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REVIEW ARTICLE

DNA BARCODING AND TRADITIONAL TAXONOMY: AN INTEGRATIVE APPROACH

[*,1]Ankita Rajpoot, [2,3]Ved Prakash Kumar, [1]Archana Bahuguna and [3]Dhyanendra Kumar

[1]Zoological Survey of India, NRC, 218, Kaulagarh Road, Dehradun 248195, Uttarakhand, India
[2]Wildlife Institute of India, Post Box # 18, Chandrabani, Dehradun 248001, Uttarakhand, India
[3]Veer Kunwar Singh University, Arrah 802301, Bihar, India

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ABSTRACT

Taxonomic classification of vertebrates and invertebrates is one of the most important and challenging method for biologists. Previously, species discovery and taxonomic classification were based on morphological features, ecology, reproductive isolation, or behavior. However, most species remain unidentified due to the slow progress and lack of expertise in the relevant field. Furthermore, there is very little morphological variation in cryptic species, making it difficult to discriminate between them. Therefore, there is a need for a highly informative, rapid, and easy procedure to resolve these issues. Integrative approach is a new and robust technique that uses for species identification, classification, and discrimination. Most taxonomists consider DNA barcoding as a threat to traditional taxonomy. However, research indicates that DNA barcoding and traditional taxonomy are complimentary to each other. Here we advocate a combined approach of traditional taxonomy and DNA barcoding called integrated taxonomy. Integrated taxonomy is the most authentic and informatic to new species discovering, taxonomic classification and research. Recently, most studies have employed integrated approach because it is not only used for taxonomic classification but also for phylogeny, phylogeography, population genetics, ecology, development, behavior, and temperature studies. It is the most useful and relevant approach for delineating novel species, biodiversity research, and conservation.

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INTRODUCTION

Taxonomy is a branch of science dealing with categorization of plants, animals, and microorganisms. A taxonomist identifies, describes and classifies species on the basis of morphological, behavioral, genetic, and biochemical observations. Currently, taxonomy is facing a threat because of the decrease in the number of experts and inadequate funding (Godfray, 2002; Mallet & Willmott, 2003). In the last 250 years, taxonomists have identified approximately 1.78 million species of animals, plants, and microorganisms; several million species still remain unidentified (Godfray, 2002; Wilson, 2003). To revitalize traditional taxonomy, advanced approaches, such as molecular taxonomy (Godfray, 2002; Tautz *et al*., 2003; Hebert *et al*., 2003), information technology, increased utilization of cyber tools (Pyle *et al*., 2008; La Salle *et al*., 2009), have been employed (Wilson, 1985). Morphological taxonomy is the traditional and fundamental method to describing the new species. The progress of traditional taxonomy has been hampered due to some problems (May, 2004). Taxonomy initiated in the 18th century, and currently, 1.5 to 2 million species have been described with an estimated 5 to 100 million species awaiting description (Wilson, 2003). Taxonomists require a lot of time to revise the classification and describe novel species; it has been estimated that another 950 years will be required to describe all the species by traditional taxonomy (Seberg *et al*., 2003) because of the lack of experts. Some species are only known by their original descriptions, the holotype and their type material lost or destroyed. Several specimens that are deposited in museums remain unidentified (Padial & De La Riva, 2007). Therefore, biologists are exploring alternatives that will seek to modernize the field of taxonomy. Within this context, DNA barcoding cannot be viewed as a threat to taxonomy because it has attracted toward biodiversity studies (Smith, 2005). It is, however, necessary to understand that this initiative is necessary to overcome all issues faced by traditional taxonomy. Therefore, it can be successively applied when morphology is insufficient (Herre, 2006). DNA taxonomy and DNA barcoding can deliver reliable species-level classifications and species identifications of biodiversity.

Corresponding author: Ankita Rajpoot,
Zoological Survey of India, NRC, 218, Kaulagarh Road, Dehradun 248195, Uttarakhand, India.

DNA barcoding is an innovative taxonomic method that uses a short genetic marker in an organism's DNA to identify specimen (Hebert *et al*., 2003). This method has been particularly successful in the identification and description of novel species various groups (Hebert *et al*., 2003; Hebert *et al*., 2004; Ward *et al*., 2005; Cywinska *et al*., 2006; Hajibabaei *et al*., 2006a, b; Smith *et al*., 2007; Borisenko *et al*., 2008; Kerr *et al*., 2009; Ferri *et al*., 2009). This method has received acceptance because of its simplicity and affordability (Padial & De La Riva, 2007). Recently, it has been established as a mature field of biodiversity sciences to bridge the conceptual gap between traditional taxonomy and different fields of molecular systematics (Miller, 2007; Hebert *et al*., 2003; Hajibabaei M, 2007; Hubert & Hanner, 2015).

In the present review, we have discussed regarding the combined approach of morphology and DNA barcodes and its reliability to identify specimen and resolve the taxonomic issues. This study aims to combine different sources of data such as morphology and barcoding to identify and describe new species. Recent reviews also support DNA barcoding as a molecular tool for taxonomy and integration of different data sources in further biological studies (Terry & Johnso, 2000; Schindel & Miller, 2005; Will *et al*., 2005; Miller, 2007; Hajibabaei *et al*., 2007; Waugh, 2007; Valentini *et al*., 2008; Casiraghi *et al*., 2010; Pires & Marinoni, 2010; Teletchea, 2010; Padial *et al*., 2010; Kato *et al*., 2012; Simon *et al*., 2013; Hubert & Hanner, 2015; Shaffer *et al*., 2015). In this ambiance, the discussion that follows seeks to emphasize to integrate traditional taxonomy and DNA barcoding called the integrative taxonomy. Following that, we will discuss the limitations of morphology. Next, we will discuss the applications of the combination of DNA barcoding and traditional taxonomy within the context of combinations of characters, an integrative approach.

Limitations of Morphological taxonomy

Taxonomy is a root for the construction of tree of life and provides the base data for species identification, which is important in conservation and ecological studies (Wilson, 2004). Although taxonomy functions as a root of other disciplines, it is one of neglected fields, suffering because of low financial investment from funding agencies and the subsequent decrease in the students' interest (Godfray, 2002; Wilson, 2003; Pries & Marinoni, 2010). Therefore, approaches to stimulate and advance taxonomy must be developed (Godfray, 2002; Hebert *et al*., 2003; Tautz *et al*., 2003; Wheeler, 2007; La Salle *et al*., 2009). The following limitations of morphology-based taxonomy have been reported by several scientists: significant variation in taxonomically important traits, whether environmentally or genetically controlled, lead to incorrect identifications (Pires & Marinoni, 2010); some life stages (e.g., eggs and young larvae/nymphs) lack any distinguishing features, i.e. a morphology-based approach to species identification is inadequate; taxonomic keys are present for certain life stages, such as adults, larvae, and pupae; however, it is difficult for non-experts to use taxonomic keys; traditional taxonomy requires a high level of expertise for species identification.

DNA-based identification methods can overcome these limitations and provide a reliable means of identification when morphological identification is difficult.

Contradiction on DNA barcoding and DNA taxonomy

DNA barcoding has been considered as a threat to traditional morphology by taxonomists (Dunn, 2003; Lipscomb *et al*., 2003; Scotland *et al*., 2003a; Sperling, 2003; Will & Rubinoff, 2004; Ebach & Holdrege, 2005a,b; Gregory, 2005; Savolainen *et al*., 2005; Cameron *et al*., 2006). DNA-based species discrimination has been known to complement traditional morphological taxonomy. Cameron *et al*. (2006) challenged the feasibility of molecular identification, the cost of DNA barcoding and laboratory procedures for tissue extraction from the preserved specimens. The use of DNA barcoding to describe novel species has also been challenged (Lee, 2004; Ebach & Holdrege, 2005b; Hebert & Gregory, 2005; Meyer & Paulay, 2005; DeSalle *et al*., 2005, 2006; Rubinoff *et al*., 2006; Cognato, 2006; Meier *et al*., 2006; Whitworth *et al*., 2007). According to Cognato (2006), phenotypic analyses are based on total similarity; they fail to recover information regarding homology and homoplasy. Technical issues of a mitochondrial gene have also faced criticisms to delineate species boundaries (Rubinoff *et al*., 2006). The use of a mitochondrial genome is imposed as the only source of data for animal classification (Funk & Omland 2003; Ebach & Holdrege, 2005a; DeSalle *et al*., 2005; Meyer & Paulay, 2005; Rubinoff *et al*., 2006; DeSalle, 2006). Species discovery involves the use of taxonomy; therefore, it cannot solely use DNA barcodes to discover novel species (DeSalle *et al*., 2005; Desalle, 2006). It requires a species concept and a corroboration system (DeSalle *et al*., 2005). Thus, no single source of data, such as DNA, morphology, ecology, reproductive isolation or behavior, can be used to discover species (Rach *et al*., 2007). DNA taxonomy system will provide a platform to assemble the taxonomic knowledge and act as a suitable tool for species identification and description (Tautz *et al*., 2003). Genetic sequences were utilized as a supplementary material for taxonomic work along with other data, such as morphology and ecology.

DNA barcoding in species identification and revising species limits

Hebert *et al*. (2003a) proposed an identification system for specimens based on "DNA barcodes". Species description and identification are the main tasks in biology because biologists can ever conduct a research until the study organism is correctly identified. DNA barcoding is a widely used technique for species description (Hebert *et al*., 2004; Ward *et al*., 2005; Cywinska *et al*., 2006; Hajibabaei *et al*., 2006a; Smith *et al*., 2007; Borisenko *et al*., 2008; Kerr *et al*., 2009; Murthy *et al*., 2015). Tautz *et al* (2002 & 2003), proposed "DNA taxonomy" in which they reported the importance of DNA sequences for species-level taxonomy. Several studies have revised species limits using genetic distances (Zhi *et al*., 1996; Chu *et al*., 1999; Tang *et al*., 2003; Sun *et al*., 2003; Shih *et al*., 2004). According to previous research, numerous studies have demonstrated the use of barcoding in inventories of diverse taxa, including bats of Southeast Asia (Francis *et al*., 2010), freshwater invertebrates (Zhou *et al*., 2009; Laforest *et al*., 2013), tropical sand flies (Azpurua *et al*., 2010; Krüger *et al*., 2011), difficult to distinguish agricultural pest moths (Roe *et al*., 2006), pollinating insects in Africa (Nzeduru *et al*., 2012), diverse radiations of tropical weevils (Pinzón-Navarro *et al*., 2010a, 2010b; Tänzler *et al*., 2012), freshwater fishes in Africa (Swartz *et al*., 2008; Lowenstein *et al*., 2011), butterflies at country scales (Dinca *et al*., 2011; Hausmann *et al*., 2011), lizard genus Podarcis (Carretero, 2008), and Siberian sturgeon (Birstein, 2009). DNA barcode data allows comparison of species concepts across geographical boundaries, such as across vast oceanic distances (Goetze, 2010; Heimeier *et al*., 2010; Plaisance *et al*., 2011; Ranasinghe *et al*., 2012). For example, Fernandez-Triana *et al*. (2011) compared changes in a local wasp community, over 70 years' old museum specimens, using barcoding. DNA barcodes were also employed in revealing the cryptic species related to different groups, such as reptiles and amphibians (Hebert *et al*, 2004; Janzen *et al*, 2005; Smith *et al*., 2007; Smith *et al*., 2008; Moura *et al*., 2008; Funk *et al*., 2011; Jaafar *et al*., 2012; Werneck *et al*., 2015). An alternative to existing phenotypic approaches is the character-based DNA barcoding (DeSalle *et al*. 2005). Immature stages of life cycles of terrestrial as well as marine plants and animals were identified through DNA barcoding (Goetze, 2010; Heimeier *et al*., 2010; Hubert *et al*., 2010; Stern *et al*., 2010; Plaisance *et al*., 2011; Ranasinghe *et al*., 2012; Meiklejohn *et al*., 2013; Ko *et al*., 2013). These applications of DNA barcoding have opened new perspectives in fields as diversified as functional ecology (Smith *et al*., 2007), taxonomy (Hebert & Gregory, 2005; Miller ,2007; Smith *et al*., 2008), biogeography (Hubert *et al*., 2012), conservation (Forest *et al*., 2007), and wildlife forensics (Wong *et al*., 2008; Holmes *et al*., 2009; Ardura *et al*., 2010).

Integrative taxonomy approach

Integrative taxonomy approach is the most useful and authentic to specimen description. One of the main objective of using integrative approach, discovery and description of new taxa, cannot be accomplished with sequence data alone (Ebach & Holdrege, 2005b) because of the superposition of intra- and inter-specific variation (Meyer & Paulay, 2005; Cognato, 2006; Meier *et al*., 2006; Whitworth *et al*., 2007). However, this is not unique to molecular data and is encountered with other sets of data, such as morphology, ecology, and other sources (Will *et al*., 2005). The problems associated with the sole use of morphological data in taxonomy work are also acceptable in the case of phenotypic plasticity, cryptic species, and identification of immature stages (Packer *et al*., 2009; Hebert *et al*., 2003). Dayrat (2005) proposed a new approach to taxonomy, which combines multiple and complementary perspectives, such as phylogeography, comparative morphology, population genetics, ecology, development, behavior, named integrative taxonomy. This presents a comprehensive approach to species discrimination and a new approach to revitalize taxonomy.

Integration of molecular and morphological data is important to reveal cryptic species groups, which were not detected when a given species was initially described, based on morphology alone. The use of DNA in addition to morphology helps the recognition of cryptic species that consequently become distinguished based on both sources of characters (Wake, 2003; Schlick-Steiner *et al*., 2006; Roe & Sperling, 2007; Milankov *et al*., 2008; Vaglia *et al*., 2008; Fisher & Smith, 2008; Wiedenbrug *et al*., 2009; Hamada *et al*., 2010; Pante *et al*.,2015). Taxa, which are proposed on the basis of integrated data, are better defined and better-supported hypotheses for the development of other studies. Accordingly, biological research should focus on multidisciplinary complex problems. The combination of different sources of data in taxonomy is already established. There are numerous studies that support the integrative taxonomy (Wilson's, 2003; Fisher & Smith, 2008; Mengual *et al*., 2006; Smith *et al*., 2008; Ward *et al*., 2009; Mengual & Thompson, 2008; Goldstein & DeSalle., 2011). The collaboration between molecular tools and taxonomy make traditional taxonomy more attractive to investment funds. This increases its impact factor, making it more likely to compete with well-funded projects in medicine and genomics. Integrative taxonomy has been proposed as a framework to bring together these conceptual and methodological developments (Padial *et al*., 2010). The incorporation of DNA and morphological data may help in overcoming the taxonomic crisis (Carvalho *et al*., 2008).

Conclusion

Nomenclature of new species is a primary and important step when describing biodiversity and is the only way to ensure that scientists are talking about the same entity, and that all the data linked to conspecific specimens but produced by different researchers can be associated in a comparative analysis (Schlick-Steiner *et al*. 2007; Patterson *et al*. 2010; Satler *et al*. 2013). We are convinced that integrative approach, when associated with formal species description, is a good way to improve the quality of species hypotheses and associated descriptions, and should therefore be encouraged. There are numerous examples of using integrative approach to improve quality and reliability of their research and solve problems regarding cryptic species, immature life stages (Terry *et al*., 2000; Smith *et al*., 2008; Wiedenbrug *et al*., 2009; Ferri *et al*., 2009; Hamada *et al*., 2010; Malekzadeh-Viayeh *et al*., 2014; Pante *et al*., 2015). Crucial taxonomic information is facilitated by a growing body of cyber infrastructures, such as species names databases, images of type specimens, digitized descriptions, and historical literature (Hubert & Hammer, 2015). Data integration overcomes the difficulties imposed by the use of only one source of data and facilitates the further research of biodiversity (Mengual *et al*., 2006; Schlick-Steiner *et al*., 2006; Roe & Sperling, 2007; Milankov *et al*., 2008; Vaglia *et al*., 2008; Smith *et al*., 2008). One of its advantages is that new species descriptions that correspond to better-supported hypotheses will facilitate future studies (Fisher & Smith, 2008; Mengual & Thompson, 2008; Kirichenko *et al*., 2015). We successfully correlated the factors, such as phylogeography, comparative morphology, population genetics, ecology, development, behavior, and temperature by the integrative approach. Thus, an integrative and evolutionary framework provides a useful platform for the study of species on the planet.

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Competing interest

The authors declare that they have no competing interest.

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