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Delimitação e descrição de espécies crípticas: lições da sistemática de *Aurelia* (Cnidaria, Scyphozoa)

Delimitation and description of cryptic species: lessons from the systematics of *Aurelia* (Cnidaria, Scyphozoa)



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Delimitation and description of cryptic species: lessons from the systematics of *Aurelia* (Cnidaria, Scyphozoa)

Dissertação apresentada ao Instituto de Biociências da Universidade de São Paulo, para a obtenção de Título de Mestre em Ciências, na Área de Zoologia.

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General Introduction

“All systematists today, whether they like it or not, are Hennigian cladists” (Wheeler, 2012: 19). This sentence highlights a consensus that appears to exist across the scientific community, that a phylogenetic relationship means a genealogical relationship, and that evidence of these relationships comes from features that are shared and derived (Hennig, 1966). These relationships have been traditionally reconstructed based on morphology, but with the technological and methodological advances that accompanied the end of the 20th century, molecular data started to be included in the framework, and is nowadays an important component of systematic studies (Wheeler, 2012). The integration of genetic data brought not only new findings, but also new questions and issues.

One of these issues arise from the idea of DNA barcodes, that was proposed as the sole mean to overcome taxonomic impediment as we go through a global diversity crisis (Hebert *et al.*, 2003). Many studies since then have adopted barcoding into their studies, but as a neo-phenetic approach, its issues soon started to be acknowledged, at least for species delimitation (Valdecasas *et al.*, 2008; Collins & Cruickshank, 2013). Coalescent theory also brings new possibilities (Knowles & Carstens, 2007), but we are yet to understand how to operationally distinguish the fine-line between population and species level divergences (Sukumaran & Knowles, 2017), and so coalescent-based methods should be used with caution. In this sense, phylogenetic systematics, even though with its caveats, still compose a major component in species delimitation (Grant *et al.*, 2006; Gómez-Daglio & Dawson, 2017).

To some extent, the scientific community seems to agree in a broader species concept, or at least in the separation of the abstract concept of species and the operation of delimiting these units (de Queiroz, 2007). Nevertheless, it seems there is still some confusion. Congruence across methods, or even operational criteria, have been suggested as evidence for species delimitation, and therefore each of the methods and criteria considered would be different lines or sources of evidence (de Queiroz, 2007; Carstens *et al.*, 2013). The use of congruence and the discovery operations it represents, has already been discussed in the context of evolutionary biology (Kluge, 1998; Grant, 2002), yet epistemological issues remain.

Alongside species delimitation, challenges also arise for species descriptions. Molecular data have revealed previously undetected diverging lineages, which could not be readily told apart by morphology (Struck *et al.*, 2017). This hidden diversity, namely

‘cryptic’, has now been uncovered in many metazoan groups (Bickford *et al.*, 2007), and it sometimes creates parallel worlds populated by candidate species without formal descriptions (Jörger & Schrödl, 2013). The transition from species delimitation to description is a first necessary step in recognizing cryptic diversity and reducing nomenclatural confusion, which in turn is important not only for conservation but for many other studies, such as understanding ecological interactions, pathogen spread and disease, and even for chemical and pharmaceutical studies (Bickford *et al.*, 2007).

Recent efforts have attempted to provide genetic data in taxonomic descriptions, although not in a standardized way (Goldstein & DeSalle, 2010). Yet, a consensus that seems to remain is that descriptions should be character-based (Bauer *et al.*, 2011), regardless if these characters are derived from morphology or molecules. Even though there may be no reason to believe that a diagnostic character underlies the nature of species, these provide a comparable framework for species hypothesis, as well as facilitates species discovery and identification (Grant *et al.*, 2006; Bauer *et al.*, 2011). Recent efforts have accepted the undertaking and have described cryptic species based on molecular diagnostic characters, opening a new window in taxonomy (e.g., Jörger and Schrödl, 2013).

In this thesis, I attempted to further discussions on issues involving species delimitation, as well as perform delimitation and formal descriptions. The scyphozoan jellyfish genus *Aurelia*, long known to potentially encompass cryptic species (Dawson & Jacobs, 2001), was used in this context. In **Chapter 1**, a discussion is presented on the misconceptions and the epistemology that encircles species delimitation, currently applied methods, and the nature of evidence and discovery operations. In **Chapter 2**, initially, morphological analyses were performed, in order to better understand morphological variation that can occur in *Aurelia* medusae. With this data, alongside molecular analyses and recorded distributions, species were delimited and described for the genus. With this study, I expect to encourage and improve the framework for cryptic species delimitation and description, which I believe will help forward not only discussions on systematics and taxonomy, but also on an important second step to understand morphological plasticity and the patterns and processes involved in generating cryptic species.

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General Discussion and Conclusion

Species delimitation and descriptions form the basis of biology (Frost & Kluge, 1994). We should take advantage of all the information at hand to perform these, but with careful scrutiny on what is useful and informative within the framework of systematics and taxonomy (Valdecasas *et al.*, 2008). Recent proposals have suggested congruency across methods, or even operational criteria, as evidence for species delimitation (de Queiroz, 2007; Carstens *et al.*, 2013). In this study, I discussed that both operational criteria and methods, if not derived from different data, represent exclusive discovery operations (Grant, 2002), and therefore have no epistemic value as evidence. They only represent the degree to which the different analyses lead to the same or different conclusions (Grant, 2002). In that sense, I advised the use of epistemological and operational justifications on the careful choice of methods, further highlighting issues with some of them, such as DNA barcoding, coalescent theory, and even phylogenetic systematics.

DNA barcoding relies on a phenetic approach that can be of heuristic value for species identification in some groups, but it is unreliable for species delimitation (Collins & Cruickshank, 2013). This unreliability was here demonstrated, as well as its further limitations even for species identification. In the case of medusozoans (Gómez-Daglio & Dawson, 2017), which includes this study with *Aurelia*, COI seems as the best barcoding candidate, which can help first assessments and the detection of cryptic species. Nevertheless, there are still overlaps between some intra- and interspecific distances, likely due to variation on molecular evolution that can occur even across congeners (Grant *et al.*, 2006; Gómez-Daglio & Dawson, 2017). This apparent overall ‘gap’ on distances can still be due to undersampling of diversity (Wiemers & Fiedler, 2007). Phylogenetic analysis, even though with its caveats, still has its place as an important tool for species delimitation (Frost *et al.*, 1998; Grant *et al.*, 2006; Gómez-Daglio & Dawson, 2017).

We reassessed the use of medusae morphological characters for species delimitation and description, and observed an overlap in morphology across regions, with considerable variation within these, and even within collection lots and species hypotheses. Also, no regional structure in variation could be found, which could lead the distinction of species locally. This potential for morphological plasticity had been previously reported not only for the medusae stage (Chiaverano *et al.*, 2016), but also for other stages of the life cycle, such as polyps and ephyrae (Gambill & Jarms, 2014;

Chiaverano & Graham, 2017). Having detected this cryptic across *Aurelia* specimens, we based our species delimitation and descriptions mostly on multi-marker molecular analyses and species distributions. The description of biodiversity, whether cryptic or not, is a necessary step to accompany delimitation, especially in the current scenario of biodiversity crisis (Pimm *et al.*, 1995).

For species descriptions, I followed the current requirements and consensus in taxonomy of reporting character-based diagnosis (ICZN, 1999; Bauer *et al.*, 2011). Molecular diagnostic characters have been previously reported in descriptions, especially in the case of cryptic species (*e.g.*, Jörger & Schrödl, 2013; Maggioni *et al.*, 2017; see review in Goldstein & DeSalle, 2010). This scenario, allows a comparable outline for proposed species hypotheses (Bauer *et al.*, 2011; Jörger & Schrödl, 2013). Nevertheless, these diagnoses are not devoid of potential shortcomings. For example, with only four character-states possible, our study demonstrates that finding unique and non-homoplastic molecular synapomorphies might only be an artifact of low sampling. In that sense, a combination of the synapomorphies found can be more informative, and future studies can address the challenges of their practical applications for species identification. The use of diagnostic characters for identification can be more reliable than barcoding, as well as consistent with constructed species hypotheses.

This does not mean that morphology should be ignored, we should strive to better understand how speciation and morphology are related, what environmental factors may regulate morphological plasticity, and patterns and processes involved in generating cryptic (Swift *et al.*, 2016; Struck *et al.*, 2017). With this study, I hope to encourage research on these questions, as well as the recognition of cryptic diversity with the due caution and rigor established in the epistemology of taxonomy, systematics, and biology.

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Resumo

A delimitação e descrição de espécies, as unidades fundamentais da biologia, tem intrigado cientistas por séculos. Suas identidades eram tradicionalmente reconhecidas com base na distribuição, ecologia e, acima de tudo, na morfologia. Dados moleculares recentemente entraram em cena, e hoje formam um importante componente na maioria dos estudos de sistemática. Muitos métodos foram rapidamente elaborados e aplicados para integrar esses dados no processo de delimitação e descrição de espécies, e foram acompanhados não só por fascinantes possibilidades e descobertas, mas também por novas questões e desafios. Algumas problemáticas epistemológicas aparecem a partir de proposições recentes que sugerem congruência entre métodos, ou mesmo critérios operacionais, como evidência para delimitação. Além disso, a descoberta de linhagens genéticas morfologicamente indistinguíveis, descritas como ‘crípticas’, tem dificultado o reconhecimento e avaliações formais da diversidade biológica. No Capítulo 1, abordei o raciocínio epistemológico que envolve as operações de descoberta, métodos e congruência para a delimitação de espécies baseada em evidências. Discutimos que a congruência entre métodos, ou mesmo critérios operacionais, se baseados nos mesmos dados, são operações de descoberta exclusivas e, portanto, não tem valor epistêmico como evidência. Questões relacionadas a alguns métodos também são destacadas, incluindo a teoria de coalescência, o código de barras de DNA, e até mesmo a sistemática filogenética. No Capítulo 2, passei para a aplicação da delimitação e descrição de espécies em *Aurelia* (Cnidaria, Scyphozoa). Uma reavaliação morfológica de medusas coletadas ao redor do globo, não revelou nenhuma estrutura geográfica nas dissimilaridades, com considerável variação morfológica entre indivíduos de um mesmo lote de coleta e até mesmo da mesma espécie hipotética. Esta plasticidade morfológica já havia sido relatada em medusas para algumas espécies de *Aurelia*, bem como nos estágios de pólipo e éfira. Considerado essa diversidade críptica, análises moleculares com múltiplos marcadores e dados da distribuição foram utilizados para delimitar e descrever espécies. Também discuti sobre a inconfiabilidade do código de barras de DNA para a delimitação de espécies, e suas limitações até mesmo para identificação. Os caracteres moleculares diagnósticos relatados não apenas preenchem os requisitos necessários para as descrições, mas também sugerem a possibilidade de seu uso prático para identificação, em lugar de utilizar o código de barras de DNA. Esperamos que este estudo encoraje futuras pesquisas não apenas na delimitação e descrição da diversidade críptica, que deve incluir uma cuidadosa avaliação dos métodos e dados utilizados, mas também sobre plasticidade morfológica e os padrões e processos envolvidos na geração dessa diversidade.

Palavras-chave: filogenética, evidência, água-viva, taxonomia, sinapomorfia.

Abstract

The delimitation and description of species, the fundamental units of biology, have puzzled scientists for centuries. Their identities were traditionally recognized based on distribution, ecology and most of all, morphology. Molecular data have recently come into play, and nowadays form an important component of most systematic studies. Many methods have been quickly devised and applied to integrate these data in the species delimitation and description process, and they have been accompanied not only by exciting possibilities and discoveries, but also by new questions and challenges. Some epistemological issues appear from recent proposals that suggest congruency across methods, or even operational criteria, as evidence for delimitation. Also, the discovery of morphologically indistinguishable genetic lineages, described as ‘cryptic’, has hindered recognition and formal assessments of biological diversity. In Chapter 1, I address epistemological reasoning that encircles discovery operations, methods and congruency for evidence-based species delimitation. We discuss that congruence across methods or operational criteria, if based on the same data, are exclusive discovery operations and therefore have no epistemic value as evidence. Issues regarding some methods are also highlighted, including coalescent theory, DNA barcoding, and even phylogenetic systematics. In Chapter 2, I move into the application of species delimitation and description in *Aurelia* (Cnidaria, Scyphozoa). A morphological reassessment of medusae specimens from across the globe revealed no geographic structure on dissimilarities, with considerable morphological variation within collection lots and even within hypothesized species. This morphological plasticity had already been reported for medusae in some *Aurelia*, as well as in the polyp and ephyra stages. Considering this crypsis, multi-marker molecular analyses and distribution records were used to delimit and describe species. I also address the unreliability of DNA barcoding for species delimitation and its limitations even for identification. The reported diagnostic molecular characters not only fill the requirements for descriptions, but also hint on the possibility of its practical uses for identification, rather than barcoding. This study should encourages future research not only on delimitation and description of cryptic diversity, which should include careful scrutiny of methods and data used, but also on morphological plasticity and the patterns and processes involved in generating crypsis.

Keywords: phylogenetics, evidence, jellyfish, taxonomy, synapomorphy.