DNA barcoding and traditional taxonomy: An integrated approach for biodiversity conservation

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DNA barcoding and traditional taxonomy: An integrated approach for biodiversity conservation

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Abstract

Biological diversity is depleting at an alarming rate. Additionally, a vast amount of biodiversity still remains undiscovered. Taxonomy has been serving the purpose of describing, naming, and classifying species for more than 250 years. DNA taxonomy and barcoding have accelerated the rate of this process, thereby providing a tool for conservation practice. DNA barcoding and traditional taxonomy have their own inherent merits and demerits. The synergistic use of both methods, in the form of integrative taxonomy, has the potential to contribute to biodiversity conservation in a pragmatic timeframe and overcome their individual drawbacks. In this review, we discuss the basics of both these methods of biological identification- traditional taxonomy and DNA barcoding, the technical advances in integrative taxonomy, and future trends. We also present a comprehensive compilation of published examples of integrative taxonomy that refer to nine topics within biodiversity conservation. Morphological and molecular species limits were observed to be congruent in ~41% of the 58 source studies. The majority of the studies highlighted the description of cryptic diversity through the use of molecular data, whereas research areas like endemism, biological invasion, and threatened species were less discussed in the literature.

Keywords: Taxonomy, DNA barcoding, Integrative taxonomy, Conservation, Biodiversity

We should preserve every scrap of biodiversity as priceless while we learn to use it and come to understand what it means to humanity.

— E. O. Wilson (1999)

Introduction

The most remarkable feature of life since its inception on Earth is its diversity in forms. The term “biodiversity” or “biological diversity”, originally coined by Walter Rosen (Wilson 1988), is defined by the Convention on Biological Diversity (CBD) as – “the variability among living organisms, from all sources, including, inter alia, terrestrial, marine and other aquatic ecosystems as well as the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems.” Biodiversity thus encompasses the diversity at gene, species, and ecosystem levels of the biosphere. It resides at the intersection of various territories of science like taxonomy, molecular biology, biogeography, ecology, evolution, genetics, and conservation biology (Khuroo et al. 2007).

The majority of life forms on earth are facing a mass extinction at an abnormal rate of approximately 1000 times the background extinction rate (Pimm et al. 2014), caused predominantly by human activities unlike the previous five mass extinction events in the earth’s history (Dirzo et al. 2014). The resulting biodiversity crisis is intense in several habitats, where endemic taxa are exposed not only to the harsh effects of habitat destruction, fragmentation, and degradation, but also to biological invasions that replace native species. In addition, the levels of biodiversity loss are unknown. Moreover, the vast majority of biological diversity still remains undiscovered although estimates of true global diversity may vary according to different indicators and between taxa (Butchart et al. 2010; Scheffers et al. 2012). Our knowledge of diversity is remarkably incomplete. There are varying opinions as to the global estimates of species (Mora et al. 2011; Costello et al. 2013); 1.2-1.5 million species are considered to be described and valid to date (Costello et al. 2013; Mora et al. 2011; Zhang et al. 2011). Recent estimates indicate that 86% of terrestrial and 80-90% of marine species remain to be described (Mora et al. 2011; Appeltans et al. 2012). There might

be daunting consequences of losing these species from the planet before their discovery (Mora et al. 2011). Hence, attention has been drawn to the importance of conserving biological diversity, which has eventually resulted in the expansion of concepts, testable hypotheses, and increased technological innovation (Singh 2002) in conservation biology.

Conservation biology often aims to assess and protect existing biological diversity and is also concerned with the sustainable use of natural resources over the long term. The assessment of biodiversity is the first step to the successful design of any conservation strategy. Identification of the organisms—combined with detailed knowledge of their life histories, species richness, endemism, rarity, and the extent of morphological and genetic variability between them—are the essential components of any biodiversity assessment. Amongst these, the identification of individual organisms via taxonomical and/or molecular means is the first step and vital for designing any conservation strategy.

In this review, we advance the position that the synergistic use of traditional taxonomy and molecular biology in the form of ‘integrative taxonomy’ could help biodiversity conservation goals. The biodiversity crisis is an issue of societal concern, and so biodiversity conservation requires strong public support and action. The dire demands of biodiversity conservation are to analyze the vast biological diversity as well as to respond quickly to fading opportunities for action. Here, we discuss the various descriptors of biodiversity, major challenges to the description of biodiversity, and the role of integrative taxonomy in circumventing these challenges. Particular emphasis is given to current analytical inputs, updated published examples of integrative taxonomy in connection to different research areas within the field of biodiversity conservation, and future trends.

Key challenges and limitations to the description of biodiversity

Taxonomy—the science of discovery, description, and classification of living organisms on earth—is a fundamental base for biodiversity informatics. Taxonomists also often are involved with specimen identification. The foundations of this discipline are laid on the significant contributions of many botanists, the most important being Carl Linnaeus. Later, Hennig re-elevated taxonomy, as phylogenetic systematics, a central field of the biological sciences (Hennig 1966). Taxonomic data are comprised of morphology, physiology, anatomy, behaviour, geography, phenology, molecular information, biological and ecological associations, imagery, and literature (Thessen and Patterson 2011). Taxonomists use these data in order to test species hypotheses for the classification of organisms. Taxonomists, thus, maintain a biological nomenclature and thereby provide an integrated biological vocabulary for communicating and describing biodiversity (Knapp et al. 2002). Taxonomy is particularly useful for understanding of species on Red Data Lists and for identification of biodiversity hotspots and keystone species for prioritizing conservation efforts (Mace 2004) as well as eventual establishment of protected areas, addressing cross-border concerns like the spread of alien invasive species (Khuroo et al. 2007) and the conservation of migratory species. Therefore, the taxonomic discipline is of immense importance for documentation, conservation, and sustainable use of biodiversity.

However, there are various difficulties to studying biodiversity using only taxonomic means. One of them is the ‘taxonomic impediment’, which refers to the disproportionately small number of trained taxonomists compared to that required for the beneficial utilization of the taxonomic enterprise for various purposes (de Carvalho et al. 2007). It is a key obstacle to the successful exploration and conservation of biodiversity (Giangrande 2003). Thus, the removal of the taxonomic impediment is crucial to conserving biodiversity. The ‘taxonomic

impediment' has forced biologists to employ other methods for the identification and preservation of biodiversity, majorly DNA taxonomy. Godfray (2002) has highlighted the major challenges to taxonomy. These include the imbalance between described and undescribed organisms on the planet and a small fraction of published systematic research, irrespective of the publication quality and, more often, published in low-circulation journals only available in specialized libraries. Apart from these, several other problems as highlighted by Khuroo et al. (2007) include the ever-obscure consensus on the species concept, nomenclatural instability (uncertainty of the synonyms of organism names), difficulty in inter-operability of taxonomic databases due to inconsistency in file formats, geographic mismatch between the number of taxonomists and biodiversity hotspots, political boundaries which restrict the scope of broad-scale studies, taxonomic bias towards exploring more lucrative species in comparison to most other organisms, stagnation of funding and training in taxonomy, lack of standardization of classification among different taxa in large domains of life, and an enormous amount of time required for biodiversity exploration by traditional taxonomists.

Additionally, traditional taxonomy alone is not useful for species delineation of several diverse and morphologically conserved groups like nematodes, earless dragons, etc. (Boufahja et al. 2015; Melville et al. 2014). Also, the morphological identification of nematodes is usually problematic owing to the high phenotypic plasticity among populations, few diagnostic characters, and the presence of cryptic species (Derycke et al. 2010; Bhadury et al. 2008; Rodrigues Da Silva et al. 2010). Boufahja et al. (2015) have highlighted the importance of using molecular resources along with traditional morphological means in the form of 'integrative taxonomy' in biodiversity assessment and conservation of African marine nematodes.

The promise and limitations of DNA barcoding for taxonomic practice

Conservation biology is underpinned by evolutionary theory (Meffe and Carroll 1997). Changes in DNA are fundamental to the evolution and adaptation of different levels of biodiversity (species, populations). From the design of wildlife reserves to the management of breeding programs, molecular techniques are crucial and therefore used extensively to address questions of conservation relevance. Hence, genetic diversity is often a basis for the design and further implementation of long-term conservation strategies. Molecular biology acts as a tool (and not in isolation) for revealing the genetic variation within and amongst the individual components of biodiversity. Molecular inputs and computational innovations should be given due consideration for expanding the taxonomic enterprise (Bisby et al. 2002). Owing to the wide acceptance of molecular sciences and shortcomings of traditional taxonomy, there has been a plea for web-based DNA taxonomy (Tautz et al. 2003).

DNA taxonomy *sensu stricto* refers to the situation wherein DNA sequences themselves act as a reference system for taxonomy (Vogler and Monaghan 2006). Theoretically, the DNA sequences of ~600bp (i.e. DNA barcodes) contains more than enough information to distinguish millions of species. The foundations of DNA taxonomy are based on the fact that most species are equally distinguishable with DNA sequences as with morphological characters. The promises of DNA taxonomy have been highlighted by Blaxter (2004). The use of DNA sequences to taxonomy approximately dates 30 years back, firstly for the delineation of bacterial species (Fox et al. 1980). The DNA sequence information facilitates easy specimen identification even by people unfamiliar with morphological details (Hebert et al. 2003). The DNA sequence data freely accessible across these online databases has helped grow the knowledge of Earth's biodiversity. The public consortia for DNA sequence searching include the International Nucleotide Sequence Database Collaboration (INSDC),

comprised of NCBI (National Center for Biotechnology Information), EMBL (European Molecular Biology Laboratory), and DDBJ (DNA Data Bank of Japan), as well as BOLD (Barcode of Life Data Systems; Ratnasingham and Hebert 2007) which was designed specifically for barcode data. However, the latter also houses locality data, sequence chromatograms, and specimen photographs, opening avenues for verification of data and new research prospects. DNA sequence databases can be envisioned as ‘the need of the hour’ for the systematics research (Savolainen et al. 2005). The molecular approach to taxonomy majorly deals with discrete molecular entities referred to as MOTUs (Molecular Operational Taxonomic Units). A MOTU, originally proposed by Floyd and co-workers, is defined as a group of sequences that differed from one another by a maximum number of base pairs in any gene amongst a number of species (Floyd et al. 2002). MOTUs are genetically defined entities, hypothesized to be useful as biodiversity units, akin to species (Blaxter 2004).

At this point, it is important to clearly distinguish DNA barcoding from DNA taxonomy. The ‘DNA barcoding’ technique is based on the idea that sequence diversity of standardized gene regions (i.e. DNA barcodes) amongst different organisms can serve as a tool to identify specimens to known species and potentially discover new ones (Hebert et al. 2003). DNA barcoding (in contrast to DNA taxonomy *sensu stricto*) supplements the traditional taxonomic process, and does not supplant the taxonomic inputs (Hajibabaei et al. 2007; Hubert and Hanner 2015) for the conservation of biological diversity. This system will open new avenues for using accumulated taxonomic knowledge, and linked biological data, and act as a convenient tool for specimen identification (to known species) as well as species discovery. Moreover, it is important to note that ‘species discovery’ and ‘specimen identification’ are two separate objectives of DNA barcoding which are often confused under the term ‘species identification’ (Collins and Cruickshank 2013). DNA-based species

discovery and specimen identification depends on distinguishing intraspecific from interspecific genetic variation. The use of the CO1 barcode in taxonomy is already well established in the study of animals and algae, but this marker is not suitable in the case of plants, where, chloroplast loci like *rbcL* and *matK* better serve the purpose (CBOL Plant Working Group 2009). However, species-level resolution for plants has been lower than for animals using these standard regions (Hollingsworth et al. 2011). The search for complementary and/or better methods has been ongoing in the plant literature (Coissac et al. 2016). DNA barcoding techniques are comprised of standard protocols with minimal field work where museum collections are used (Chase et al. 2007). The barcode sequences would provide a unique biological nomenclature in a universally accessible format across the widespread scientific community. The latter is done in two specific ways: 1) if identification is obtained through a match to reference sequences; 2) if a barcode-based nomenclature is used based up a formal MOTU system like that used in packages like jMOTU (Jones et al. 2011) and now revolutionized by a newer BIN system developed by Ratnasingham and Hebert (2013). The DNA barcodes provide a unique ‘horizontal genomics’ perspective with broad implications (Hajibabaei et al. 2007). One of the chief arguments of DNA barcoders is its efficiency in quick identification of specimens to species, and in this context the target of barcoding 500K species was met in August 2015 as mentioned on the iBOL website (<http://ibol.org/>). Hebert et al. (2003) outlined some weaknesses of morphotaxonomy like phenotypic plasticity, morphologically overlooked species, lack of taxonomic keys or diagnostic character to identify immature specimens of species, and lack of expertise in taxonomical identification (since it is restricted to specialists), which can be overcome by successful application of DNA barcoding. DNA barcoding can be used as a means to revitalize traditional taxonomy if it is used in conjunction with ecological, morphological, and other genetic studies (e.g. Creer et al. 2010).

The potential of DNA barcoding is far beyond the perspectives of only databasing the sequences; there exists a wealth of analytical and bioinformatics tools to serve the purpose of deriving many meaningful conclusions (Ji et al. 2013; Kress et al. 2008; Sheth and Thaker 2015; Bhargava and Sharma 2013). Ratnasingham and Hebert (2013) have developed a structured registry based on MOTUs for species delimitation using BIN (Barcode Index Numbers). The BIN system clusters sequences using a novel algorithm that was thoroughly tested in several well-known animal groups in order to produce MOTUs that closely correspond to species. Moreover, as these clusters show high concordance with species, this system can be used to delineate species when taxonomic information is deficient. Hence, this system provides the species-level information needed to strengthen biodiversity science, and it is also helpful in overcoming the taxonomic impediment. DNA barcoding essentially provides the ease of specimen identification using simple molecular protocols, irrespective of the specimen's life stage and place of collection as well as non-availability of taxonomic expertise (Teletchea 2010). The simplicity and precision of the method has enabled its application in various ways, such as studies of invasive species, identification of botanicals, detection of species substitution in seafoods, biomonitoring of ecosystem health, etc. (Adamowicz 2015).

However, species delimitation using DNA barcoding is much more controversial (Desalle 2006; Dick and Webb 2012) than specimen identification. The task of species delimitation should be accomplished carefully with the DNA barcoding approach. Collins and Cruickshank (2013) listed seven common problems in barcoding studies (and suggested solutions for each), including highlighting the lack of universality of applying the same

classification method for species delimitation of different groups as well as lack of uniform genetic distance thresholds between different taxonomic groups.

One of the major reasons that DNA barcodes may fail to distinguish previously recognized species is species paraphyly corresponding to gene trees, which is seen in many cases (Funk and Omland 2003; Thalmann et al. 2004; Tautz et al. 2003) and observed more frequently in plants than animals (Fazekas et al. 2009). Additionally, there are several additional potential limitations of using organellar DNA for species delimitation such as the retention of ancestral polymorphisms, uniparental mode of inheritance (resulting in cases of hybridization or introgression being overlooked), selection on any organellar DNA segment (as the whole genome is one linkage group), and paralogy resulting from transfer of mtDNA gene copies to the nucleus (Moritz and Cicero 2004). Moreover, Laiou et al. (2013) also showed certain limitations of DNA barcoding, like the imperfect discrimination capacity of the barcode loci in light of rapid threshold-based MOTU delineation methods currently in use and that reference databases are especially incomplete for some taxonomic groups and habitat types, while working on some economically important woody plant genera in the Mediterranean basin. These limitations can increase ambiguity in specimen identifications from DNA barcodes and thereby complicate conservation efforts. However, there are numerous examples in the literature where there exists close correspondence between species recognized through traditional means and DNA barcode clusters, making barcoding useful for biodiversity surveys (Sangster et al. 2015; Wijayathilaka et al. 2016; Hiebert and Maslakova 2015; etc.)

The successful application of DNA barcoding is based on 250 years of taxonomic groundwork. Hence, DNA barcoding complements taxonomy and in no case is a substitute

for the same (Hebert and Gregory 2005; Hajibabaei et al. 2007; Ebach and Holdrege 2005). More recently, with the advancements in next-generation sequencing (NGS) platforms (Shendure and Ji 2008; Glenn 2011), a new technique, termed DNA metabarcoding (discussed in Cristescu 2014), has emerged, which aims at the identification of biotic communities (containing individuals from multiple species) from single samples (containing mixed-species collections of organisms) or from environmental DNA (eDNA) taken directly from environmental samples, such as water or soil. It extends DNA-based single-specimen identification to identification of communities of individuals belonging to many groups of species with distinct roles in the ecosystem (Taberlet et al. 2012a). Environmental DNA (eDNA) is defined as small fragments of DNA left behind by organisms in the environment (Taberlet et al. 2012b). It has recently emerged as a tool for conservation and monitoring past and present biodiversity (Thomsen and Willerslev 2015; Lacoursière-Roussel et al. 2016). Environmental DNA metabarcoding has an immense potential to enhance data acquisition and interpretation in biodiversity research (Ji et al. 2013; Cristescu 2014), hence shedding light on the community or syn-ecological aspects of biodiversity conservation.

Integrative taxonomy in biodiversity assessment and conservation

The virtues and vices of DNA barcoding, as well as those of traditional taxonomy, have led to several researchers advocating an integrative approach to exploring biodiversity and fighting the biodiversity crisis (Figure 1). Dayrat (2005) has made a formal suggestion for integrative taxonomy. ‘Integrative taxonomy’ is defined as the science that aims to delimit the units of life's diversity from multiple and complementary perspectives (phylogeography, comparative morphology, population genetics, ecology, development, behaviour, etc.). It accelerates the traditional taxonomic routine by incorporating other characters in addition to morphological aspects for species delineation, thereby improving correspondence between species units and

evolutionary units and also facilitating specimen identification practices. Many studies have highlighted the importance of integrated molecular and morphological approaches to biodiversity-based studies (Larsen 2001; Boero 2010; Heethoff et al. 2011). More rigorous delimitation using integrative approaches can cause an increase or decrease in species at different times, with increase by the discovery of cryptic species, as evident in the literature, and decrease by the revelation of conspecificity of nominal species, often ending a long-standing taxonomic dispute (Schlick-Steiner et al. 2010). Molecular data in co-operation with taxonomic research can minimize the severity of the taxonomic impediment and also the time for exploring biological diversity, thereby providing more scope for conservation efforts. Although an initial time investment in the integrative approach is needed during the species delimitation phase, time can be saved later by performing routine identifications using molecular approaches. Also, DNA barcoding offers taxonomists/ phylogeneticists the opportunity to expand greatly the global inventory of biological diversity. It requires bridging the gaps between taxonomists and molecular biologists in careful curation of DNA databases from voucher specimens correctly identified by the expert taxonomic community, resulting in an amalgamated effort of taxonomists, molecular biologists, and bioinformaticians to conserve the depleting biological diversity.

There exist examples in the literature where traditional taxonomy has contradicted DNA barcoding (Treewick 2008; Resch et al. 2014) and vice versa (de Boer et al. 2014; Mutanen et al. 2015); additionally, there are also examples where neither morphological nor molecular data are complete by themselves (Page and Hughes 2011) and others where they complement each other (e.g. Chan et al. 2014). Recently, Galimberti et al. (2012) proposed a synergistic synthesis of classical taxonomic approaches (e.g. morphology, biogeography) and molecular characteristics into discrete units called Integrated Operational Taxonomic Units (IOTUs).

They defined IOTUs as groups of organisms confirmed by at least two approaches, the first of which is molecular-based and the other has a taxonomic line of evidence. They tested this approach in poorly investigated Italian echolocating bats, which require more conservation efforts. In their study, they found that out of 31 molecular entities, 26 corresponded to the morphologically assigned species, two were morphologically cryptic MOTUs, and three were IOTUs based on morphological, molecular, and behavioural evidence. Their study reflected that IOTUs were more informative for approximating evolutionary species units than the general OTUs (Operational Taxonomic Units) and the more recent MOTUs (Molecular Operational Taxonomic Units). However, the morphological evidence corroborated with the molecular evidence in the identifications of >80% of the specimens.

There are many studies in the literature where molecular evidence is more informative than most other lines of evidence for revealing evolutionary species, and there are also examples where the reverse is true. For example, in a study of the species status of a predatory mite-*Typhlodromus pyri* by Marie-Staphane et al. (2012), using morphology and molecular (nuclear and mitochondrial) markers, there was strong agreement of morphological evidence only with the nuclear marker, while unexpectedly high genetic distances were observed with the mitochondrial markers probably due to their organellar origin. This case study highlighted the difficulty to conclude the species status using only mitochondrial markers and genetic distances and showed the necessity of applying multiple approaches for species definition. Moreover, many studies explain the fact that the mitochondrial barcode data—as any single locus—is not enough in itself for designating species (Lumley and Sperling 2010; Martinsson et al. 2013; Draper et al. 2015). It is more suited for both identifying deeply divergent (matrilinal) lineages and rapidly assigning individuals to the rank of species after boundaries have been delimited based on integration of all other sources of evidence (Funk and Omland

2003; Moritz and Cicero 2004; Scheffer et al. 2005; Kaila and Ståhls 2006). In yet another interesting and different study by Mengual et al. (2006), DNA sequences from taxa sampled from a geographically restricted region revealed both conflict and congruence with the taxonomic information derived from morphological and molecular characters (and the need for integrative taxonomy); i.e., morphological evidence alone failed to recognize three probable cryptic species, while the COI barcode sequences failed to distinguish 2 species from others in the dataset. Similarly, in a study by Smith et al. (2008) in parasitoid wasps and their caterpillar hosts, all the lines of evidence in integration were unavoidable to ascertain the real parasitoid biodiversity and host specificity, as any data source alone by itself was incomplete to draw a reliable picture.

‘Species’ form the basic taxonomic rank while considering the ecosystem-scale conservation required to preserve significant environmental, ecological, and evolutionary (e.g. adaptation, speciation) processes, to form the basis for both biodiversity assessments and for management (e.g. Kekkonen and Hebert 2014). The classification of a group of individuals or populations to one or several species requires the explicit use of a species concept. Various concepts have been proposed to date, the most commonly used ones are: biological (which relies on the reproductive isolation of species) and phylogenetic species concepts (Agapow et al. 2004). However, an evolutionary concept given by Simpson (1951) describes ‘species as distinct entities having unique evolutionary role, tendencies, and historical fate’. A more recent unified species concept (given by de Queiroz 2007) could be attained by treating existence as a separately evolving metapopulation lineage as the only necessary property of species and former secondary species criteria, such as different properties acquired by lineages during the course of divergence (e.g., intrinsic reproductive isolation, diagnosability, monophyly), as different lines of evidence (operational criteria) relevant to assessing lineage

separation. While applying the data from different lines of evidence in any integrative approach, species boundaries may differ for each data type (Padial and de la Riva 2010) and species concept (Tan et al. 2008). Such differences do not hamper species delineation, although being explicit about the selected concept is important; instead, they enable biologists to take different lines of argumentation into account, leading to well-substantiated conclusions regarding the delineation of the studied species. Also, different species concepts and methods can be used to strengthen each other, so as to reach consistent conclusions on species boundaries (e.g., Jarman and Elliott 2000).

Integrative taxonomy plays an important role in various aspects of conservation of biological diversity, primarily in the description of species. It helps the preservation of endemic, rare, and threatened biodiversity in nature. The role of integrative taxonomy in species delimitation has been explained in more detail through a thorough survey of literature by Pante et al. (2014). Bickford et al. (2007) consider two or more species to be 'cryptic' if they are, or have been, classified as a single nominal species because they are at least superficially morphologically indistinguishable. Cryptic species require special consideration in conservation planning as the occurrence of cryptic complexes in already-endangered nominal species presents several problems: (i) species already considered endangered or threatened might be composed of multiple species that are even rarer than previously supposed; and (ii) the different species might require different conservation strategies (Schönrogge et al. 2002). Melville et al. (2014) used integrative taxonomy for delimitation of the endangered earless dragons in the *Tympanocryptis tetraporophora* species complex. Arribas et al. (2013) also used an integrative approach to delimit beetle species in the *Enochrus falcarius* species complex. They delimited the complex into four species including three new species. Among these, *Enochrus falcarius* is not considered to be of conservation concern, because till then, it

had been regarded as a single broadly distributed species in the Mediterranean region. However, the four entities delimited within this species complex displayed characteristics that categorised them as vulnerable taxa. Such newly discovered cryptic species present opportunities to study important mechanisms of speciation, mate recognition, and conservation management. Moreover, the loss of cryptic evolutionary lineages reduces evolutionary potential and ends ongoing diversification processes in nature, eventually affecting future biodiversity.

On the other hand, alien invasive species are major causes of biodiversity loss as they replace the native species in an ecosystem, thereby causing an imbalance in the prevailing biodiversity. Identification of such alien species will aid in prioritizing the conservation strategies for the native species of an ecosystem. Apart from identification, a large number of species owe their initial discovery to integrative taxonomic efforts.

Yet another interesting aspect of using integrative taxonomy is assessment of the species richness with respect to geographical scales. In some cases, barcoding alone could help in estimation of species richness in poorly studied taxa and areas (Costion et al. 2011; Mutanen et al. 2013; Stahlhut et al. 2013, etc.). However, integrative taxonomy is also efficient in measuring species richness (Gill et al. 2014). But, it is not only enough to calculate species richness; the evolutionary relationships between species must be understood (Hendry et al. 2010). Phylogenetic reconstructions have been historically used as a tool in systematics, examining relationships among species and at higher-level taxonomic classifications. But, with the introduction of nucleotide sequencing, they have been used in assessment of regional biodiversity as well as in studying the genetic patterns at different taxonomic levels (Sinclair et al. 2005).

A review of the literature with respect to the use of integrative taxonomy for biodiversity conservation

Available literature was reviewed in order to assess the current role of integrative taxonomy for biodiversity assessment and conservation purposes. We performed a literature survey in Google Scholar and Pubmed Central in March 2016. The search keywords included “Integrative taxonomy+ biodiversity” or “molecular+morphological”. The time span was restricted to 2006-2016 as it follows the formal introduction of integrative taxonomy by Dayrat (2005). From the resulting articles, the majority of the articles were removed as they did not fit the context of this review; these included methodological and theoretical articles, review studies, opinions, commentaries, etc. The source studies included in the literature review were the ones which integrated more than one line of evidence for species delimitation, including morphology, barcodes, behaviour, ecology, etc. The inclusion criteria for the papers were: clear mention of sample size in either species/specimen numbers, as well as those which included at least one of the nine most frequently mentioned biodiversity topics in literature (mentioned below).

The list presented in Table 1 includes the papers screened from the literature survey. Table 1 highlights a thorough literature survey on use of integrative taxonomy with respect to different research topics within biodiversity conservation (A-I). ‘A’ indicates those papers dealing with description of a new species in a specific geographical area (e.g. Heethoff et al. 2011); ‘B’ includes those studies which highlight the finding of cryptic species (e.g. Soldati et al. 2014); ‘C’ includes those studies which involve species delineation aspects (e.g. Ruocco et al. 2012); ‘D’ includes those studies which show the use of integrative taxonomy for invasive species (e.g. deWaard et al. 2011); ‘E’ includes those studies which mention phylogenetic diversity (e.g. Puillandre et al. 2014); ‘F’ includes those studies where the

comparison of molecular and morphological identification is mentioned (e.g. Nitta 2008); ‘G’ are those studies which highlight endemism (e.g. Manghisi et al. 2014); ‘H’ includes those studies which describe the protection of threatened and/or endangered species (e.g. Melville et al. 2014); and ‘I’ includes those studies which mention discovery of novel species in science (e.g. Glaw et al. 2010). Moreover, overlaps of these ‘biodiversity topics’ (A-I) were observed in most of the source studies. Also, we noted whether or not the morphological and molecular species limits were in agreement, in each of the selected studies.

From the 58 screened papers, we found that 24 (~41%) showed accordance between the molecular and other means of identification, while 34 (~59%) showed discordance. Furthermore, the included papers were also analyzed with respect to the relative percent occurrence of different biodiversity topics in the source studies (Figure 2). The percent relative occurrence of the various biodiversity indicator terms (A-I) (mentioned in Table 1) from the 58 source studies of the reviewed literature were compared in order to show the areas to which integrative taxonomic efforts are currently most contributing to biodiversity assessment and conservation. The majority of the studies highlighted the cryptic diversity estimates and species discovery aspects of biodiversity conservation. Many studies also included the topic of endemic species diversity. Only a few studies included invasive species or threatened and rare species, indicating that urgent attention is required on these overlooked areas for immediate conservation efforts. The literature ensemble also shows that the number of molecular species units is higher than morphological ones in most of the cases, which indicates that in such cases more conservation areas may be required than originally thought in the geographic ranges under consideration, given that cryptic and recently diverged species were frequently reported to be allopatric (e.g. Martinsson et al. 2013; Oliver et al. 2009). These unique DNA-based barcode lineages, despite not being supported as species by

traditional taxonomic and integrative means, also need to be protected, as they reflect evolutionarily significant diversity as well. This, in turn, points toward an urgent need for cautious conservation plans and efforts towards protecting this hidden biodiversity too.

Conclusion

To date, although a lot of work has been done already and is being conducted with respect to faunal diversity using integrative taxonomy, there are fewer reports on the floral side. DNA barcoding has been problematic in plants because land plant phylogenetic markers seem to have too little variation to determine species limits (Kress et al. 2005). Also, there has been a debate regarding the universal use of any one locus in plants, like COI in case of animals (Cowan and Fay 2012). However, *rbcL* and *matK* along with several other loci like ITS have so far been used by plant barcoders (CBOL PWG 2009; Li et al. 2011), and, increasingly, usage of expanded portions of the genome is advocated (Hollingsworth et al. 2016).

Integrative taxonomy is inevitable to describe and conserve the depleting biodiversity. Accurate data using various lines of evidence are critical for determining basic parameters of protection, such as species distributions and threat levels to design effective conservation plans (Rondinini et al. 2006). Higher molecular to morphological species as evident from various studies included in Table 1 require higher conservation efforts than originally thought for the ones described. It is definitely useful to consider to protect the molecular units as well, in case they are separate species and also to help to preserve the evolutionary potential of these species. Also, nominal morphological species already considered endangered or threatened may comprise additional other species, each of which is often rarer than their 'parent species', making them more susceptible to extinction (Hedges and Conn 2012). Without published descriptions, this biodiversity is essentially 'off the conservation radar'

and therefore often ignored in conservation plans. On the other hand, sometimes erroneous decisions pertaining to conservation may be made if taxonomic status is incorrectly assigned. It could lead to ignorance of an endangered species that prevents its conservation plans as well as legal protection of different populations of a common species erroneously considered as distinct species or hybridization issues in conservation management (reviewed in Frankham et al. 2010).

Hence, an integrative approach will permit researchers to use the speed and standardization of molecular data, while also using traditional species names to be able to invoke current laws and policy. Much current law and policy on endangered species is linked to standard species names. MOTU does not currently have a formal status under the law. Thus, a harmonized approach can be necessary in order for conservation action to be taken.

As mentioned earlier, as compared to the terrestrial diversity, a vast majority of marine biodiversity still needs to be explored. The identification, naming, and documentation of species could be made automated using the combination of DNA barcoding and digital image processing (Vogler et al. 2007; La Salle et al. 2009). These approaches could be especially helpful for the preliminary screening of hyperdiverse groups like small arthropods and marine nematodes, and for geographical areas facing imminent habitat destruction (requiring immediate conservation priorities). More work on marine diversity will be seen in the coming years as the work is currently in progress in various parts of the world including that under the aegis of the Global Marine Biodiversity Project launched by the Smithsonian Institution in 2012. Hence, more attention is anticipated in the coming years, with the application of integrative taxonomy in these overlooked areas of biodiversity, where conservation measures are imperative.

Thus, the integration of taxonomical inputs with DNA barcoding efforts will be useful for overcoming the taxonomic impediment and conservation of biodiversity. It is a logical solution to the debate on conservation biology versus molecular biology. We advance the view that there is no scope for absolute monopoly of any fields for sustainable use and development of biological diversity. A combinatorial approach 'alone' can be the ultimate solution for the conservation of biodiversity.

Draft

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Table 1: Survey of Literature with respect to the use of integrative taxonomy in connection to different indicators of biodiversity conservation (A: Description of new species in a geographical area; B: Cryptic species; C: Species delineation; D: Invasion biology; E: Phylogenetic diversity; F: Comparison of molecular and morphological identification; G: Endemism; H: Protection of threatened and endangered species; I: Species discovery)

* Species boundaries can differ even if the species count is the same

Sr. No.	Taxa	Areas covered	Sample size (No. of morphological species/ No. of molecular species)	Molecular and morphological species limits in agreement*	Reference
1	Acari: Trhypochthoniidae	A, C, E, F	3/3	Yes	Heethoff et al. (2011).
2	Acari: Phytoseiidae	B, C, E, F	3/1	No	Marie-Stephane et al. (2012).
3	Annelida: Clitellata: Hormogastridae	C, E, F, I	20/21	No	Novo et al. (2012).
4	Anura: Mantellidae	B, H, I	58 / 70	No	Glaw, et al. (2010)
5	Anura: Strabomantidae	B, C, E, I	6/8	No	Padial and Riva (2009).
6	Araneae: Dysderidae	B, C, E, G, I	3/3	Yes	Macias-Hernandez et al. (2010).
7	Bivalvia: Corbiculidae	C, D, E, F	3/3	Yes	Pigneur et al. 2011
8	Branchiopoda: Spinicaudata	C, I	8/11	No	Schwentner et al. (2011).
9	Chondrichthyes: Myliobatiformes: Myliobatidae	C, I	2/3	Yes	Ruocco et al. (2012).
10	Coleoptera: Cerambycidae: Saperdini	C, E	2/2	Yes	Kvamme et al. (2012).

11	Coleoptera: Tenebrionidae:	B, C, E, G,	6/10	No	Soldati et al. (2014).
14	Ulomini	I			
12	Diptera: Chironomidae:	B, C, E, F	11/14	No	Silva and Wiedenbrug (2014).
	Orthocladiinae				
13	Diptera: Drosophilidae: Phortica	A,B, C, I	3/3	Yes	An et al. (2015).
14	Diptera: Syrphidae	B, C, E	2/6	No	Milankov et al. (2008).
15	Diptera: Tephritidae	D, E, F	3/2	No	Schutze et al. (2014).
16	Gastropoda: Conoidea	C, E, G, I	2/3	Yes	Puillandre et al. (2014)
17	Halymeniales: Rhodophyta	B, C, E, G, I	12/11	No	Manghisi et al. (2014).
18	Hymenophyllales: Hymenophyllaceae	C, E, F	12/12	Yes	Nitta, J. H. (2008).
19	Hymenoptera, Halictidae	A, B, F, I	1/5	No	Gibbs, J. (2009).
20	Hymenoptera: Apidae: Meliponini	C, E, F, I	3/4	No	Koch H. (2010).
21	Hymenoptera: Apidae: Xylocopinae	C, E, I	1/3	No	Rehan and Sheffield (2011).
22	Lepidoptera: Gelechiidae	B, E, I	14/14	Yes	Huemer and Hebert (2011).
23	Lepidoptera: Tortricidae	B, C, E, F	5/2	No	Lumley and Sperling (2010).
24	Nudibranchia: Polyceridae	A, C, E, F, I	16/16	Yes	Pola et al. (2006).
25	Nymphalidae: Satyrinae: Euptychiina	B, C, E, F	12/8	No	Seraphim et al. (2014).
26	Oligochaeta: Acanthodrilidae, Octochaetidae	A, E, G, I	3/3	Yes	Boyer et al. (2011).
27	Platyhelminthes: Proseriata	B, E, F, I	3/4	No	Casu et al. (2009).
28	Porifera: Demospongiae	A, C, E, I,	10/10	Yes	Cárdenas et al. (2009).

29	Rotifera: Monogononta	C, E, F	2/4	No	Malekzadeh-Viayeh et al. (2014).
30	Taeniidae: Cyclophyllidea	B,C, F	1/3	No	Galimberti et al. 2012
31	Squamata: Varanidae	A, B, C, E, I	3/5	No	Welton et al. 2014
32	Amphibia: Anura	A, B, C, F, G, I,	76/76	Yes	Rosa et al. 2012
33	Hymenoptera: Formicidae	A, B, E, F, G, I	51/51	Yes	Smith and Fisher 2009
34	Odonata: Libellulidae	B, C, E, F, I	6/7	No	Damm et al. 2010
35	Araneae: Dysderidae	A, B, C, E, F, G, I	3/2	No	Rezac et al. 2014
36	Jungermannioptera: Porellales	C, E, F, I	10/8	No	Heinrichs et al. 2015
37	Typhlopidae: Scolecophidia: Ramphotyphlops	A, B, C, F	27/56	No	Marin et al. 2013
38	Lepidoptera : Noctuidae : Apameini	B, C, E, F, G, I	10/10	Yes	Le Ru et al. 2014
39	Diptera: Syrphidae	B, C, E, F, G	3/7	No	Francuski et al. 2011
40	Lepidoptera: Geometridae	B, C, D, E	400/423	No	deWaard et al. 2011
41	Hymenoptera: Chalcidoidea: Encyrtidae	B, C, F	1/3	No	Chesters et al. 2012
42	Agamidae: Tympanocryptis	B, C, E, F, H	1/4	No	Melville et al. 2014
43	Clitellata: Naididae	B, C, E, F, I	2/6-7	No	Martinsson et al. 2013

44	Diplodactylus, Gekkota	A, B, C, E, F	13/29	No	Oliver et al. 2009
45	Diptera: Syrphidae	B, C, E, F	17/19	No	Mengual et al. (2006)
46	Rodentia: Muridae	C, E, F, I	6/10	Yes	Demos et al. 2014
47	Lepidoptera: Noctuidae: Sesamiina	C, E, I	1/7	Yes	Kergoat et al. 2015
48	Araneae: Mesothelae: Liphistiidae	A, B, C, E, F, G, I	6/6	Yes	Xu et al. 2015
49	Bryophyta: Lembophyllaceae	A, C, E, F, G, I	6/4	No	Draper et al. 2015
50	Nemertea: Heteronemertea	B, C, I	5/5	Yes	Hiebert and Maslakova 2015
51	Annelida: Sabellidae	A, B, C, I	4/9	No	Capa and Murray 2015
52	Porifera: Homoscleromorpha	C, E, F, I	3/3	Yes	Ruiz et al. 2014
53	Muridae, Gerbillinae	C, F	2/2	Yes	Ndiaye et al. 2014
54	Aves: Fringillidae: Fringilla	C, H	2/2	Yes	Sangster et al. 2015
55	Anura: Bufonidae	A, C, E, F, I	6/10	No	Rojas et al. 2016
56	Anura: Microhylidae	A, C, F, I	2/2	Yes	Wijayathilaka et al. 2016
57	Diptera: Syrphidae	A, C, F, I	3/3	Yes	Nedeljković et al. 2015
58	Braconidae: Doryctinae	B, C, E, F	100/78	No	Ceccarelli et al. 2012

List of Figures

Figure 1: Integrative taxonomy in biodiversity conservation. Taxonomical classification comprises of species delimitation based upon various lines of evidence like behaviour, anatomy, DNA sequences, habitat, physiology, and morphology of the species under consideration. Moreover, DNA barcoding involves sequencing standardized genetic regions, typically followed by sequence clustering and comparison of sequences to existing databases, thereby facilitating species delimitation and specimen identification, respectively. The combination of both these methods in the form of ‘integrative taxonomy’, with added advantages of both methods, could play a vital role in biodiversity monitoring and conservation purposes. The various biodiversity topics which are dealt successfully by integrative taxonomy include elucidation of cryptic species, species discovery, endemism, elucidation of species richness in protected areas, protection of threatened and endangered flora and fauna, phylogenetic diversity, and invasion biology. The usage of standardized genetic regions greatly facilitates quantification and comparison of diversity across regions. A newer technique called ‘metabarcoding’ is used in order to know the diversity of organisms present in bulk samples.

Figure 2: The percent relative occurrence of the biodiversity topics in the literature survey carried out in this study

The percent relative occurrence of the various biodiversity indicator terms (A-I) (mentioned in Table 1) from the 58 source studies of the reviewed literature were used to create a chart in order to show the highly exploited areas for biodiversity assessment and conservation purposes by integrative taxonomic efforts. The values total more than 100% because many studies cover multiple research topics.

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