774), has been estimated at 186–189 million years (Cañestro et al., 2002), whereas the origin of the whole radiation of extant Brassicaceae (3709 species; Warwick et al., 2006) is probably not older than 40 million years (Couvreur et al., 2010; Franzke et al., 2011), and perhaps even younger, around 16 million years (Franzke et al., 2009). This can be compared to the 22.4 million years through which the hummingbirds (338 living species) have been apparently radiating from their last common ancestor (McGuire et al., 2014). Still very long times, indeed, if compared to the 100 000 years, or so, within which the cichlids of Lake Victoria have radiated into a species flocks of five hundred species at least (Verheyen et al., 2003; Genner et al., 2007).

GENES INVOLVED IN SPECIATION

Research on the genes more directly involved in speciation is attracting increasing interest, but convincing generalizations are still difficult to obtain.

Problems in fixing the boundary between two closely related taxa that broadly, but not completely exhibit the character of distinct species are often due to the fact that some parts of their genome are more readily and extensively affected by introgression, whereas other parts are much more resilient. A classic case – Carrion Crow (*Corvus corone* Linnaeus, 1758) vs. Hooded Crow (*Corvus cornix* Linnaeus, 1758) – has been carefully investigated by Poelstra et al. (2014). These authors have found that only a small number of narrow genomic islands are not affected by gene flow. As mirrored by these birds' livery, gene expression divergence between them is concentrated in pigmentation genes expressed in gray versus black feather follicles. Despite its limited genetic basis, this trait is critically important, however, as it affects mate choice and thus color-mediated prezygotic isolation.

In pairs of stick insect populations adapted to different host plants and undergoing parallel speciation, Soria-Carrasco et al. (2014) found thousands of small genomic regions, most of which unique to individual population pairs, to be significantly diverging between populations. These authors have also detected parallel genomic divergence across population pairs involving an excess of coding genes with specific molecular functions.

STABILITY OF SPECIES IN THE FACE OF INTROGRESSION

While the existence of introgression between locally sympatric related species is well documented in a large number of animals and plant species pairs, very little is known about the long-term effects of a gene flow continuing over centuries. A recent study of two widely hybridizing tree species, the white spruce (*Picea glauca* (Moench) Voss) and Engelmann spruce (*P. engelmannii* Parry ex Engelm.) in western North America, suggests that these two species have a long history of hybridization and introgression, dating to at least 21 000 years ago, nevertheless they still maintain their distinct species identity (De La Torre et al., 2014).

The boundaries between closely related species are sometimes permeable in one direction only. For example, brown bear (*Ursus arctos* Linnaeus, 1758) and polar bear (*Ursus maritimus* Phipps, 1774) are genetically distinct, but evidence of polar bear genes has been found in the brown bear population of the Admiralty, Baranof and Chicagof Islands off Alaska, whereas no evidence of brown bear genes has been found in the local polar bear population (Cahill et al., 2015). Another example of asymmetric introgression has been recently described between a pair of freshwater fish, the North American darters *Etheostoma caeruleum* Storer, 1845 and *Etheostoma spectabile* (Agassiz, 1854) (Zhou & Fuller, 2014).

HYBRIDIZATION

Opportunities for hybridization between closely related biological species are not restricted to species pairs that have being diverging only in recent time, witness a fern from the French Pyrenees (*Cystocarpium* x *roskamianum* Fraser-Jenk), a recently formed hybrid whose parental lineages diverged from each other ca. 60 million years ago, and are currently classified in different genera (*Cystopteris* and *Gymnocarpium*) (Rothfels et al., 2015).

Due to both climatic and biological reasons, hybrid zones are not fixed in space. Detailed evidence of moving hybrid zones has summarized by Buggs (2007) for the following pairs of taxa (nomenclature updated where necessary):

MAMMALIA
Cervus nippon nippon Temminck, 1838 - *Cervus elaphus* Linnaeus, 1758

AVES
Poecile carolinensis (Audubon, 1834) - *Poecile atricapillus* (Linnaeus, 1766)
Hippolais polyglotta (Vieillot, 1817) - *Hippolais icterina* (Vieillot, 1817)
Vermivora pinus (Linnaeus, 1766) - *Vermivora chrysoptera* (Linnaeus, 1766)
Corvus corone corone Linnaeus, 1758 - *Corvus corone cornix* Linnaeus, 1758
Quiscalus quiscula quiscula (Linnaeus, 1758) - *Quiscalus quiscula versicolor* Vieillot, 1819

SQUAMATA
Pholidobolus montium (Peters, 1863) - *Pholidobolus affinis* (Peters, 1863)
Sceloporus tristichus (Cope, 1875) - *Sceloporus cowlesi* Lowe et Norris, 1956

AMPHIBIA
Pseudophryne bibroni Günther, 1859 - *Pseudophryne semimarmorata* Lucas, 1892
Triturus cristatus Laurenti, 1768 - *Triturus marmoratus* (Latreille, 1800)
Plethodon glutinosus (Green, 1818) - *Plethodon jordani* Blatchley, 1901

OSTEICHTHYES
Pseudorasbora parva (Temminck et Schlegel, 1846) - *Pseudorasbora pumila* Miyadi, 1930

INSECTA
Heliconius hydara Hewitson, 1867 - *Heliconius erato petiverana* (E. Doubleday, 1847)
Anartia fatima (Fabricius, 1793) - *Anartia amathea* (Linnaeus, 1758)
Solenopsis invicta Buren, 1972 - *Solenopsis richteri* Forel, 1909
Orchelimum nigripes Scudder, 1875 - *Orchelimum pulchellum* Davis, 1909
Allonemobius socius (Scudder, 1877) - *Allonemobius fasciatus* (De Geer, 1773)
Limnoporus dissortis (Drake et Harris, 1930) - *Limnoporus notabilis* (Drake et Hottes, 1925)
Geomydoecus aurei Price et Hellenthal, 1981 - *Geomydoecus centralis* Price et Hellenthal, 1981

CRUSTACEA

Orconectes rusticus (Girard, 1852) - *Orconectes propinquus* (Girard, 1852)

ANGIOSPERMAE

Helianthus annuus L. - *Helianthus bolanderi* A. Gray

Mercurialis annua L. diploid - *Mercurialis annua* L. hexaploid

Occasionally, the peculiar geographical distribution of a set of populations offers the opportunity to investigate different stages of an ongoing speciation process. This happens with the so-called ring species, where the two extremes, say A and E, of a series of progressively differentiated populations have recently come in contact but fail to interbreed. This happens generally when the whole complex is distributed, ring-like, around an inhospitable area, such as very high mountains, or an exceedingly arid area. Ring species are extremely rare in plants: recently, Cacho & Baum (2012) have presented the Caribbean slipper spurge (*Euphorbia tithymaloides*) as the first example among the flowering plants. More numerous are the zoological examples, as summarized by Irwin et al. (2001). These authors listed seventeen examples where the populations at the opposite ends of the chain overlap without any sign of hybridization, or nearly so. In many cases the two extreme forms have been given distinct specific names, whereas in other cases taxonomists still treat all the populations involved in the ring as belonging to the same Linnaean species: one example, among a number of possible ones, of the danger of inferring evolutionary status from simply considering the current taxonomic status (i.e., the nomenclature) of a set of populations.

Irwin et al.'s (2001) list includes a number of birds: Crested Honey-buzzard *Pernis ptilorhyncus* (Temminck, 1821) and Barred Honey-buzzard *P. celebensis* Wallace, 1868; Herring Gull *Larus argentatus* Pontoppidan, 1763 and Lesser Black-backed Gull *L. fuscus* Linnaeus, 1758 (with some hybridization); Ringed Plover *Charadrius hiaticula* Linnaeus, 1758 and Semipalmated Plover *C. semipalmatus* Bonaparte, 1825; Collared Kingfisher *Todiramphus chloris* (Boddaert, 1783) and Micronesian Kingfisher *T. cinnamominus* (Swainson, 1821); Eurasian Skylark *Alauda arvensis* Linnaeus, 1758, Japanese Skylark *A. japonica* Temminck et Schlegel, 1848 and Oriental Skylark (*A. gulgula* Franklin, 1831); Greenish Warbler *Phylloscopus trochiloides* (Sundevall, 1837); Chiffchaff *Phylloscopus collybita* (Vieillot, 1817) and Mountain Chiffchaff (*P. sindianus* W. E. Brooks, 1880); Sulawesi Triller *Lalage leucopygialis* Walden, 1872, Pied Triller *L. nigra* (J. R. Forster, 1781), and White-shouldered Triller *L. sueurii* (Vieillot, 1818); Brown Thornbill *Acanthiza pusilla* (Shaw, 1790) and Tasmanian Thornbill *A. ewingii* Gould, 1844; Large Tree-finch *Camarhynchus psittacula* Gould, 1837 and Medium Tree-finch *C. pauper* Ridgway, 1890.

The other taxa in the list are rodents (Deer Mouse *Peromyscus maniculatus* (Wagner, 1845); Pocket Mice *Perognathus amplus* Osgood, 1900 and *P. longimembris* (Coues, 1875), a bee *Hoplitis producta* (Cresson, 1864), a group of butterflies *Junonia coenia* Hübner, [1822] and *J. genoveva* (Cramer, 1780)/*J. evarete* (Cramer, 1782)) and a fruit fly (*Drosophila paulistorum* Dobzhansky et Pavan, 1949).

In the case of the salamander *Ensatina eschscholtzii* Gray, 1850, some hybridization between the end forms of the ring has been reported, and past but still recognizable hybridization has been found in the ring of the Japanese pond frogs *Rana nigromaculata* Hallowell, 1861 and *R. brevipoda* Ito, 1941.

In still other cases, there is no reproductive isolation between the two, now overlapping, terminal forms of the ring; as a consequence, a hybrid zone is formed. The cases listed by Irwin et al. (2001) include birds Crimson Rosella *Platycercus elegans* (Gmelin, 1788), Adelaide Rosella *P. adelaidae* Gould, 1841, Yellow Rosella *P. flaveolus* Gould, 1837, Great Tit *Parus major* Linnaeus, 1758, a mammal House Mouse *Mus musculus* Linnaeus, 1758 and two millipedes *Rhymogona silvatica* (Verhoeff, 1894) and *R. cervina* (Verhoeff, 1910).

Several ring species (putative ones as well as confirmed ones) have been extensively studied over the last few years. No wonder, the actual interrelationships among the involved populations are often more complex than in the simple model outlined above. For example, in the case of the Greenish Warbler *Phylloscopus trochiloides* (Sundevall, 1837) species complex Alcaide et al. (2014) have recently revisited the status, and the history, of the ring of populations distributed around Tibet. The two extreme, reproductively isolated forms co-existing in central Siberia are connected through a southern chain of populations showing a gradient of genetic and phenotypic traits. The authors demonstrate that the gene flow has been interrupted in the past at more than one location around the ring, whereas the two Siberian forms have occasionally interbred. Eventually, this little bird displays a continuum from slightly divergent contiguous populations to almost fully reproductively isolated species.

RETICULATION

Patterns of hybridization and introgression among closely related taxa take sometimes a reticulated structure. A recently investigated example involving the biogeographical history of the Eurasian species of *Fraxinus* has revealed the occurrence of an ancient reticulation between European and Asian species as well as other ancient reticulation events between *F. angustifolia* Vahl and *F. excelsior* L. and the other species of the section *Fraxinus*. Some of these events would have occurred during the Miocene, following climatic variations that may have led these species to expand their distribution range, eventually coming into contact (Hinsinger et al., 2014).

SPECIATION REVERSED

Incomplete speciation and ongoing gene flux between partially isolated populations may cause divergence to be stopped and even reversed. Well-documented cases of reversed speciation are, however, very limited. An example has been described by Bhat et al. (2014) for the European whitefish *Coregonus lavaretus* (Linnaeus, 1758), of Lake Skrukkebukta in Northern Norway. This freshwater fish is highly polymorphic and in several lakes it has independently differentiated into sympatric morphs that specialize on different food (plankton vs. benthos) and are to some extent reproductively isolated and genetically differentiated. In 1993, Lake Skrukkebukta was invaded by another *Coregonus* species, the vendace *Coregonus albula* (Linnaeus, 1758). A zooplanktivorous specialist, this fish displaced the planktivorous whitefish from the pelagic niche pushing it into the benthic habitat already inhabited by the benthivorous whitefish morphs. As a consequence, within three generations (15 years) the genetic differentiation between the two whitefish morphs has dramatically dropped: the invasion of a superior trophic competitor has thus caused incipient speciation to reverse. An overview of cases of speciation reversal was provided a few years ago by Seehausen et al. (2008).

REFERENCES

Alcaide M., Scordato E.C., Price T.D. & Irwin D.E., 2014. Genomic divergence in a ring species complex. Nature, 511: 83–85.

Arioli M., Jakob C. & Reyer H.U., 2010. Genetic diversity in water frog hybrids (*Pelophylax esculentus*) varies with population structure and geographic location. Molecular Ecology, 19: 1814–1828.

Astrin J.J., Stüben P.E., Misof B., Wägele J.W., Gimnich F., Raupach, M.J. & Ahrens, D., 2012. Exploring diversity in cryptorhynchine weevils (Coleoptera) using distance-, character- and tree-based species delineation. Molecular Phylogenetics and Evolution, 63: 1–14.

Ball S.L., Hebert P.D.N., Burian S.K. & Webb J.M., 2005. Biological identifications of mayflies (Ephemeroptera) using DNA barcodes. Journal of the North American Benthological Society, 24: 508–524.

Bernardi M. & Minelli A., 2011. Il concetto di specie e la paleontologia: una rassegna introduttiva. Rendiconti Online della Società Geologica Italiana, Volume speciale 13: 1–24.

Bhat S., Amundsen P.-A., Knudsen R., Gjelland K.Ø., Fevolden S.-E., Bernatchez L. & Præbel K., 2014. Speciation reversal in European whitefish (*Coregonus lavaretus* (L.)) caused by competitor invasion. PLoS ONE, 9(3): e91208.

Buggs R.J.A., 2007. Empirical study of hybrid zone movement. Heredity, 99: 301–312.

Cacho N.I. & Baum D.A., 2012. The Caribbean slipper spurge *Euphorbia tithymaloides*: the first example of a ring species in plants. Proceedings of the Royal Society B, 279: 3377–3383.

Cahill J.A., Stirling I., Kistler L., Salamzade R., Ersmark E., Fulton T.L., Mathias Stiller M., Green R.E. & Shapiro B., 2015. Genomic evidence of geographically widespread effect of gene flow from polar bears into brown bears. Molecular Ecology, DOI: 10.1111/mec.13038.

Cain A.J., 1954. Animal species and their evolution. Hutchinson University Library, London, ix+190 pp.

Cañestro C., Albalat R., Hjelmqvist L., Godoy L., Jörnvall H. & Gonzalez-Duarte R., 2002. Ascidian and amphioxus Adh genes correlate functional and molecular features of the ADH family expansion during vertebrate evolution. Journal of Molecular Evolution, 54: 81–89.

Carson L.H., 1957. The species as a field for gene recombination. In: E. Mayr, Editor, The species problem. American Association for the Advancement of Science, Washington: 23–38.

Christiansen D.G., 2009. Gamete types, sex determination and stable equilibria of all-hybrid populations of diploid and triploid edible frogs (*Pelophylax esculentus*). BMC Evolutionary Biology, 9: 135.

Christidis L., Goodman S.M., Naughton K., Appleton B., 2014. Insights into the evolution of a cryptic radiation of bats: dispersal and ecological radiation of Malagasy *Miniopterus* (Chiroptera: Miniopteridae). PLoS ONE, 9(3): e92440.

Claridge M.F., Dawah H.A. & Wilson M.R. (Eds.), 1997. The species: the units of biodiversity. Chapman & Hall, London: xvi+439 pp.

Clark A.G. & Messer P.W., 2015. Conundrum of jumbled mosquito genomes. Science, 347: 27–28.

Couvreur T.L.P., Franzke A., Al-Shehbaz I.A., Bakker F.T., Koch M.A. & Mummenhoff K., 2010. Molecular phylogenies, temporal diversification, and principles of evolution in the mustard family (Brassicaceae). Molecular Biology and Evolution, 27: 55–71.

Coyne J.A. & Orr H.A., 2004. Speciation. Sinauer Associates, Sunderland, MA: xiii+545 pp.

Cracraft J., 1983. Species concepts and speciation analysis. Current Ornithology, 1: 159–187.

Cracraft J., Feinstein J., Vaughn J. & Helm-Bychowski K., 1998. Sorting out tigers (*Panthera tigris*): mitochondrial sequences, nuclear inserts, systematics, and conservation genetics. Animal Conservation, 1: 139–150.

Darwin C., 1859. On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life. Murray, London: x+502 pp.

De La Torre A.R., Roberts D.R. & Aitken S.N., 2014. Genome-wide admixture and ecological niche modelling reveal the maintenance of species boundaries despite long history of interspecific gene flow. Molecular Ecology, 23: 2046–2059.

De Queiroz K. & Donoghue M.J., 1988. Phylogenetic systematics and the species problem. Cladistics, 4: 317–338.

deWaard J.R., Landry J.-F., Schmidt B.C., Derhoussoff J., McLean J.A. & Humble L.M., 2009. In the dark in a large urban park: DNA barcodes illuminate cryptic and introduced moth species. Biodiversity and Conservation, 18: 3825–3839.

Dobzhansky T., 1935. A critique of the species concept in biology. Philosophy of Science, 2: 344–355.

Dobzhansky T., 1937. Genetics and the origin of species. Columbia University Press, New York, xvi+364 pp.

Dobzhansky T., 1950. Mendelian populations and their evolution. American Naturalist, 84: 401–418.

Dobzhansky T., 1970. Genetics of the evolutionary process. Columbia University Press, New York, ix+505 pp.

Erwin T.L., 1982. Tropical forests: their richness in Coleoptera and other arthropod species. Coleopterists Bulletin, 36: 74–75.

Fontaine M.C., Pease J.B., Steele A., Waterhouse R.W., Neafsey D.E., Sharakhov I.V., Jiang X., Hall A.B., Catteruccia F., Kakani E., Mitchell S.N., Wu Y.-C., Smith H.A., Love R.R., Lawniczak M.K., Slotman M.A., Emrich S.J., Hahn M.W. & Besansky N.J., 2015. Extensive introgression in a malaria vector species complex revealed by phylogenomics. Science, 347: 1258524.

Franzke A., German D., Al-Shehbaz I.A. & Mummenhoff K., 2009. *Arabidopsis* family ties: molecular phylogeny and age estimates in the Brassicaceae. Taxon, 58: 425–437.

Franzke A., Lysak M.A., Al-Shehbaz I.A., Koch M.A. & Mummenhoff K, 2011. Cabbage family affairs: the evolutionary history of Brassicaceae. Trends in Plant Science, 16: 108–116.

Genner M.J., Seehausen O., Lunt D.H., Joyce D.A., Shaw P.W., Carvalho G.R. & Turner G.F., 2007. Age of cichlids: new dates for ancient lake fish radiations. Molecular Biology and Evolution, 24: 1269-1282.

George T.N., 1956. Biospecies, chronospecies and morphospecies. In: P.C. Sylvester-Bradley, Editor, The species concept in paleontology. The Systematics Association, London: 123–137.

Graf J.-D. & Polls Pelaz M., 1989. Evolutionary genetics of the *Rana esculenta* complex. In: R.M. Dawley & J.P. Bogart (Eds.), Evolution and ecology of unisex vertebrates. New York State Museum, Albany, NY: 289–302.

Grassle J.F. & Maciolek N.J., 1992. Deep-sea species richness: regional and local diversity estimates from quantitative bottom samples. American Naturalist, 139: 313–341.

Greenstone M.H., Vandenberg N.J. & Hu J.H., 2011. Barcode haplotype variation in North American agroecosystem lady beetles (Coleoptera: Coccinellidae). Molecular Ecology Resources, 11: 629–637.

Groves C. & Grubb P., 2011. Ungulate taxonomy. The Johns Hopkins University Press, Baltimore: ix+317 pp.

Günther R., 1991. Europäische Wasserfrösche (Anura, Ranidae) und biologisches Artkonzept. Mitteilungen aus dem Zoologischen Museum in Berlin, 67: 39–53.

Hausmann A., Haszprunar G. & Hebert P.D.N., 2011a. DNA barcoding the geometrid fauna of Bavaria (Lepidoptera): successes, surprises, and questions. PLoS ONE, 6: e17134.

Hausmann A., Haszprunar G., Segerer A.H., Speidel W., Behounek G. & Hebert P.D.N., 2011b. Now DNA-barcoded: the butterflies and larger moths of Germany (Lepidoptera: Rhopalocera, Macroheterocera). Spixiana, 34: 47–58.

Hinsinger D.D., Gaudeul M., Couloux A., Bousquet J., Frascaria-Lacoste N., 2014. The phylogeography of Eurasian *Fraxinus* species reveals ancient transcontinental reticulation. Molecular Phylogenetics and Evolution, 77: 223–237.

Hodkinson I.D. & Casson D., 1991. A lesser predilection for bugs: Hemiptera (Insecta) diversity in tropical rain forests. Biological Journal of the Linnean Society, 43: 101–109.

Huemer P., Mutanen M., Sefc K.M. & Hebert P.D.N., 2014. Testing DNA barcode performance in 1000 Species of European Lepidoptera: large geographic distances have small genetic impacts. PLoS ONE, 9(12): e115774.

International Institute for Species Exploration, 2011. SOS – State of Observed Species. Arizona State University, Tucson, AZ; available at http://www.esf.edu/species/SOS.htm

International Institute for Species Exploration, 2012. Retro SOS 2000-2009. Arizona State University, Tucson, AZ; available at http://www.esf.edu/species/SOS.htm

Irwin D.E., Irwin J.H. & Price T. D., 2001. Ring species as bridges between microevolution and speciation. Genetica, 112-113: 223-243.

Linnaeus C., 1758. Systema Naturae per regna tria Naturae secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Editio decima, reformata. Laurentius Salvius, Holmiae, Tomus I: [4]+1-823+[1] pp.; Tomus II: [4]+825–1384 pp.

Lücking R., Dal-Forno M., Sikaroodi M., Gillevet P.M., Bungartz F., Moncada B., Yánez-Ayabaca A., Chaves J.L., Coca L.F. & Lawrey J.D., 2014. A single macrolichen constitutes hundreds of unrecognized species. Proceedings of the National Academy of Science of the United States of America, 111: 11091–11096.

Mallet J., 2013. Species, concepts of. In: S.A. Levin, Editor, Encyclopedia of biodiversity, Volume 6. Waltham, MA, Academic Press: 679–691.

Marotta R., Crottini A., Raimondi E., Fondello C. & Ferraguti M., 2014. Alike but different: the evolution of the T*ubifex tubifex* species complex (Annelida, Clitellata) through polyploidization. BMC Evolutionary Biology, 14:73.

May R.M., 1988. How many species are there on earth? Science, 241: 1441–1449.

Mayr E., 1940. Speciation phenomena in birds. American Naturalist, 74: 249–278.

Mayr E., 1942. Systematics and the origin of species from the viewpoint of a zoologist. Columbia University Press, New York: xiv+334 pp.

Mayr E., 1963. Animal species and evolution. The Belknap Press of Harvard University Press, Cambridge: xiv+797 pp.

Mayr E., 1970. Populations, species, and evolution: an abridgment of animal species and evolution. The Belknap Press of Harvard University Press, Cambridge: xv+453 pp.

Mayr E. & Ashlock P.D., 1991. Principles of systematic zoology. McGraw-Hill, New York: xx+475 pp.

Mazak J.H. & Groves C.P., 2006. A taxonomic revision of tigers (*Panthera tigris*) of Southeast Asia. Mammalian Biology, 71: 268–287.

McGuire J.A., Witt C.C., Remsen J.V. Jr, Corl A., Rabosky D.L. Altshuler D.L. & Dudley R., 2014. Molecular phylogenetics and the diversification of hummingbirds. Current Biology, 24: 1–7.

Meier R. & Willmann R., 2000. The Hennigian species concept. In: Q.D. Wheeler, R. Meier, Editors, Species concepts and phylogenetic theory: a debate. Columbia University Press, New York: 30–43.

Minelli A., 2000. The ranks and the names of species and higher taxa, or, a dangerous inertia of the language of natural history. In: M.T. Ghiselin & A.E. Leviton, editors, Cultures and institutions of natural history. Essays in the history and philosophy of science. California Academy of Sciences, San Francisco: 339–351.

Mora C., Tittensor D.P., Adl S. & Simpson A.G.B., 2011. How many species are there on earth and in the ocean? PLoS Biology, 9 e1001127.

Nygren A., 2014. Cryptic polychaete diversity: a review. Zoologica Scripta, 43: 172–183.

Paterson H.E.H., 1979. A comment on 'mate recognition systems.' Evolution, 34: 330-331.

Paterson H.E.H., 1985. The recognition concept of species. In: E.S. Vrba, Editor, Species and speciation (Transvaal Museum Monograph 4). Transvaal Museum, Pretoria: 21–29.

Percy D.M., Argus G.W., Cronk Q.C., Fazekas A.J., Kesanakurti P.R., Burgess K.S., Husband B.C., Newmaster S.G., Barrett S.C.H. & Graham S.W., 2014. Understanding the spectacular failure of DNA barcoding in willows (*Salix*): does this result from a trans-specific selective sweep? Molecular Ecology, 23: 4737–4756.

Phillips-Rodríguez E., Powell J.A., Hallwachs W. & Janzen D.H., 2014. A synopsis of the genus *Ethmia* Hübner in Costa Rica: biology, distribution, and description of 22 new species (Lepidoptera, Gelechioidea, Depressariidae, Ethmiinae), with emphasis on the 42 species known from Área de Conservación Guanacaste. ZooKeys, 461: 1–86.

Pinzón-Navarro S., Barrios H., Múrria C., Lyal C.H.C. & Vogler A.P., 2010. DNA-based taxonomy of larval stages reveals huge unknown species diversity in neotropical seed weevils (genus *Conotrachelus*): relevance to evolutionary ecology. Molecular Phylogenetics and Evolution, 56: 281–293.

Pleijel F., 2000. Phylogenetic taxonomy, a farewell to species, and a revision of *Heteropodarke* (Hesionidae, Polychaeta, Annelida). Systematic Biology, 48: 755–789.

Pleijel F. & Rouse G.W., 1999. Least-inclusive taxonomic unit: a new taxonomic concept for biology. Proceedings of the Royal Society of London B, 267: 627–630.

Plötner J., 2005. Die westpaläarktischen Wasserfrösche. Laurenti-Verlag, Bielefeld, 160 pp.

Poelstra J.W., Vijay N., Bossu C.M., Lantz H., Ryll B., Mueller I., Baglione V., Unneberg P., Wikelski M., Grabherr M.G., Wolf J.B.W. 2014. The genomic landscape underlying phenotypic integrity in the face of gene flow in crows. Science, 344: 1410–1414.

Raupach M.J., Astrin J.J., Hannig K., Peters M.K., Stoeckle M.Y. & Wägele, J.-W., 2010. Molecular species identifications of Central European ground beetles (Coleoptera: Carabidae) using nuclear rDNA expansion segments and DNA barcodes. Frontiers in Zoology, 7: 26.

Raupach M.J., Hannig K. & Wägele J.W., 2011. Identification of Central European ground beetles of the genus *Bembidion* (Coleoptera: Carabidae) using DNA barcodes: a case study of selected species. Angewandte Carabidologie, 9: 63–72.

Raupach M.J., Hendrich L., Küchler S.M., Deister F., Morinière J. & Gossner M.M., 2014. Building-up of a DNA barcode library for true bugs (Insecta: Hemiptera: Heteroptera) of Germany reveals taxonomic uncertainties and surprises. PLoS ONE, 9(9): e106940.

Regan C., 1926. Organic evolution. Report of the British Association for the Advancement of Science 1925: 75–86.

Ridley M., 1989. The cladistic solution to the species problem. Biology and Philosophy, 4: 1–16.

Riedel A., Sagata K., Surbakti S., Tänzler R. & Balke M., 2013. One hundred and one new species of *Trigonopterus* weevils from New Guinea. ZooKeys, 280: 1–150.

Riedel A., Tänzler R., Balke M., Rahmadi C. & Suhardjono Y.R., 2014. Ninety-eight new species of *Trigonopterus* weevils from Sundaland and the Lesser Sunda Islands. ZooKeys, 467: 1–162.

Rohland N., Reich D., Mallick S., Meyer M., Green R.E., Georgiadis N.J., Roca A.L. & Hofreiter M., 2010. Genomic DNA sequences from mastodon and woolly mammoth reveal deep speciation of forest and savanna elephants. PLoS Biology, 8 (12): e1000564.

Rosen D.E., 1978. Vicariant patterns and historical explanation in biogeography. Systematic Zoology, 27: 159–188.

Rothfels C.J., Johnson A.K., Hovenkamp P.H., Swofford D.L., Roskam H.C., Fraser-Jenkins C.R., Windham M.D. & Pryer K.M., 2015. Natural hybridization between genera that diverged from each other approximately 60 million years ago. American Naturalist, 185: 433–442.

Scheffers B.R., Joppa L.N., Pimm S.L. & Laurance W.F., 2012. What we know and don't know about Earth's missing biodiversity. Trends in Ecology and Evolution, 27: 501–510.

Seehausen O., Takimoto G., Roy D. & Jokela J., 2008. Speciation reversal and biodiversity dynamics with hybridization in changing environments. Molecular Ecology, 17: 30–44.

Simpson G.G., 1951. The species concept. Evolution, 5: 285–298.

Simpson G.G., 1961. Principles of animal taxonomy. Columbia University Press, New York: xii+247 pp.

Smith M.A. & Fisher B.L., 2009. Invasion, DNA barcodes, and rapid biodiversity assessment using the ants of Mauritius. Frontiers in Zoology, 6: 31.

Sneath P.H.A., 1976. Phenetic taxonomy at the species level and above. Taxon, 25: 437–450.

Soria-Carrasco V., Gompert Z., Comeault A.A., Farkas T.E., Parchman T.L., Johnston J.S., Buerkle C.A., Feder J.L., Bast J., Schwander T., Egan S.P., Crespi B.J. & Nosil P. ,2014. Stick insect genomes reveal natural selection's role in parallel speciation. Science, 344: 738–742.

Spolsky C. & Uzzell T., 1986. Evolutionary history of the hybridogenetic hybrid frog *Rana esculenta* as deduced from mtDNA analyses. Molecular Biology and Evolution, 3: 44–56.

Ståhls G. & Savolainen E., 2008. MtDNA COI barcodes reveal cryptic diversity in the *Baetis vernus* group (Ephemeroptera, Baetidae). Molecular Phylogenetics and Evolution, 46: 82–87.

Stork N.E., 1988. Insect diversity: facts, fiction and speculation. Biological Journal of the Linnean Society, 35: 321–337.

Strutzenberger P., Brehm G. & Fiedler K., 2011. DNA barcoding-based species delimitation increases species count of *Eois* (Geometridae) moths in a well-studied tropical mountain forest up to 50%. Insect Science, 18: 349–362.

Stur E. & Borkent A., 2014. When DNA barcoding and morphology mesh: Ceratopogonidae diversity in

Finnmark, Norway. ZooKeys, 463: 95–131.

Templeton A.R., 1989. The meaning of species and speciation: a genetic perspective. In: D. Otte, J.A. Endler, Editors, Speciation and its consequences. Sinauer, Sunderland: 3–27.

Van Valen L., 1976. Ecological species, multispecies, and oaks. Taxon, 25: 233-239.

Verheyen E., Salzburger W., Snoeks J. & Meyer A., 2003. Origin of the superflock of cichlid fishes from Lake Victoria, East Africa. Science, 300: 325–329.

Warwick S.I., Francis A. & Al-Shehbaz I.A., 2006. Brassicaceae: species checklist. Plant Systematics and Evolution, 259: 249–258.

Winterbottom R., Hanner R.H., Burridge M. & Zur M., 2014. A cornucopia of cryptic species - a DNA barcode analysis of the gobiid fish genus *Trimma* (Percomorpha, Gobiiformes). ZooKeys, 381: 79–111.

Witt J., Threloff D.S., Doug L. & Hebert P.D.N., 2006. DNA barcoding reveals extraordinary cryptic diversity in an amphipod genus: implications for desert spring conservation. Molecular Ecology, 15: 3073–3082.

Woodcock T.S., Boyle E.E., Roughley R.E., Kevan P.G., Labbee R.N., Smith A.B.T., Goulet H., Steinke D. & Adamowicz S.J., 2013. The diversity and biogeography of the Coleoptera of Churchill: insights from DNA barcoding. BMC Ecology, 13: 40.

Zachos F.E., Apollonio M., Barmann E.V., Festa-Bianchet M., Gohlich U., Habel J.C., Haring E., Kruckenhauser L., Lovari S., McDevitt A.D., Pertoldi C., Rossner G.E., Sanchez-Villagra M.R., Scandura M. & Suchentrunk F., 2013. Species inflation and taxonomic artefacts. A critical comment on recent trends in mammalian classification. Mammalian Biology, 78: 1–6.

Zaldívar-Riverón A., Martínez J.J., Ceccarelli F.S., De Jesús-Bonilla V.S., Rodríguez-Pérez A.C., Reséndiz-Flores A. & Smith M.A., 2010. DNA barcoding a highly diverse group of parasitoid wasps (Braconidae: Doryctinae) from a Mexican nature reserve. Mitochondrial DNA, 21(S1): 18–23.

Zhang Z.-Q., 2011. Accelerating biodiversity descriptions and transforming taxonomic publishing: the first decade of Zootaxa. Zootaxa, 2896: 1–7.

Zhang Z.-Q., Christenhusz M.J.M., Esser H.-J., Chase M.W., Vorontsova M.S., Lindon H., Monro A. & Lumbsch H.T., 2014. The making of world's largest journal in systematic botany. Phytotaxa, 191: 001–009.

Zhou M. & Fuller R.C., 2014. Reproductive isolation between two darter species is enhanced and asymmetric in sympatry. Journal of Fish Biology, 84: 1389–1400.

Zhou X., Robinson J.L., Geraci C.J., Parker C.R., Flint Jr. O.S., Etnier D.A., Ruiter D., DeWalt R.E., Jacobus L.M. & Hebert P.D.N., 2011. Accelerated construction of a regional DNA-barcode reference library: caddisflies (Trichoptera) in the Great Smoky Mountains National Park. Journal of the North American Benthological Society, 30: 131–162.