


CURRICULUM AND EDUCATION

Open Access



Using copepods to develop a didactic strategy for teaching species concepts in the classroom

Camila Moraes Gomes¹, Davidson Sodr ^{1,2}, Rauqu rio Marinho da Costa^{1,3}, Andr  Magalh es⁴, Renata Furtado do Ros rio¹, Stephen Francis Ferrari⁵, Grazielle Fernanda Evangelista Gomes¹, Iracilda Sampaio¹ and Marcelo Vallinoto^{1,6*} 

Abstract

While there is little doubt that the species is the lowest independent evolutionary unit, understanding the many different species concepts is a difficult task, even for university students. In the present study, we propose a didactic sequence that involves fieldwork, laboratory analyses, experimental cultures, and computational work in an integrated strategy for the comprehension of the phenetic, ecological, biological, and phylogenetic species concepts. This activity is based on the observation of the morphological, ecological, biological, and phylogenetic characteristics of samples of two copepod crustaceans, *Acartia tonsa* Dana, 1849 and *Acartia liljeborgi* Giesbrecht, 1889 (Copepoda, Calanoida). These species were the focus of a simple practical that contributes to the effective comprehension of the four species concepts mentioned above, using straightforward methods that can be standardized easily in the laboratory and classroom. The practical activities developed for the didactic sequence presented here not only made the classes more interesting and motivational, but also contributed to the more effective assimilation of the content, as well as the more effective consolidation of the knowledge presented in the class. It is important to note that these activities can be developed at different educational levels (i.e., undergraduate and graduate students), and can be applied to other types of organism (e.g., amphibians, insects or other copepods), as long as their characteristics are adequate for the systematic exploration of the four species concepts included here.

Keywords: Education, Practical activities, Copepods, Didactic strategies

Introduction

University students often face a number of difficulties when learning basic concepts in Biology (Lazarowitz and Penso 1992; Tekkaya et al. 2001), which is frequently due to the adoption of teaching methods based on simple memorization (Cimer 2012). Other, more participative teaching methods may not only contribute to a more

effective acquisition of knowledge, but can also help to develop practical skills (Jeronen et al. 2016). Given this, learning through the development of projects and experiments, in which the educator takes on the role of supervisor, can have a highly positive effect on the student (Palmberg et al. 2015), and the adoption of this type of approach can improve significantly the understanding of fundamental concepts in Biology.

Adequate comprehension of the concept of species—the fundamental evolutionary unit (Mayr 2004; Gao and Rieseberg 2020)—is essential to learning and research in many fields of Biology. In particular, the species concept is fundamental to studies of biodiversity and the

*Correspondence: mvallino@ufpa.br

¹ Laboratory of Evolution, Coastal Studies Institute, Universidade Federal do Par , Alameda Leandro Ribeiro, s/n, Aldeia, 68.600-000, Bragan a, PA, Brazil

Full list of author information is available at the end of the article



  The Author(s) 2022. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

elaboration of conservation policies and legislation, which demand the reliable quantification of species-level taxa (Agapow et al. 2004; de Queiroz 2007; Stanton et al. 2019; Hosegood et al. 2020). The importance of the species concept for Biology is equivalent to the difficulty of defining a concept that applies equally well to all the possible scenarios observed in nature (Rossello-Mora 2003). This has resulted in the proposal of a large number of different concepts, based on a range of different criteria, which may generate conflicting results, hampering both the adequate application of the concepts, in practise, and the understanding of their theoretical fundamentals (Hey 2006; Zachos 2016).

The unified concept, which defines the species as the smallest possible independent evolutionary unit, was proposed in an attempt to resolve this problem. This concept considers that two organisms represent distinct species when their populations follow different evolutionary trajectories (de Queiroz 2005, 2007). When this criterion is adopted, any evidence whatsoever of separation is considered to be relevant to the classification of the units, and distinct properties may be important for the delimitation of species (de Queiroz 2007). Although this concept has been adopted in some recent studies, there is considerable controversy with regard to the exact moment at which two lineages would be considered to have separated in evolutionary terms (Zachos 2016), a problem that exemplifies the ongoing debate on the species concept (Zachos 2018, 2019; Gippoliti 2019).

Depending on the type of approach, at least 32 different species concepts have been proposed up to now, and each of these proposals is based on a different set of classification criteria (Zachos 2016). This enormous diversity of concepts limits the ability of the educator to cover the whole range of approaches in the classroom, which means that, typically, only the best-known and most widely-used concepts are presented in textbooks and, in turn, in classes (Ridley 2003; Herron and Freeman 2014). In general, the most popular concepts are the biological (Mayr 1942), ecological (van Valen 1976), phenetic (Michener 1970; Sokal and Crovello 1970), and phylogenetic species concepts (Hennig 1966; Mishler and Donoghue 1982; Mishler and Brandon 1987).

The biological species concept is based on the reproductive isolation of populations (Mayr 1942), while the ecological concept evaluates the environmental conditions to which the species is adapted and the ways in which it interacts with this environment through its ecological niche (van Valen 1976). The phenetic species concept is based primarily on the analysis of diagnostic characters, while the phylogenetic concept focuses on the monophyletism of lineages, which can be determined by the analysis of DNA sequences (de Queiroz 2007).

While a number of studies demonstrate the application of these different concept (Razkin et al. 2017; Finot et al. 2018; Galen et al. 2018; Kajtoch et al. 2018; Nwankwo et al. 2018), incongruities often arise between the different approaches (Roca et al. 2001; Fišer et al. 2018; Pastori et al. 2018).

For example, Lee (2000) verified a number of incongruities in the data on geographic distance, genetic divergence, reproductive isolation, and the morphological differentiation of the copepod *Eurytemora affinis* Poppe, 1980 (Crustacea, Maxillopoda, Copepoda) from a number of different localities in the northern hemisphere. This analysis of 38 populations identified eight genetically divergent groups, which were morphologically indistinguishable. The study also confirmed reproductive incompatibility between genetically proximate and morphologically identical populations. In a review of cryptic amphipod species (Crustacea: Malacostraca: Peracarida), Fišer (2018) found that 109 morphological species actually encompassed a mean of 304 cryptic species, with estimates ranging from 295 to 315, depending on the approach.

Cryptic species, in particular, represent a major problem for the interpretation of biological diversity, and the application of the different species concepts (Kotsakiozi et al. 2018; Figueroa et al. 2020). All in all, these examples (which are just a few of the many similar studies) reinforce the difficulties faced by many researchers to delimit species and estimate the total number of taxa in existence (de Queiroz 2007). They also epitomise the fundamental practical challenges facing the teachers of this important component of the biological sciences in the classroom.

Despite the extensive body of published material on species concepts (de Queiroz 2005, 2007; Aldhebiani 2018; Zachos 2018), many students, and even educators, in some cases, are unable to gauge the practical importance of these concepts for the understanding of biodiversity (Palmberg et al. 2018). This is reflected in many common deficiencies in the assimilation of this knowledge and the consolidation of the learning process. If learning is defined as a change in the cognition, behavior, and attitudes of the individual that results from their experiences (Klein 2018), practical activities may often have a decisive impact on the assimilation of knowledge by the student (Randler and Bogner 2006; Price et al. 2016; Pope et al. 2017).

Randler and Bogner (2006) demonstrated that teaching students how to recognize and identify species can improve the consolidation of the key concepts necessary for the development of practical activities. Given this, we propose a didactic sequence based on practical activities that involve two copepod species, *Acartia tonsa* Dana, 1849 and *Acartia lilljeborgi* Giesbrecht, 1889 (Copepoda:

Calanoida), which are common on the Brazilian coast, as a strategy that aims to improve the understanding of the most widely-used species concepts by undergraduate students.

The choice of the two species used to demonstrate the practical application of species concepts was due to their ample distribution and sympatry, which facilitates the collection of samples and, in particular, the existence of traits appropriate for the application of the phenetic, ecological, biological, and phylogenetic species concepts. It is important to note that, while *A. tonsa* and *A. lilljeborgi* were used as the model for the present study, the practical activities proposed here can just as easily be developed with any number of other copepod species from different regions of the world, as well as other organisms or even microorganisms that satisfy the basic prerequisites for the distinction of the different concepts (as outlined below).

These activities were tested during classes in the discipline of Evolution, part of the undergraduate course in Biological Sciences at the Bragança campus of the Federal University of Pará, in northern Brazil. The approach was evaluated in terms of the effectiveness of the methods, which were discussed in class, and the performance of the student in the final exams.

Materials and methods

The practical activities presented here can be developed with either undergraduate or graduate students, attending courses in the biological and other natural sciences. The activities can be developed in two to four weeks, following the initial collection of the samples, although they do require basic equipment, including plankton nets, bottles, and stereoscopic microscopes (other equipment is specified below), and access to a teaching laboratory or similar working space.

As the activities described here involve a specific type of organism (in the present case, zooplankton), it is also important to provide the students with a theoretical overview of the biology of these organisms prior to the development of the didactic sequence. This overview can include topics such as the definition of the organisms, their ecological role and importance, the classification of species (biological, ecological, and phylogenetic characteristics), and methods for the collection of samples.

Collection of the biological material

Samples of zooplankton containing *A. tonsa* and *A. lilljeborgi* can be collected easily from most estuaries located within the geographic distribution of the two species, which includes the whole of the Brazilian coast (Fig. 1). It is important to note, however, that *A. tonsa* represents a species complex (Figueroa et al. 2020), so the practical

described here focuses on the cryptic species found in South America. The activities described here can be developed equally successfully using other copepod species, as long as they satisfy the criteria (see below) necessary for the differentiation of the species concepts.

The samples were collected by horizontal trawls of the subsurface water using a conical plankton net with a 120–200 µm mesh or by filtering a known volume of water (collected in plastic containers or using a suction pump) through a net of the same mesh size. The samples were processed in three ways, being: (i) fixed in 4% formaldehyde and stored in transparent plastic containers for the morphological identification of the organisms, (ii) fixed in 95% ethanol for the extraction of DNA, and (iii) stored on ice in a controlled environment to avoid the death of the specimens (which are taken immediately to the laboratory for culture).

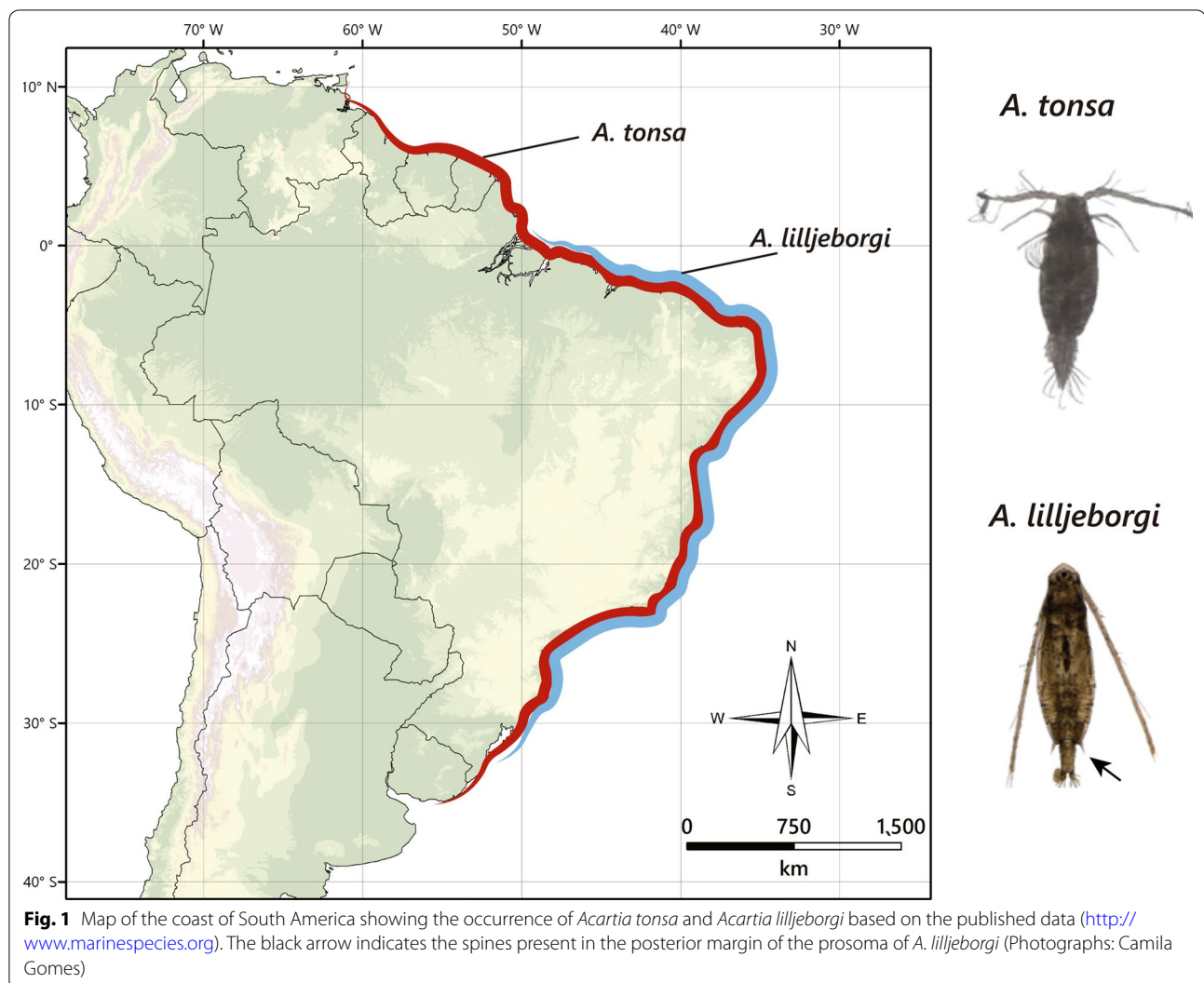
The morphological identification of copepods is normally based on specimens fixed in formaldehyde, given that, when fixed in ethanol, the carapace becomes transparent and, when viewed under a magnifying glass, the heat emitted by the bulb used to illuminate the specimens causes the ethanol to evaporate, resulting in a shimmer effect that hampers the viewing of the morphological traits. Specimens fixed in ethanol can nevertheless be used for the extraction of DNA, which tends to be degraded in formaldehyde (Tokuda et al. 1990).

The copepods stored on ice, which reduces the metabolism of these animals, are required for experimental culture in the laboratory. Storing on ice is important to cannibalism (Hansen et al. 2018), and this temporary immobility also facilitates the morphological identification of the specimens.

The genetic material of the specimens can be obtained using a specific lysis buffer containing detergents, following the protocol described by Lee and Frost (2002). Once the DNA has been extracted, the mitochondrial COI gene (the Cytochrome *b* gene can also be used) can be amplified by Polymerase Chain Reaction (PCR) using the primers and protocol described by Gomes (2018). The positive PCRs can be sequenced for analysis.

The COI sequences obtained in the laboratory can be analyzed together with GenBank sequences by the students using personal computers, who can learn basic approaches of genetic analysis, including the edition and alignment of the DNA sequences in MEGA X (Kumar et al. 2018), which can also be used to construct phylogenetic trees and calculate genetic distances (*p*). The phylogenetic trees can be edited and visualized in Figtree 1.4 (Rambaut and Drummond 2014).

For the culture of the copepods, the adult males and females of the two species must be identified and separated. The simplest way of doing this is to sort the



specimens by size, given that the females are typically larger (body length: 0.90–1.50 mm) than the males (1.00–1.10 mm). It is important at this stage not to select fecundated females, which would lead to false positive results during the tests of reproductive isolation (Plough et al. 2018). To ensure this, the females must be isolated from the males for at least 2 days (Holste and Peck 2006), which would be long enough to guarantee that any fecundated eggs hatch, resulting in the appearance of nauplii in the containers, given that these copepods have a relatively short generation time, of approximately 28 days (Mauchline 1998).

For the practical, the males and females must be separated and rearranged in groups of 10 or 12 individuals, half of which should be of each sex. These groups must be kept alive long enough to guarantee the experimental pairing of the males and females. The culture conditions are as follows:

1. The copepods are raised in sterile containers filled with filtered seawater obtained from the collecting site, in order to guarantee that the salinity of the captive environment is the same as that in the wild (Støttrup et al. 1986).
2. The detritus, feces, and leftover food should be syphoned daily from the containers to maintain the quality of the water (Støttrup et al. 1986; Plough et al. 2018);
3. The containers should be maintained under a 12/12 h light/dark photoperiod (Kaviyarasan and Santhanam 2019), and the organisms must be provisioned daily, given that food availability favors the hatching of the eggs (Hansen et al. 2018);
4. The copepods should be provisioned with marine microalgae, such as *Rhodomonas salinas* Hill and Wetherbee 1989 (Zhang et al. 2013; Arndt and Sommer 2014; Plough et al. 2018) or *Rhodomonas bal-*

tica Karsten 1898 (Costa and Fernández 2002; Costa et al. 2008). However, any other species known to be consumed by *Acartia* could be used to provision the copepods.

Results

Practical 1: The phenetic species concept

The phenetic species concept can be demonstrated through the identification of the morphological characteristics of the samples of *A. tonsa* and *A. lilljeborgi* using a stereoscopic microscope, Petri dishes, and identification keys (Boltovskoy 1999). The students should be provided with the identification key at the beginning of the exercise, to enable them to familiarize themselves with the morphological traits used to identify the species. Alternatively, the students can be directed to observe the specimens and identify their phenotypic differences without using an identification key. This would demonstrate the observational capacity of the students, although the key would need to be applied at the end of the exercise, to enable the identification of the species. The principal morphological traits used to distinguish *A. tonsa* and *A. lilljeborgi* are shown in Fig. 1.

Practical 2: The ecological species concept

As *A. tonsa* prefers more brackish water (salinity of 15–22) and *A. lilljeborgi*, more salty water, i.e., salinity of 25–35 (Montú and Goeden 1986; Cervetto et al. 1999; Magalhães et al. 2015), the density of the two species tends to vary systematically over the course of the year, in accordance with the fluctuation in the salinity of estuarine and coastal waters between the rainy and the dry seasons. As the two species coexist throughout the year, the ecological species concept can be demonstrated through the comparison of the counts of the two species in the samples collected during the rainy and dry seasons. As proposed above, then, copepod samples should be obtained either prior to the class or during the semester, to provide comparative data.

The students should also receive data on the salinity (the primary ecological variable) of the water at the sites from which the samples were collected. The copepod abundance and salinity should be plotted graphically to allow the students to visualize the relationship between the two variables.

The findings of the study of Magalhães et al. (2015) should be presented here to provide an example of the expected pattern. This study recorded a higher density of *A. tonsa* during the rainy season, and an increase in the abundance of *A. lilljeborgi* during the dry season, in the Taperaçu Estuary, in northern Brazil (Fig. 2). This pattern can be confirmed easily in practise by the students, given

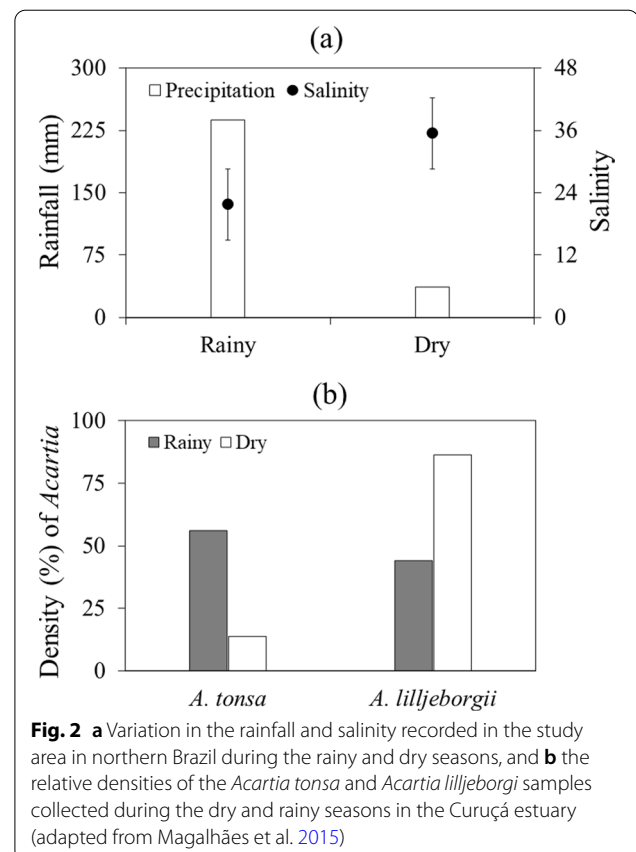
that *A. tonsa* and *A. lilljeborgi*, together with *Pseudodiaptomus marshalli*, Wright S., 1936, are the principal copepod species found in this estuary.

Practical 3: The phylogenetic species concept

The phylogenetic species concept can be demonstrated through the analysis of the genetic material obtained from the copepods collected by the students. It is important to note here that, if genetic analyses are not possible, COI sequences of the two *Acartia* species (or any other species used in this practical) can be obtained for analysis from GenBank. The phylogenetic tree obtained from the analysis of the sequences produced by the students during the present study, together with those obtained from GenBank, is shown in Fig. 3. This tree shows clearly that the *A. tonsa* (Ato) and *A. lilljeborgi* (Ali) clades are reciprocally monophyletic, and that the samples can be easily differentiated from each other, given that their genetic divergence is greater than 16%.

Practical 4: The biological species concept

The biological species concept can be presented using tests of reproductive isolation. For this, the captive



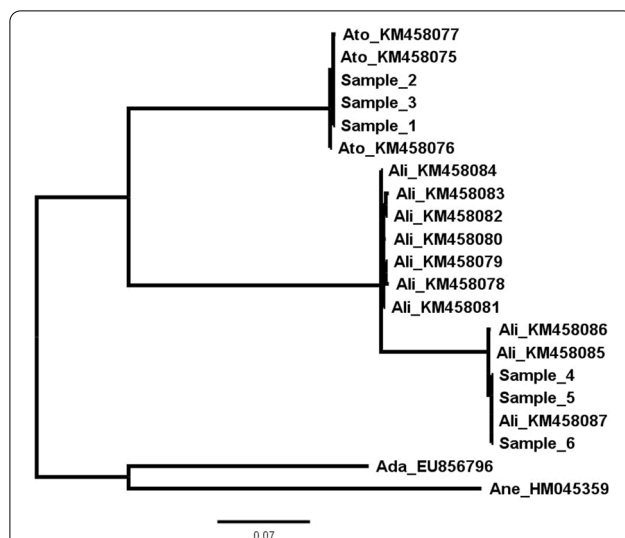


Fig. 3 Neighbor-joining tree based on sequences of the COI gene of the *A. tonsa* (Ato) and *A. lilljeborgi* (Ali) samples collected during the present study. *Acartia negligens* (Ane) and *Acartia danae* (Ada) were included as the outgroups. The samples collected for molecular identification are identified as “sample”, and the number at the end of each species code is the GenBank accession number

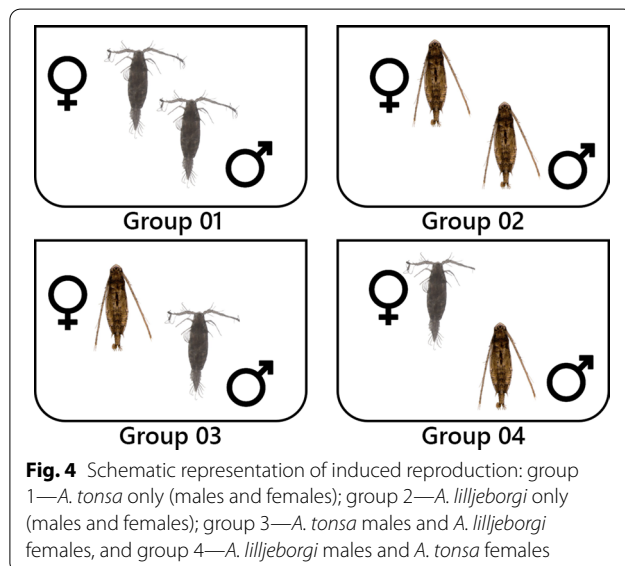


Fig. 4 Schematic representation of induced reproduction: group 1—*A. tonsa* only (males and females); group 2—*A. lilljeborgi* only (males and females); group 3—*A. tonsa* males and *A. lilljeborgi* females, and group 4—*A. lilljeborgi* males and *A. tonsa* females

copepods collected during the class, must be organized in four different containers corresponding to: group 1—*A. tonsa* only (males and females); group 2—*A. lilljeborgi* only (males and females); group 3—*A. tonsa* males and *A. lilljeborgi* females, and group 4—*A. lilljeborgi* males and *A. tonsa* females (Fig. 4).

Reproduction should only occur in groups 1 and 2, that is, between the males and females of the same species. This can be confirmed within 5–10 days, and the

experimental culture does not need to be maintained any longer than this. Reproductive efficiency can be evaluated through the analysis of groups 1 and 2, which can be designated as the positive controls.

Testing the didactic sequence proposed here

As assessment is an integral part of the teaching–learning process, and is essential for the establishment of an effective teaching approach (Leenknecht et al. 2021), it was essential to evaluate the outcome of the didactic sequence presented here. This evaluation was conducted primarily through the establishment of discussion groups, which allowed the students to reflect on the activities, and debate the themes presented. A more systematic evaluation was conducted using a written test which required the students to discuss what they learned about the four species concept presented during the didactic sequence, and discuss the themes included in the sequence. This was followed by diagnostic evaluation of the capability of the students to differentiate *A. tonsa* and *A. lilljeborgi*. The students received all the material – photographs, phylogenetic trees, graphs, etc. – presented during the activities.

Discussion

Although technological tools are becoming increasingly important for the accumulation and dissemination of knowledge, in particular in the educational field (Shim et al. 2003; Al-Azawei et al. 2017), the assimilation of some concepts remains a challenge, and may lead to persistent errors on the part of the students (Lazarowitz and Lieb 2006). The principal bottlenecks for the assimilation of knowledge include the difficulty of linking the learned content systematically with the external reality and, in particular, a lack of interest or motivation on the part of the students, with regard to the content (Tenenbaum et al. 2011). Given this, the development of practical activities, such as those presented here, can be an extremely valuable didactic tool. In addition to providing a much more effective contextualization of the content, these activities can convert the classes into a far more dynamic and mentally-stimulating process (Pajares and Schunk 2001; Bonney 2015). The need for interaction among the students may greatly increase their motivation and, eventually, contribute to their academic success (Pajares 1996, 2002; Pajares and Schunk 2001).

During the didactic sequence presented here, the students were involved in activities that enabled them to (i) identify and understand the morphological (practical 1) and genetic (practical 3) differences between the two study species, (ii) to demonstrate the influence of environmental factors on their occurrence in the wild (practical 2), and (iii) to confirm their reproductive isolation

(practical 4). By exploring the different species concepts in practise, the students were not only able to reinforce their understanding of their content presented in the classroom, but were also motivated to discuss the different approaches presented in class and their contribution to the understanding of biological phenomena.

In addition, the experiences accumulated by the students during the practical activities were likely to stimulate a number of questions, such as “what are the mechanisms responsible for the reproductive isolation of closely-related species that occur in sympatry?” or “if interbreeding does occur, what will happen to the hybrids?” related to post-zygotic incompatibility, that is, the Dobzhansky-Muller model of hybrid incompatibility (Coyne and Orr 2004). This type of association between theory and practise is important to guarantee that the processes understood during the didactic sequence can be extrapolated by the student to other types of organism, such as other copepods, insects, toads, or even mammals and birds (Carneiro et al. 2010; Sequeira et al. 2011; Poelstra et al. 2014; Taylor et al. 2014; Seixas et al. 2018; König et al. 2019; Lima et al. 2019). This will amplify their understanding of the concepts and ensure the consolidation of this knowledge (Ausubel 1963).

Given this, other examples can be presented in the form of explanatory texts or published papers, allowing the students to decide which of the species concepts they have learned could be applied (or not) to the study organisms, in this case, the toads of the genus *Rhinella* (see Sequeira et al. 2011; Acevedo et al. 2016; Sodré et al. 2018; Bessa-Silva et al. 2020). It is important to note here that the organisms chosen for these exercises were selected because of the specific knowledge of some of the co-authors of the present study. In other words, any other type of organism could be selected by the educator, according to their scientific specialty, to ensure the best possible discussion of the subject and the material. Finally, the educator also has the option of testing the knowledge of the students before and after the species concept practicals, in order to evaluate the quality of the teaching.

The performance of the students in the final test also indicated that their assimilation of the species concept was superior to that of the classes of previous years, which were not exposed to the didactic sequence presented here.

While questions on the species concept may be resolved easily by an experienced educator or researcher, they may not be so obvious to the typical undergraduate student. This is especially the case when the content is presented in a typical textbook manner, which may often employ terminology that is hard for the student to digest, especially when language is a barrier (Duran

et al. 1998). Clearly, any approach that brings the content closer to the everyday reality of the student and involves the hands-on resolution of problems will improve comprehension, contribute to the development of practical abilities, and ultimately ensure the more effective consolidation of knowledge (Pajares 1996, 2002; Pajares and Schunk 2001; Bonney 2015).

It is important to note here that, while two *Acartia* species were used as the model in the present study, the activities proposed here can just as easily be developed with any other type of organism, in accordance with the expertise of the educator, and the available of resources (in some more complex cases), such as the materials needed for the raising and breeding of the study organisms. Insects can be an excellent model for this practical, given their low maintenance costs, but any other type of organism can be used, including vertebrates such as mammals or amphibians, depending on the educator's own personal experience or access to resources. Even so, it is essential that the chosen organism satisfies the prerequisites for the systematic analysis of species concepts. That is, organisms with adequate levels of morphological, ecological, and genetic divergence, as well as reproductive isolation, although the inclusion of species able to hybridize may also be useful, especially for the demonstration of Haldane's rule (Orr 1997; Hay-Roe et al. 2007).

Conclusions

The use of practical activities as a complement to classroom content can be an important didactic tool, capable of both motivating the students and contributing to their assimilation of complex topics. These activities may not only ensure the more effective assimilation of complex ideas, such as the different species concepts, but also motivate the student to develop new abilities and the capacity to discuss academic content more knowledgeably.

Abbreviations

COI: Cytochrome Oxidase subunit I; Cyt b: Cytochrome b; PCR: Polymerase Chain Reaction; Ato: *Acartia tonsa*; Ali: *Acartia lilljeborgi*.

Acknowledgements

We would like to thank the UFPA Laboratory of Evolution and the Institute of Coastal Studies (IECOS) for technical support and infrastructure, and the undergraduate students of Biology and Natural Science courses of IECOS/UFPA for their support during the development of this study.

Authors' contributions

MV, DS and RMC designed the study; CG, RF and AM designed the experiments, CG, SF, IS, GFEG and MV wrote the manuscript. All authors read and approved the final manuscript.

Funding

This study was financed by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) through a graduate scholarship to CG and research productivity (PQ) fellowships to MV (308217/2019-5) and SFF (310852/2017-0).

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

All the students were previously informed that the results of their participation, as a group, would be used as the basis of the manuscript presented here. However, any data obtained directly by the students, such as answers to test questions, have not been provided in the text. All the biological samples were obtained in accordance with the requirements of Brazilian environmental legislation, and their collection was authorized by the Chico Mendes Federal Institute for Biodiversity Conservation (ICMBio), through license number 63712-1.

Competing interests

The authors confirm that they have no competing interests.

Author details

¹Laboratory of Evolution, Coastal Studies Institute, Universidade Federal do Pará, Alameda Leandro Ribeiro, s/n, Aldeia, 68.600-000, Bragança, PA, Brazil. ²Universidade Federal Rural da Amazônia, Campus de Capitão Poço, PA, Brazil. ³Marine Sciences Institute (ICM/CSIC), Passeig Marítim de la Barceloneta, 37-49. E-08003, Barcelona, Spain. ⁴Universidade Federal Rural da Amazônia, Campus de Capanema, PA, Brazil. ⁵Laboratory of Conservation Ecology, Universidade Federal de Sergipe, São Cristóvão, Brazil. ⁶Centro de Investigação em Biodiversidade and Recursos Genéticos, CIBIO-InBIO, Universidade do Porto, Laboratório Associado, Campus Agrário de Vairão, 4485-661 Vairão, Portugal.

Received: 12 November 2021 Accepted: 13 January 2022

Published online: 20 January 2022

References

- Acevedo AA, Lampo M, Cipriani R. The cane or marine toad, *Rhinella marina* (Anura, Bufonidae): two genetically and morphologically distinct species. *Zootaxa*. 2016. <https://doi.org/10.11646/zootaxa.4103.6.7>.
- Agapow P-M, Bininda-Emonds OR, Crandall KA, Gittleman JL, Mace GM, Marshall JC, Purvis A. The impact of species concept on biodiversity studies. *Q Rev Bio*. 2004. <https://doi.org/10.1086/383542>.
- Al-Azawei A, Parslow P, Lundqvist K. Investigating the effect of learning styles in a blended e-learning system: An extension of the technology acceptance model (TAM). *Australas J Educ Technol*. 2017. <https://doi.org/10.14742/ajet.2741>.
- Aldhebiani AY. Species concept and speciation. *Saudi J Biol Sci*. 2018. <https://doi.org/10.1016/j.sjbs.2017.04.013>.
- Arndt C, Sommer U. Effect of algal species and concentration on development and fatty acid composition of two harpacticoid copepods, *T. isbe* sp. and *T. achidius* discipes, and a discussion about their suitability for marine fish larvae. *Aquac Nutr*. 2014. <https://doi.org/10.1111/anu.12051>.
- Ausubel DP. The psychology of meaningful verbal learning. New York: Grune and Stratton; 1963.
- Bessa-Silva A, Vallinoto M, Sampaio I, Flores-Villela OA, Smith EN, Sequeira F. The roles of vicariance and dispersal in the differentiation of two species of the *Rhinella marina* species complex. *Mol Phyl Evol*. 2020. <https://doi.org/10.1016/j.ympev.2019.106723>.
- Boitovskoy D. South Atlantic Zooplankton. Leiden: Backhuys; 1999. p. 149–212.
- Bonney KM. Case study teaching method improves student performance and perceptions of learning gains. *J Microbiol Biol Educ*. 2015. <https://doi.org/10.1128/jmbe.v16i1.846>.
- Carneiro M, Blanco-Aguilar JA, Villafuerte R, Ferrand N, Nachman MW. Speciation in the European rabbit (*Oryctolagus cuniculus*): islands of differentiation on the X chromosome and autosomes. *Evol*. 2010. <https://doi.org/10.1111/j.1558-5646.2010.01092.x>.
- Cervetto G, Gaudy R, Pagano M. Influence of salinity on the distribution of *Acartia tonsa* (Copepoda, Calanoida). *J Exp Mar Biol Ecol*. 1999. [https://doi.org/10.1016/S0022-0981\(99\)00023-4](https://doi.org/10.1016/S0022-0981(99)00023-4).
- Cimer A. What makes biology learning difficult and effective: Students' views. *Educ Res Rev*. 2012. <https://doi.org/10.5897/ERR11.205>.
- Costa R, Fernández F. Feeding and survival rates of the copepods *Euterpina acutifrons* Dana and *Acartia grani* Sars on the dinoflagellates *Alexandrium minutum* Balech and *Gyrodinium corsicum* Paulmier and the *Chrytophyta* *Rhodomonas baltica* Karsten. *J Exp Mar Biol Ecol*. 2002. [https://doi.org/10.1016/S0022-0981\(02\)00132-6](https://doi.org/10.1016/S0022-0981(02)00132-6).
- Costa RM, Pereira LCC, Fernández F. Effects of toxic *Alexandrium minutum* strains on the feeding and survival rates of pelagic marine copepods *Acartia grani* and *Euterpina acutifrons*. *Hydrobiologia*. 2008. <https://doi.org/10.1007/s10750-008-9536-4>.
- Coyne JA, Orr HA. Speciation. Sunderland, MA: Sinauer Associates, Inc; 2004.
- de Queiroz K. A unified concept of species and its consequences for the future of taxonomy. *Proc Calif Acad Sci*. 2005;56(Suppl 1):196–215.
- de Queiroz K. Species concepts and species delimitation. *Syst Biol*. 2007. <https://doi.org/10.1080/10635150701701083>.
- Duran BJ, Dugan T, Weffer R. Language minority students in high school: The role of language in learning biology concepts. *Sci Educ*. 1998. [https://doi.org/10.1002/\(SICI\)1098-237X\(199806\)82:3%3C311::AID-SCE2%3E3.0.CO;2-F](https://doi.org/10.1002/(SICI)1098-237X(199806)82:3%3C311::AID-SCE2%3E3.0.CO;2-F).
- Figuerola NJ, Figuerola DF, Hicks D. Phylogeography of *Acartia tonsa* Dana, 1849 (Calanoida: Copepoda) and phylogenetic reconstruction of the genus *Acartia* Dana, 1846. *Mar Biodivers*. 2020. <https://doi.org/10.1007/s12526-020-01043-1>.
- Finot VL, Soreng RJ, Giussani LM, Muñoz RG. A multivariate morphometric delimitation of species boundaries in the South American genus *Nicorae-poa* (Poaceae: Pooideae: Poeae). *Plant Syst Evol*. 2018. <https://doi.org/10.1007/s00606-018-1499-y>.
- Fišer C, Robinson CT, Malard F. Cryptic species as a window into the paradigm shift of the species concept. *Mol Ecol*. 2018. <https://doi.org/10.1111/mec.14486>.
- Galen SC, Nunes R, Sweet PR, Perkins SL. Integrating coalescent species delimitation with analysis of host specificity reveals extensive cryptic diversity despite minimal mitochondrial divergence in the malaria parasite genus *Leucocytozoon*. *BMC Evol Biol*. 2018. <https://doi.org/10.1186/s12862-018-1242-x>.
- Gao L, Rieseberg LH. While neither universally applicable nor practical operationally, the biological species concept continues to offer a compelling framework for studying species and speciation. *Natl Sci Rev*. 2020. <https://doi.org/10.1093/nsr/nwaa108>.
- Gippoliti S. Species delimitation in mammals: A comment on Zachos (2018). *Mamm Biol*. 2019. <https://doi.org/10.1016/j.mambio.2018.05.007>.
- Gomes CM, Costa KG, Ferreira GS, Costa RM, Asp NE, Barros B, Vallinoto M. Hidden diversity in cryptic Brazilian lineages of *Acartia* (Copepoda, Calanoida, Acartiidae). *J Sea Res*. 2018. <https://doi.org/10.1016/j.seares.2018.08.005>.
- Hansen BW, Boesen E, Brodnick OB, Corfixen NL, Jepsen PM, Larsen SM, Læssøe CD, Munch PS, Nielsen PK, Olesen J. Interactions between populations of the calanoid copepod *Acartia tonsa* Dana and the harpacticoid copepod *Tisbe holothuriae* Humes in mixed cultures of live feed for fish larvae. *Aquac Res*. 2017. <https://doi.org/10.1111/are.13581>.
- Hay-Roe MM, Lamas G, Nation JL. Pre-and postzygotic isolation and Haldane rule effects in reciprocal crosses of *Danaus erippus* and *Danaus plexippus* (Lepidoptera: Danainae), supported by differentiation of cuticular hydrocarbons, establish their status as separate species. *Biol J Linn Soc*. 2007. <https://doi.org/10.1111/j.1095-8312.2007.00809.x>.
- Hennig W. *Phylogenetic Systematics*. Urbana, IL: University of Illinois Press; 1966.
- Herron J, Freeman S. *Evolutionary analysis*. 5th ed. Boston: Pearson Education; 2014.
- Hey J. On the failure of modern species concepts. *Trends Ecol Evol*. 2006. <https://doi.org/10.1016/j.tree.2006.05.011>.
- Holste L, Peck MA. The effects of temperature and salinity on egg production and hatching success of Baltic *Acartia tonsa* (Copepoda: Calanoida): a laboratory investigation. *Mar Biol*. 2006. <https://doi.org/10.1007/s00227-005-0132-0>.
- Hosegood J, Humble E, Ogden R, de Bruyn M, Creer S, Stevens GM, Abudaya M, Bassos-Hull K, Bonfil R, Fernando D. Phylogenomics and species

- delimitation for effective conservation of manta and devil rays. *Mol Ecol*. 2020. <https://doi.org/10.1111/mec.15683>.
- Jeronen E, Palmberg I, Yli-Panula E. Teaching methods in biology education and sustainability education including outdoor education for promoting sustainability—A literature review. *Educ Sci*. 2016. <https://doi.org/10.3390/educsci7010001>.
- Kajtoch Ł, Montagna M, Wanat M. Species delimitation within the *Bothryorhynchapion weevils*: Multiple evidence from genetics, morphology and ecological associations. *Mol Phylogenetics Evol*. 2018. <https://doi.org/10.1016/j.ympev.2017.12.022>.
- Kaviyarasan M, Santhanam P. A Technique on the Culture and Preservation of Marine Copepod Eggs. *Basic and Applied Zooplankton Biology*: Springer; 2019. p. 197–208.
- Klein SB. *Learning: Principles and applications*. Boston: Sage Publications; 2018. p. 528.
- König K, Zundel P, Krimmer E, König C, Pollmann M, Gottlieb Y, Steidle JL. Reproductive isolation due to prezygotic isolation and postzygotic cytoplasmic incompatibility in parasitoid wasps. *Ecol Evol*. 2019. <https://doi.org/10.1002/ece3.5588>.
- Kotsakiozi P, Jablonski D, Ilgaz Ç, Kumlutaş Y, Avcı A, Meiri S, Itescu Y, Kukushkin O, Gvoždík V, Scillitani G. Multilocus phylogeny and coalescent species delimitation in Kotschy's gecko, *Mediodactylus kotschy*: Hidden diversity and cryptic species. *Mol Phylogenetics Evol*. 2018. <https://doi.org/10.1016/j.ympev.2018.03.022>.
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol Biol Evol*. 2018. <https://doi.org/10.1093/molbev/msy096>.
- Lazarowitz R, Lieb C. Formative assessment pre-test to identify college students' prior knowledge, misconceptions and learning difficulties in biology. *Int J Sci Math Educ*. 2006. <https://doi.org/10.1007/s10763-005-9024-5>.
- Lazarowitz R, Penso S. High school students' difficulties in learning biology concepts. *J Biol Educ*. 1992. <https://doi.org/10.1080/00219266.1992.9655276>.
- Lee CE. Global phylogeography of a cryptic copepod species complex and reproductive isolation between genetically proximate "populations." *Evol*. 2000. <https://doi.org/10.1111/j.0014-3820.2000.tb01245.x>.
- Lee CE, Frost BW. Morphological stasis in the *Eurytemora affinis* species complex (Copepoda: Temoridae). *Hydrobiologia*. 2002. <https://doi.org/10.1023/a:1021293203512>.
- Leenknecht M, Wijnia L, Köhlen M, Fryer L, Rikers R, Loyens S. Formative assessment as practice: the role of students' motivation. *Assess Eval High Educ*. 2021. <https://doi.org/10.1080/02602938.2020.1765228>.
- Lima TG, Burton RS, Willett CS. Genomic scans reveal multiple mito-nuclear incompatibilities in population crosses of the copepod *Tigriopus californicus*. *Evol*. 2019. <https://doi.org/10.1111/evo.13690>.
- Magalhães A, Pereira LCC, Costa RM. Relationships between copepod community structure, rainfall regimes, and hydrological variables in a tropical mangrove estuary (Amazon coast, Brazil). *Helgol Mar Res*. 2015. <https://doi.org/10.1007/s10152-014-0421-4>.
- Mauchline J. *The Biology of Calanoid Copepods* *Advances in Marine Biology*. New York: Elsevier; 1998. p. 710.
- Mayr E. *Systematics and the Origin of Species from the Viewpoint of a Zoologist*. New York: Columbia University Press; 1942. p. 372.
- Mayr E. What makes biology unique?: considerations on the autonomy of a scientific discipline. Cambridge: Cambridge University Press; 2004. p. 246.
- Michener CD. Diverse Approaches to Systematics. *Evol Biol*. 1970;4:1–38.
- Mishler BD, Brandon RN. Individuality, pluralism, and the phylogenetic species concept. *Biol Philos*. 1987. <https://doi.org/10.1007/bf00127698>.
- Mishler BD, Donoghue MJ. Species concepts: a case for pluralism. *Syst Zool*. 1982. <https://doi.org/10.2307/2413371>.
- Montú M, Goeden IM. Atlas dos Cladocera e Copepoda (Crustacea) do estuário da lagoa dos Patos (Rio Grande, Brasil). *Revista Nerítica*. 1986. <https://doi.org/10.5380/rn.v1i2.41190>.
- Nwankwo EC, Pallari CT, Hadjoannou L, Ioannou A, Mulwa RK, Kirschel AN. Rapid song divergence leads to discordance between genetic distance and phenotypic characters important in reproductive isolation. *Ecol Evol*. 2018. <https://doi.org/10.1002/ece3.3673>.
- Orr HA. Haldane's rule. *Annu Rev Ecol Syst*. 1997. <https://doi.org/10.1146/annurev.ecolsys.28.1.195>.
- Pajares F. Self-efficacy beliefs in academic settings. *Rev Educ Res*. 1996. <https://doi.org/10.3102/00346543066004543>.
- Pajares F. Gender and perceived self-efficacy in self-regulated learning. *Theory Pract*. 2002. https://doi.org/10.1207/s15430421tip4102_8.
- Pajares F, Schunk DH. Self-beliefs and school success: Self-efficacy, self-concept, and school achievement. In: Riding RJ, Rayner SG, editors. *Perception*. London: Ablex Publishing; 2001. p. 11:239–266.
- Palmberg I, Berg I, Jeronen E, Kärkkäinen S, Norrgård-Sillanpää P, Persson C, Vilkonis R, Yli-Panula E. Nordic-Baltic student teachers' identification of and interest in plant and animal species: The importance of species identification and biodiversity for sustainable development. *J Sci Teacher Educ*. 2015. <https://doi.org/10.1007/s10972-015-9438-z>.
- Palmberg I, Hermans M, Jeronen E, Kärkkäinen S, Persson C, Yli-Panula E. Nordic Student Teachers' Views on the Importance of Species and Species Identification. *J Sci Teacher Educ*. 2018. <https://doi.org/10.1080/1046560X.2018.1468167>.
- Pastori T, Eggers L, de Souza-Chies TT, Chauveau O. Iterative taxonomy based on morphological and molecular evidence to estimate species boundaries: a case study in *Cypella* (Iridaceae: Iridoideae). *Plant Syst Evol*. 2018. <https://doi.org/10.1007/s00606-018-1536-x>.
- Plough L, Fitzgerald C, Plummer A, Pierson J. Reproductive isolation and morphological divergence between cryptic lineages of the copepod *Acartia tonsa* in Chesapeake Bay. *Mar Ecol Prog Ser*. 2018. <https://doi.org/10.3354/meps.12569>.
- Poelstra JW, Vijay N, Bossu CM, Lantz H, Ryll B, Müller I, Baglione V, Unneberg P, Wikelski M, Grabherr MG. The genomic landscape underlying phenotypic integrity in the face of gene flow in crows. *Science*. 2014. <https://doi.org/10.1126/science.1253226>.
- Pope DS, Rounds CM, Clarke-Midura J. Testing the effectiveness of two natural selection simulations in the context of a large-enrollment undergraduate laboratory class. *Evolution*. 2017. <https://doi.org/10.1186/s12052-017-0067-1>.
- Price RM, Pope DS, Abraham JK, Maruca S, Meir E. Observing populations and testing predictions about genetic drift in a computer simulation improves college students' conceptual understanding. *Evolution*. 2016. <https://doi.org/10.1186/s12052-016-0059-6>.
- Rambaut A, Drummond AJ. FigTree version 1.4, available at <http://tree.bio.ed.ac.uk/software/figtree/>. 2014.
- Randler C, Bogner FX. Cognitive achievements in identification skills. *J Biol Educ*. 2006. <https://doi.org/10.1080/00219266.2006.9656038>.
- Razkin O, Gómez-Moliner BJ, Vardinoyannis K, Martínez-Ortí A, Madeira MJ. Species delimitation for cryptic species complexes: case study of *Pyramidula* (Gastropoda, Pulmonata). *Zool Scr*. 2017. <https://doi.org/10.1111/zsc.12192>.
- Ridley M. *Evolution*. New York: Wiley-Blackwell; 2003. p. 778.
- Roca AL, Georgiadis N, Pecon-Slattery J, Obrien SJ. Genetic evidence for two species of elephant in Africa. *Science*. 2001. <https://doi.org/10.1126/science.1059936>.
- Rossello-Mora R. Opinion: the species problem, can we achieve a universal concept? *Syst Appl Microbiol*. 2003. <https://doi.org/10.1078/07320203322497347>.
- Seixas FA, Boursot P, Melo-Ferreira J. The genomic impact of historical hybridization with massive mitochondrial DNA introgression. *Genome Biol*. 2018. <https://doi.org/10.1186/s13059-018-1471-8>.
- Sequeira F, Sodré D, Ferrand N, Bernardi JAR, Sampaio I, Schneider H, Vallinoto M. Hybridization and massive mtDNA unidirectional introgression between the closely related Neotropical toads *Rhinella marina* and *R. schneideri* inferred from mtDNA and nuclear markers. *BMC Evol Biol*. 2011. <https://doi.org/10.1186/1471-2148-11-264>.
- Shim K-C, Park J-S, Kim H-S, Kim J-H, Park Y-C, Ryu H-I. Application of virtual reality technology in biology education. *J Biol Educ*. 2003. <https://doi.org/10.1080/00219266.2003.9655854>.
- Sodré D, Rocha T, Rendeiro F, Vallinoto M. A case of prolonged amplexus between males of *Rhinella mirandaribeiroi* and *R. marina* on Marajó Island, state of Pará. *Brazil Herpetol Notes*. 2018;11:127–8.
- Sokal RR, Crovello TJ. The biological species concept: a critical evaluation. *Am Nat*. 1970;104:127–53.
- Stanton DW, Frandsen P, Waples RK, Heller R, Russo IRM, Orozco-Wengel PA, Pedersen C-ET, Siegmund HR, Bruford MW. More grist for the mill? Species delimitation in the genomic era and its implications for conservation. *Conserv Genet*. 2019. <https://doi.org/10.1007/s10592-019-01149-5>.

- Støttrup JG, Richardson K, Kirkegaard E, Pihl NJ. The cultivation of *Acartia tonsa* Dana for use as a live food source for marine fish larvae. *Aquaculture*. 1986. [https://doi.org/10.1016/0044-8486\(86\)90028-1](https://doi.org/10.1016/0044-8486(86)90028-1).
- Taylor SA, White TA, Hochachka WM, Ferretti V, Curry RL, Lovette I. Climate-mediated movement of an avian hybrid zone. *Curr Biol*. 2014. <https://doi.org/10.1016/j.cub.2014.01.069>.
- Tekkaya C, Özkan Ö, Sungur S. Biology concepts perceived as difficult by Turkish high school students. *Hacettepe Üniversitesi Eğitim Fakültesi Dergisi*. 2001;21:145–50.
- Tenenbaum JB, Kemp C, Griffiths TL, Goodman ND. How to grow a mind: Statistics, structure, and abstraction. *Science*. 2011. <https://doi.org/10.1126/science.1192788>.
- Tokuda Y, Nakamura T, Satonaka K, Maeda S, Doi K, Baba S, Sugiyama T. Fundamental study on the mechanism of DNA degradation in tissues fixed in formaldehyde. *J Clin Pathol*. 1990. <https://doi.org/10.1136/jcp.43.9.748>.
- van Valen L. Ecological species, multispecies, and oaks. *Taxon*. 1976. <https://doi.org/10.2307/1219444>.
- Zachos FE. (New) Species concepts, species delimitation and the inherent limitations of taxonomy. *J Genet*. 2018. <https://doi.org/10.1007/s12041-018-0965-1>.
- Zachos FE. Species delimitation in mammals: a response to Gippoliti. *Mamm Biol*. 2019. <https://doi.org/10.1016/j.mambio.2018.05.008>.
- Zachos FE. Species concepts in biology historical development, theoretical foundations and practical relevance. New York: Springer; 2016. doi:<https://doi.org/10.1007/978-3-319-44966-1>.
- Zhang J, Wu C, Pellegrini D, Romano G, Esposito F, Ianora A, Buttino I. Effects of different monoalgal diets on egg production, hatching success and apoptosis induction in a Mediterranean population of the calanoid copepod *Acartia tonsa* (Dana). *Aquaculture*. 2013. <https://doi.org/10.1016/j.aquaculture.2013.02.032>.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

