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Lesson

What is Speciation, How Does It Occur, and Why Is It Important for Conservation?

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Abstract

Speciation provides a framework for classifying biodiversity on Earth and is a central concept in evolutionary biology. To help undergraduate students learn about speciation, we designed a student-centered lesson that uses active-learning techniques (e.g., clicker questions, small group work, and whole class discussion) and compares multiple species concepts (morphological, biological, and phylogenetic) using giraffes as an example. Giraffes were chosen as the focus of this lesson because they are familiar and have broad appeal to students; are in danger of becoming extinct; and have ecological, economic, and cultural importance. Students also learn about contemporary giraffe conservation issues and the current debate in the literature regarding the total number of giraffe species. Students then apply their knowledge by working in small groups on speciation scenarios that highlight organisms across the tree of life. Student understanding is assessed using multiple-choice pre/post-test questions, in-class clicker questions with peer discussion, and exam questions. Here we provide details about the lesson and report that student learning is improved.

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Supporting Materials: Supporting Files S1. Speciation and Conservation – Presentation Slides with Instructor Notes; S2. Speciation and Conservation – Discussion After Application Worksheet Activity; S3. Speciation and Conservation – Student Worksheet Packet; S4. Speciation and Conservation – Clicker Questions; S5. Speciation and Conservation – Pre/Post-Test Questions; S6. Speciation and Conservation – Exam Questions; and S7. Speciation and Conservation – References Table.

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Learning Goal(s)

- Students will explore the process of speciation by comparing definitions of a species, describing the mechanisms of speciation, and exploring how speciation affects conservation issues.

Learning Objective(s)

- LO-1: Students will be able to explain why defining species may be difficult and examine scenarios that illustrate common challenges in distinguishing species.
- LO-2: Students will be able to describe three commonly accepted ways in which species are defined (morphological, biological, and phylogenetic) and use these definitions to evaluate scientific evidence.
- LO-3: Students will be able to determine whether or not speciation is likely to occur in different scenarios and describe how this likelihood is influenced by the mechanisms of evolutionary change (natural selection, genetic drift, gene flow, mutation).
- LO-4: Students will be able to recognize global issues (e.g., conservation) for which it is important to know whether similar organisms are one or more species.

INTRODUCTION

Speciation – the evolution of discrete biological lineages (1) – is a central concept in evolutionary biology. It is comprised of complex evolutionary processes that produce the distinct groups that we call species (1). Speciation occupies the intersection of micro- and macro-evolutionary patterns and spans both deep and contemporary time scales, making it an important topic for any course on evolutionary biology. Knowledge about this concept is also critical for making decisions about conservation issues.

While students can easily recognize distinct species, it can be difficult for students to understand the complexity of the interconnected processes that produce them (2). Speciation may cause confusion among students for several reasons, such as the multiple – and sometimes conflicting – ways in which a species can be defined. For example, there are a number of species concepts that are often used, including morphological (in which organisms that have distinguishable phenotypic characteristics are classified as members of different species); biological (in which organisms that are capable of interbreeding in nature and producing fertile offspring are classified as members of the same species); and phylogenetic (in which organisms that have inherited traits from a recent common ancestor that uniquely distinguishes them from other groups are classified as members of the same species). Student confusion can also arise given the variation in how mechanisms of evolutionary change (natural selection, genetic drift, gene flow, and mutation) can influence speciation, and the use of inconsistent terminology by experts (3,4). This complexity makes teaching about speciation a challenge and can result in inconsistent strategies to explain this crucial concept.

While nearly all college evolution courses address speciation (5), learning materials focus predominantly on species concepts and fossil evidence for speciation events, rather than the speciation process itself. Published lessons range in focus, including delimiting species according to the biological species concept (2,6,7), exploring morphological differences between groups (8), examining the influence of biogeography on speciation (9), and learning about the role of natural selection and genetic drift (2,10). Notably, there are no concept assessments/inventories targeting students' understanding of speciation available in the literature, though some assessments (9-12) have addressed students' knowledge of speciation within a wider breadth of concepts. Variations in the way we teach speciation highlight the need for integrative lesson plans that clearly articulate details of the speciation process, with formative and summative assessments of students' understanding. To meet this goal, the key is to emphasize a lack of gene flow between populations as the central role in speciation (1,13) while probing students' thinking on the subject and their command of the material (5).

To meet these needs, we designed a student-centered lesson for an undergraduate introductory evolution class, which uses active-learning techniques: clicker questions, small group activities, and whole class discussions. This lesson integrates the evolutionary processes underlying speciation using the example of African giraffes (Giraffa sp.). African giraffes were chosen because they are a species familiar to the students; are in danger of becoming extinct; and have ecological, economic, and cultural significance. Students learn about the speciation process and a subset of the most commonly used species concepts (morphological, biological, and phylogenetic) in the context of a case study with real-world implications for giraffe conservation. This strategy immerses biological concepts within issues of social relevance to improve learning retention (14,15), while highlighting the work of international researchers from diverse backgrounds in the biological sciences to foster inclusive learning (16). Students then explore speciation concepts via a worksheet activity that features multiple taxa. Taken together, this lesson offers a conceptually holistic approach that explores a broad range of taxa (e.g., mammals, plants, birds) for teaching speciation.

Intended Audience

This lesson was implemented in an undergraduate introductory evolution course for students who are not majoring in the biological sciences, but would also be appropriate to teach as part of the evolution unit in an introductory biology course for majors. The course in which we tested this lesson was offered through the Department of Ecology and Evolutionary Biology at Cornell University. Students in this course ranged in undergraduate education level (from first-year through senior class standing), and the majority of students had no prior background in biology beyond high school.

The lesson was taught as part of the regular semester course that does not have an associated lab. We originally piloted the lesson in a summer course of seven students, but all data presented in this manuscript are from the regular semester course of 50 students. While we taught this lesson at a Ph.D.-granting research university, the content is transferable to other institutions and can be implemented in a variety of class sizes.

Required Learning Time

This lesson is designed to be taught over two 75-minute class periods and includes out-of-class pre and post-tests (see Table 1 for the progression through the lesson with approximate timing). The lesson could also be taught over three 50-minute class periods. The first class period could include Activities 1-4 in Table 1, the second class period Activities 5-8, and the last class period Activities 9-10. Alternatively, if instructors wish to teach this in a two-hour lab block, they could provide some of the background information and clicker questions (Activities 1-4 in Table 1) in a lecture class period and complete Activities 6-10 in the laboratory section.

Prerequisite Student Knowledge

Before participating in this lesson, students should be capable of reading a phylogenetic tree and thinking about mechanisms of evolutionary change. Terms associated with mechanisms of evolutionary change include gene flow (movement of genetic material from one population to another); mutation (change in the DNA); genetic drift (changes in genotype frequency within a population through a random process); and natural selection (the non-random process by which biological traits become more or less common in a population as a result of the differential reproductive success). More information on these terms and ideas can be found here: https://evolution.berkeley.edu/evolibrary/article/evo_14. Previous work using concept assessment/inventories reveals that students often struggle with these concepts (12,17-20), so revisiting them in the context of this speciation unit may be helpful for their learning.

Prerequisite Teacher Knowledge

We recommend that the instructor reads about speciation – and the mechanisms that drive it (e.g., mutation, natural selection, genetic drift, and lack of gene flow) – at a level typically presented in introductory biology textbooks. We also suggest that the instructor is aware of potential conceptual difficulties that students may have about evolution, especially in connection with genetic drift and natural selection. One way to explore those difficulties is to read manuscripts associated with concept assessments/inventories because these papers often discuss common conceptual difficulties identified during the instrument development and response validation process. Useful concept assessments/inventories for consultation include Ecology and Evolution-Measuring Achievement and Progression in Science (Eco-Evo MAPS) (12), General Biology-Measuring Achievement and Progression in Science (GenBio-MAPS) (17), Conceptual Assessment of Natural Selection (CANS) (18), Assessing Contextual Reasoning about Natural Selection (ACORNS) (19), and the Genetic Drift Inventory (GeDI) (20).

We also encourage the instructor to read about the Uganda Wildlife Authority (https://ugandawildlife.org/news-events/news/item/465-uwa-successfully-translocates-19-giraffes) and the Giraffe Genome Project (https://giraffegenome.science.psu.edu/#/), which will provide the instructor with the most up-to-date information on giraffe conservation efforts and feature scientists from a range of backgrounds. Additionally, we recommend the instructor checks the latest conservation status of giraffes and any other species they wish to highlight [e.g., the International Union for Conservation of Nature (IUCN) Red List of Threatened Species webpage at https://www.iucnredlist.org/]. The most up-to-date conservation status of giraffes can be found at https://www.iucnredlist.org/species/9194/136266699. The IUCN currently considers all giraffes to belong to the same species: Giraffa camelopardalis.

The instructor may find it helpful to read the primary literature referenced throughout the lesson, especially (21-25). Other literature referenced in the lesson includes (26-43); more information about each of these references is included in the instructor notes in Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, as well as in Supporting File S7. Speciation and Conservation – References Table).

Finally, the instructor should be comfortable with using technology (e.g., for administering pre/post tests, clicker questions, and online prompts), managing discussion groups (in this lesson, students formed self-selected groups of 3-5 students), facilitating productive conversations with one student talking at a time and the rest of the class listening, and eliciting student responses. Note: if using clickers is not a feasible option, the instructor can display or print off questions and use other polling techniques such as colored cards (index cards where answer choice A is written on one colored card, answer choice B is written on another colored card, and so on), raising hands (the instructor can say "raise your hand if you selected answer choice A," "raise your hand if you selected answer choice B," and so on), or online services such as PollEverywhere.com. The instructor also has flexibility in how to administer the surveys (including the pre/post tests and online prompt); if online options are not feasible, these questions can be administered as handouts and collected before the first day of the lesson and after the last day of the lesson. We recommend students fill the pre/post tests out as individuals.

SCIENTIFIC TEACHING THEMES

Active Learning

Students engage in a variety of active learning activities throughout this lesson, including answering and asking questions, discussing clicker questions, interacting in small groups, exploring data, and working on a worksheet (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes; Supporting File S3. Speciation and Conservation – Student Worksheet Packet). All slides marked with an orange bar along the top indicate an active-learning activity (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes).

To help instructors visualize the use of active learning techniques in this lesson, author L.A.G. evaluated the instructional practices of author M.K.S. by analyzing video recordings of the class periods using the Classroom Observation Protocol for Undergraduate STEM (COPUS) (44). This observation protocol uses a series of codes to characterize instructor and student behaviors in the classroom (44,45). The summary of COPUS results (Figure 1) highlights the diversity of student-centered teaching practices and high level of student participation in this lesson.

For example, throughout the lesson more than 35% of the codes were "students talking to the class" (asking and answering questions, engaging in whole class discussions) and more than 30% of the codes were "students working" (working individually, answering clicker questions, and working in groups). Similarly, for the instructor, more than 75% of the codes were "guiding" (posing questions to students, asking clicker questions, following up with students, moving and guiding, and engaging in one-on-one discussions with students). This lesson is categorized as "student-centered" (cluster 7) using http://www.copusprofiles.org/ (46).

Assessment

Before lesson: Students take a short online pre-test consisting of five multiple-choice questions to assess their prior knowledge about speciation and interpreting figures (Supporting File S5. Speciation and Conservation – Pre/Post-Test Questions) using a course management system. We recommend that students receive a small number of participation points (less than 1% of their course grade) for completing the pre-test. If an online course management system is not available, instructors can print the questions, hand them out, and have students circle the answers or use a bubble sheet to record the answers. Each question aligns with at least one of the lesson's four learning objectives. Since the pre-test is a measure of student understanding prior to the lesson, students should be asked to not consult any outside resources when taking it and should be told in advance that their answers will not be graded for correctness. The pre-test questions should not be discussed during the lesson because the same questions will be used after the lesson as the post-test.

During lesson: Students engage in a variety of formative assessments, including in-class clicker questions (Supporting File S4. Speciation and Conservation – Clicker Questions), think-pair-share opportunities (e.g., where students think about a question,

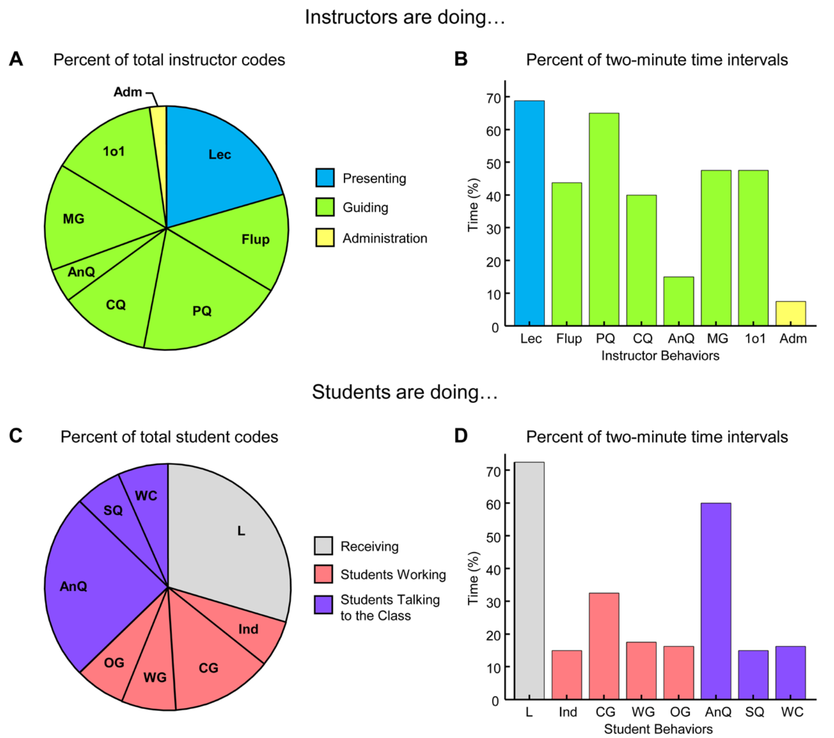

Figure 1. Frequency of active learning. The COPUS was used to document the instructional practices used in the lesson. Collapsed instructor codes include Presenting (Lec, Lecturing), Guiding (FlUp, Following-up; PQ, Posing Question; CQ, Clicker Question; AnQ, Answering Question; MG, Moving and Guiding; 1o1, One-on-one instruction), and Administration (Adm, Administration). Collapsed student codes include Receiving (L, Listening), Students Working (Ind, Individual Work; CG, Clicker Question Discussion; WG, Worksheet Group Work; OG, Other Group Work), and Students Talking to the Class (AnQ, Answering Questions; SQ, Student Question; WC, Whole Class Discussion). All students were observed at the same time, and the average observed behavior was recorded. The total class time was divided into a series of two-minute segments. A) Percent of total instructor codes and B) Percent of two-minute time intervals instructor behaviors were observed; C) Percent of total student codes and D) Percent of two-minute time intervals student behaviors were observed. The percent of two-minute time intervals (B and D) add up to more than 100% because more than one code can be observed in a two-minute time interval.

turn to someone near them, and share their ideas), collaborative group discussions, and a worksheet activity (Supporting File S3. Speciation and Conservation – Student Worksheet Packet). These formative assessments provide timely feedback to the instructor and help students self-evaluate their own learning.

Immediately after lesson: After the speciation lesson is complete (i.e., after all class sessions for this lesson), students take a short online post-test that is identical to the pre-test (Supporting File S4. Speciation and Conservation – Pre/Post-Test Questions) using a course management system. We recommend that students receive a small number of participation points (less than 1% of their course grade) for completing the post-test. If an online course management system is not available, the post-test questions can be administered via paper hardcopies, as suggested for the pre-test.

Exam: The instructor can also measure longer-term student retention by including questions about speciation on an exam (Supporting File S6. Speciation and Conservation – Exam Questions). The exam questions are different from the pre/post-test questions and address the learning objectives.

Inclusive Teaching

This lesson contains a diverse array of inclusive active-learning strategies that help to build classroom community. When participating in these activities, students work collaboratively in small groups (of 3-5 students that they select) on several formative assessments, which provides them with the opportunity to make their voices heard, draw on each other's strengths, and assist each other with data interpretation (47). The small group questions are either discussed as an entire class or asked about in follow up clicker questions (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes; Supporting File S2. Speciation and Conservation – Discussion After Application Worksheet Activity). Students earn points for participating in the clicker questions and are not scored based on selecting the correct answer. This grading policy supports the message that students should think about the questions and get help when they are incorrect, rather than worry about the grade. More information about using this scoring scheme can be found in a recent review about using clicker questions in biology courses (48). For the summative assessment, we employed a two-stage exam design (49,50) at the end of the semester, where students first answer the questions as individuals, turn in their individual answers, and then work in small randomly-assigned groups to answer the questions again. The group part inspired lively discussions where students explain their thinking and provided each other with immediate feedback.

We also designed the lesson to emphasize diverse perspectives and frame speciation in a way that endeavors to be both relevant and engaging. Before the start of the lesson, students are asked to submit photos of animals that are symbolically significant to their own cultures or identities, which are then incorporated into the lecture slides (Slide 13 of Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes). This assignment has several potential benefits, including that students feel valued in seeing their chosen animal represented on the slide and learn about their peers' backgrounds. To build upon this personal investment, we focused our lesson around the giraffe, which invites strong public engagement from communities and conservationists across the globe.

To be mindful of students who may have a color vision deficiency, we carefully designed the figures in the slides (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes; Supporting File S2. Speciation and Conservation – Discussion After Application Worksheet Activity) to be distinct in terms of color and shape and tested them through a colorblindness simulator (https://www.color-blindness.com/coblis-color-blindness-simulator/).

Students are also exposed to scientists from diverse backgrounds throughout the lesson (e.g., Slides 21, 97, and 98 of Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes). These scientists differ in terms of age, ethnicity, gender, geographical location, and academic/professional backgrounds. Our goal here is to break down societal barriers to inclusion in science by promoting the message that there are multiple paths to becoming a scientist (51).

Students' socioeconomic diversity was also an important consideration in our lesson design; students are not required to purchase any textbooks or other supplementary materials to successfully participate in the lesson. Moreover, all formative and summative assessment questions that accompany this lesson are derived from the lecture slides and in-class activities.

LESSON PLAN

The intention of this lesson is to expose students to the process of speciation, convey the challenges in defining species by comparing multiple species concepts (morphological, biological, and phylogenetic), explore the mechanisms of evolutionary change that impact speciation, and connect speciation to conservation issues.

Pre-class Preparation

The instructor administers a series of pre-test questions (Supporting File S5. Speciation and Conservation – Pre/Post-Test Questions; also refer to the Assessment section), which students should complete before the lesson begins. Each of these questions targets at least one of the lesson's learning objectives (Table 2). The purpose of the pre-test is to serve as a baseline assessment. The instructor also creates and administers an online prompt (e.g., via a course management system) before the start of the lesson. The prompt says: "Please upload a photo of an animal that is symbolically significant to your own culture or identity."

The instructor should print out one copy of the Student Worksheet Packet (Supporting File S3. Speciation and Conservation – Student Worksheet Packet) for each student. These figures reproduce best in color; however, if printing capabilities are restricted, we suggest printing a grayscale copy for each student, with a corresponding set of color copies for every two or three groups.

Progressing Through the Lesson: Class Session I

1. Introduction and assessing prior knowledge

The lesson begins with the instructor orienting students' thinking towards the types of characteristics that define a species. Students individually answer a series of four rapid-fire clicker questions (CQ1-4) that ask them to identify whether two organisms belong to the same species (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slides 2-9; Supporting File S4. Speciation and Conservation – Clicker Questions). These questions are designed to become increasingly challenging. While the majority of students will likely be able to correctly categorize the animals as different species in CQ1 (elephant vs. frog) and CQ2 (shark vs. dolphin), CQ3 (two very different-looking dogs) and CQ4 (two different species of birds which look very similar) may evoke a spread of answers. At the end of the rapid-fire clicker questions, students are asked to consider why some organisms are classified as the same species despite looking very different (e.g., the dogs in CQ3), while others are classified as different species despite appearing very similar (e.g., the woodpeckers in CQ4) (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 10).

2. Learning outcomes and symbolically significant animals

The instructor introduces students to the learning goals and objectives for the lesson (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slides 11-12). To deepen students' emotional investment in the lesson, the instructor displays photos of animals that students have submitted as symbolically significant to their cultures or identities (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 13). In the introductory evolution class for which this lesson was developed, students often selected animals that represented a country where they had family ties (e.g., bald eagle, panda bear), had religious significance (e.g., tiger, lamb), and were parts of legends that they learned as children (e.g., butterflies, snakes). To help students reflect on these images, the instructor asked questions such as: "What do you notice about the organisms? What organisms are most common? Does anyone want to share why they included their organism?" The instructor may wish to include a giraffe on this slide, as the giraffe will be the focus animal throughout the lesson. To transition to the next section of the lesson, the instructor asks students to imagine the impact of all of these animals becoming extinct (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 14).

3. Why are giraffes important, and can we save them from extinction?

This section of the lesson focuses on the giraffe as a species that is currently in decline and heading towards extinction. The instructor asks students to consider why giraffes are important and what they might contribute to their native ecosystem (GDQ1, Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 15). Here, students can express their ideas in a think-pair-share approach (i.e., students individually reflect before discussing with a partner and later have the opportunity to share with the entire class). After gathering ideas from the class, the instructor provides additional examples of giraffes' contributions to society (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 16).

Next, the instructor shows that giraffes are in danger of extinction, with a total population decline of roughly 30% in only

30 years (https://giraffeconservation.org/giraffe-conservation-status/) (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 17). To prime student thinking about conservation, the instructor asks students to brainstorm factors that may have contributed to the population decline of giraffes (GDQ2, Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 18). After gathering responses from the class, the instructor briefly explains additional drivers of decline.

According to the IUCN Red List of Threatened Species, the current conservation status of the giraffe (as one species, *Giraffa camelopardalis*) is "vulnerable" (52). To help students understand the conservation status of giraffes compared to other species, the instructor displays the full IUCN conservation scale and give examples of species classified within each category, ranging from "least concern" to "extinct" (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 19). After describing some common conservation efforts to protect species from extinction (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 20), the instructor segues into a specific example featuring giraffe translocation in Uganda (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 21). Here, students answer and discuss a clicker question identifying possible challenges that could arise upon translocation of giraffes (CQ5, Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slides 22-23; Supporting File S4. Speciation and Conservation – Clicker Questions). We recommend using a peer instruction approach during clicker questions throughout the lesson (53), where students vote individually at first, and then discuss the question with a partner before re-voting. The instructor follows up by explaining how translocations can produce both beneficial and costly reproductive outcomes (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 24). The instructor emphasizes the importance of distinguishing species in maintaining biodiversity and conservation (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 25).

4. How do we understand speciation in giraffes?

To further expand on the idea that conservationists need to distinguish species, students individually answer a poll that asks them to predict the number of giraffe species (CQ6, Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 26; Supporting File S4. Speciation and Conservation – Clicker Questions). A range of responses will likely be generated. The instructor does not provide an immediate answer; rather, the instructor explains that in order to answer this question, the class must first explore how speciation works and determine what factors define a species (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 27). The instructor next displays an outline (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slides 28-29) that students can use as a guide as they discuss the speciation process and species concepts. The instructor reviews the four mechanisms of evolutionary change (gene flow, mutation, genetic drift, and natural selection; Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 30) and asks students to pay attention to how each of these four mechanisms affects the speciation process in giraffes.

The instructor explains that the speciation process begins with a population of a particular species (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 31). To help orient the students, the slide contains the starting elements of a flowchart that will build over the following slides. Each gray circle represents one organism. The instructor next introduces the speciation process in giraffes, which began with a single ancestral population (cluster of gray circles) on the continent of Africa.

The instructor introduces the first step in the speciation process: namely, something that causes the original population of a species to separate into two or more similar isolated populations (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 32). The drawing on the right-hand side of the slide illustrates the separation of the ancestral population of giraffes into two isolated populations ("A" and "B") over time. Moreover, members of each of these newly-isolated populations are still depicted as gray circles, indicating that they still share similar characteristics. Students discuss and share factors that may cause a population to separate (GDQ3, Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slides 32-33). While students may identify that this separation could be attributed to a geographic or physical barrier, it is important for students to understand that separation could also arise from other factors, such as behavioral isolation. Therefore, the instructor should be prepared to supplement students' answers with additional possibilities, including different courtship behaviors, breeding seasons, and periods of activity (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 33). Note that we have avoided defining the terms "allopatric" and "sympatric" speciation throughout this lesson because the course is designed for students who are not majoring in the biological sciences; if instructors would like to include these terms, Slide 33 is a logical place to do so. Finally, the instructor should emphasize that while many factors may drive one population to separate from another initially, one key commonality is that isolated populations rarely interbreed and therefore, are characterized by restricted gene flow.

To demonstrate a "real-world" application of this step in the speciation process, and to summarize this information in a different way, the instructor again revisits the giraffe example. Here, the instructor explains that changes in climate (~2 MYA) disrupted giraffes' suitable habitat, leading to multiple spatially-isolated populations (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 34). Since these isolated populations rarely encountered each other, gene flow was rare or non-existent (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 35). The instructor concludes by emphasizing that limiting gene flow is a prerequisite for speciation. Students may find it helpful to revisit the "Mechanisms of Change in Speciation" slide (duplicated in Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 36), where the instructor can now display a red "X" (indicating "absence/limited") beside the term "migration (gene flow)."

The instructor introduces mechanisms of evolutionary change that lead to an accumulation of genetic and phenotypic differences (Supporting File S1. Speciation and Conservation

– Presentation Slides with Instructor Notes, Slide 37) and asks students to consider what may happen to these isolated populations of giraffes over time. After soliciting ideas from the class, the instructor reveals illustrations of isolated giraffe populations that have evolved fixed genetic and/or phenotypic differences between each other over time (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 38). While these illustrations show clear differences in morphology of these giraffes, the instructor may wish to emphasize that there are other types of differences, such as behavior.

Next, students answer and discuss a clicker question regarding which mechanisms of evolutionary change can contribute to fixed genetic and/or phenotypic differences between isolated populations (CQ7, Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slides 39-40; Supporting File S4. Speciation and Conservation – Clicker Questions). The instructor explains that these fixed differences could be a result of mutation, natural selection and/or genetic drift, (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 41) and that these mechanisms of change act separately on each isolated population (i.e., the evolution of population "A" is independent to that of population "B") (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 42). The lack of gene flow is responsible for preventing the mixing of alleles between these isolated populations, thus creating the circumstances under which speciation may occur (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 43). Students answer and discuss a clicker question asking them to identify which mechanism of change most likely contributed to the evolution of camouflage-like coat patterns in giraffes (CQ8, Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slides 44-45). Students then hypothesize how specific mechanisms of change [e.g., natural selection (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 46) and genetic drift (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slides 47-48)] may influence speciation in giraffes. Finally, the instructor revisits the "Mechanisms of Change in Speciation" slide (duplicated in Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 49) to summarize that mutation, genetic drift, and/or natural selection are sufficient to drive the speciation process. A green checkmark (indicating "potential presence") is displayed beside each of these three terms.

Next, students consider a scenario where the different isolated populations of giraffes are brought back into contact (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 50). Here, the instructor introduces the final step of the speciation process: namely, when genetic and phenotypic differences reach a point where the populations may be considered separate species (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 51). The instructor can explain that this is a difficult question to address, as the answer depends on how we define a species.

5. How do we define a species?

This section of the lesson has two key purposes: 1) to introduce students to the morphological, biological, and phylogenetic species concepts used to define a species (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 52), and 2) to convey appropriate applications and limitations of each species concept.

The instructor begins by defining the morphological species concept, in which two populations are classified as different species if they have distinguishable phenotypic characteristics (e.g., physical appearance or internal anatomy) (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 53). The instructor next presents photos of two giraffes (each representing a separate population) and their corresponding skulls; one has two ossicones ("horns"), while the other has three (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 54). Based on this information, students answer and discuss a clicker question asking them to determine whether these two giraffe populations belong to the same species based on the morphological species concept (CQ9, Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slides 54-55; Supporting File S4. Speciation and Conservation – Clicker Questions). Here, students conclude that differences in the number of ossicones between these giraffe populations distinguish them as different species under the morphological species concept. The instructor presents additional ways to differentiate giraffe species using the morphological species concept, such as via different fur patterns or fur coloration (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 56). Here, the instructor can disclose an important caveat: classifying species based on appearance alone can be difficult. For example, the dogs in the earlier clicker question (CQ3, Supporting File S4. Speciation and Conservation – Clicker Questions) look different but are the same species.

The instructor next asks the class to brainstorm types of organisms in which morphological traits may be the most useful way of classifying species (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 56). It took some time for our students to generate answers but they eventually included: extinct species (e.g., fossil taxa), species that have been classified in the past (pre-DNA evidence), species that contain DNA that is difficult to extract, and taxa with unknown interbreeding capabilities. This discussion transitions into the next slide, which focuses on using the morphological species concept when examining the fossil record (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 57). Here, the instructor displays a simplified evolutionary tree featuring two modern species (okapi and giraffe) and photos of their respective vertebrae. If scientists discovered a fossilized vertebra (e.g., from the extinct *Samotherium*) that exhibited clear morphological differences from that of the modern okapi and giraffe, then they could determine that this vertebra likely belonged to a member of a different species. Finally, the instructor summarizes some of the limitations of the morphological species concept (Supporting File S1:. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 58) and suggests that students consider other species concepts when more information is present (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 59).

Next, the instructor introduces the biological species concept, in which two populations are classified as the same species if they can interbreed in nature and produce fertile, viable offspring (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slides 60-61). Here, students revisit CQ9 but now must use the biological species concept to determine whether these two giraffe populations belong to the same species, given the new information that these giraffes can mate and produce fertile offspring (CQ10, Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slides 62-63; Supporting File S4. Speciation and Conservation – Clicker Questions). While students had previously classified these two giraffe populations as different species by the morphological species concept, students now conclude that these giraffe populations are the same species according to the biological species concept. This apparent contradiction primes the instructor to emphasize that it is not always straightforward to determine whether two populations belong to the same species because different species concepts can lead to different conclusions (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 64). The instructor then presents some limitations of the biological species concept, such as the exclusion of species that reproduce asexually and the impracticality of testing whether or not every pair of organisms can reproduce and have fertile offspring (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 65). Students are next asked to brainstorm other information that can be used to distinguish between species, such as molecular evidence.

Finally, the instructor defines the phylogenetic species concept, in which two populations are classified as the same species if they have inherited traits from a recent common ancestor that uniquely distinguishes them from other groups (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slides 66-67). As a quick review, the instructor can remind students that the tip of each branch indicates a separate species, which are clustered distinctly from an outgroup that falls outside the clade being studied. In particular, the instructor should emphasize that the nodes represent speciation events in evolutionary history (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 68) and that each node designates the location of a common ancestor (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 69). The instructor presents a hypothetical scenario where DNA is sampled from two populations of giraffes that are located so far apart that they do not interact (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 70). Students answer and discuss a clicker question asking them to predict which phylogenetic tree best represents that these giraffes have evolved into two different species (CQ11, Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slides 71-72; Supporting File S4. Speciation and Conservation – Clicker Questions).

Students next determine the node in which the speciation event occurred between these two distinct giraffe species (CQ12, Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slides 73-74; Supporting File S4. Speciation and Conservation – Clicker Questions). The instructor reviews the location of the speciation event on the tree, as well as the common ancestor for each respective species (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slides 75-76). Finally, the instructor presents a new phylogenetic tree (in which all giraffes now belong to the same species) and asks students how to define these species according to the phylogenetic species concept (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slides 77-79).

Progressing Through the Lesson: Class Session II

6. Data exploration

The second class session begins with the instructor revisiting CQ6, where students were asked to predict the number of giraffe species (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 81; Supporting File S4. Speciation and Conservation – Clicker Questions). Here, the instructor announces that students are now equipped to discuss this question, as they have learned how speciation works and what defines a species.

The instructor then introduces the data exploration activity by explaining that the students will apply their knowledge to three scenarios of possible speciation in giraffes. Students self-select to form groups of three and within each group, students number themselves between 1 and 3 (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 82). All 1's are designated experts in the morphological species concept, all 2's are designated experts in the biological species concept, and all 3's are designated experts in the phylogenetic species concept (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 83). The instructor explains that students will discuss the number of giraffe species within each of these three scenarios based on the definitions of their assigned species concept: morphological, biological, or phylogenetic. For each scenario, students work in their groups to answer two prompts: 1) which experts can contribute to a discussion of these data, and 2) given these data, what is your estimate of the number of giraffe species? One goal of this activity is for students to distinguish when they have sufficient data to evaluate speciation from the perspective of a certain species concept, and when they need more information.

The first scenario shows a map of Africa, with six populations of giraffes geographically dispersed throughout the continent (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 84) (21). A representative image of the coat pattern for each giraffe population is shown. Students who are assigned experts in the morphological species concept will likely conclude that there are five species based on the five different coat patterns. Students who represent the biological species may conclude that the populations that are geographically proximal to each other are most likely to interbreed. However, students would need more evidence about mating to evaluate the number of giraffe species based on the biological species concept. Students who are assigned experts in the phylogenetic species concept need more data to come to a conclusion.

The second scenario shows a phylogenetic tree from the DNA of four populations of giraffes living in the wild (with each tip labeled as Population 1-4; Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 85) (22). Students who are assigned experts in the phylogenetic species concept should conclude that there are up to four species of giraffe. Students who represent the biological species may determine that more data are needed to evaluate the number.

Students who are assigned experts in the morphological species concept had already classified the giraffe species as distinguished by coat pattern from the information provided in Data Set #1 (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 84). As this prompt does not provide additional morphological information, these student experts will likely not re-evaluate their previous conclusion.

In the final scenario, students consider a situation where geographically isolated giraffe populations do not interbreed; however, some populations of giraffes will interbreed in captivity (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 86). This information is accompanied by the same map of Africa as in Data Set #1, which depicts the geographical distribution of six giraffe populations (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 84) (21). Students who are assigned experts in the biological species concept will have the most to contribute to this prompt. These students may remark that the prompt does not mention whether these giraffes have intrinsic barriers to reproduction, so perhaps they are just a single group; however, more data are needed to determine a precise number of species (e.g., which of these giraffes can interbreed with others, and can they produce fertile offspring?). Students assigned the morphological species concept have already classified the giraffe species as distinguished by coat pattern from the information provided in Data Set #1 (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 84), and students assigned the phylogenetic species concept have already reached a conclusion based on the information presented in Data Set #2 (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 85). As this prompt does not provide additional morphological or phylogenetic information, these students will likely not re-evaluate their previous conclusions.

Students answer a clicker poll to indicate whether their group members agree on the same number of giraffe species across all three data sets (CQ13, Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 87; Supporting File S4. Speciation and Conservation – Clicker Questions). We expect there to be disagreement. To follow up, students use a clicker to select the number of giraffe species that has the most support among their group (CQ14, Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 88; Supporting File S4. Speciation and Conservation – Clicker Questions). Again, we expect a broad spread of responses to this question. We encourage the instructor to validate students' diverse opinions here by explaining that there is no one correct answer, as scientists have not reached a consensus on the number of giraffe species (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 89).

7. Literature debate on number of giraffe species and the Giraffe Genome Project

This section begins with the instructor featuring three articles in the primary literature to demonstrate that the number of giraffe species is an ongoing topic of debate among experts in the field. The dialogue in the accompanying slides (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slides 90-96) also illustrates how scientists use arguments from evidence to advance knowledge. The instructor first summarizes an excerpt from a manuscript by Fennessy et al. (22), which asserts that there are four species of giraffe as concluded from genetic analyses (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 90). The instructor next introduces correspondence by Bercovitch et al. (23), which disagrees with the conclusions presented in the 2016 manuscript by Fennessy et al. (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 91). Finally, the instructor summarizes a rebuttal (24) that Fennessy et al. wrote, in which they defend their original stance.

To give students the opportunity to apply the species concepts to some of the key arguments raised in the articles, the instructor next presents two summarized examples of dissenting viewpoints between the groups (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slides 92-95). Please note that we chose to paraphrase the primary literature here, as these readings may not be accessible to students who are not majoring in the biological sciences.

To frame the first argument, the instructor presents differing opinions regarding breeding behaviors as a way to classify species (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 92). Here, students talk with their peers to identify that the biological species concept is involved in this argument (GDQ4, Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 93). To give students more practice in identifying species concepts in the primary literature, the instructor next summarizes the arguments regarding fur patterns as a way to classify species (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 94). Students discuss and share that the morphological species concept is involved in this argument (GDQ5, Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slides 95).

The instructor provides paraphrased text of a final disagreement in a follow-up rebuttal (25), where Fennessy and colleagues further defend their original conclusion of four giraffe species (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 96). The unresolved controversy over the number of giraffe species demonstrates that defining a species can be challenging and is not always a straightforward process. The instructor explains that other scientists are also actively working to resolve this disagreement. Here, the instructor introduces the Giraffe Genome Project, where scientists from Africa and the United States are collaborating to understand giraffe genetic and physiological differences (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 97). This slide also includes photos of the two leaders of the Giraffe Genome Project, which also serves as an opportunity to highlight the diversity of scientists. Finally, the instructor encourages students to consider how outcomes from this project (i.e., new information regarding the number of giraffe species) can further inform conservation efforts. For example, if there is more than one species of giraffe, then they may have multiple statuses on the IUCN Red List that would result in different conservation plans.

8. The bigger picture

This section of the lesson serves to further broaden students' views of speciation. Here, the instructor explains that distinguishing and identifying species is important for everyone, not just scientists (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 98). To illustrate this point, the instructor shows images of farmers differentiating crops from weeds, fishers identifying different species of fish, a medical professional distinguishing between different types of microorganisms, and a young adult differentiating poison ivy from innocuous plants during a hike (33).

The instructor revisits the IUCN conservation scale introduced on Slide 19, accompanied by the examples of species classified within each category, ranging from "least concern" to "extinct," (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 99). Here, the instructor provides a quote by evolutionary biologist Jody Hey to help students reflect on the impacts of classifying species on preserving biodiversity (33). To provide a concrete example, the instructor highlights the giraffe's "vulnerable" conservation status and reminds students that the IUCN currently treats the giraffe as one species (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 99). The instructor next asks students to consider if the conservation status of the giraffe would change if the IUCN were to recognize multiple species of giraffe, each with a small population size. Here, students will likely rationalize that each of these smaller populations will be at higher risk for extinction than the one larger population (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 99). The instructor can wrap up this discussion by emphasizing that different classifications of a species can dramatically affect conservation practices to protect that species from extinction.

9. Application worksheet activity

In this section of the lesson, students work in small groups to evaluate six case studies of potential speciation across different systems. To begin, the instructor distributes one worksheet packet to each student (Supporting File S3. Speciation and Conservation – Student Worksheet Packet). The instructor next displays the instructions for the application worksheet activity (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 100). Here, the instructor explains that students will remain in their groups of three students, read the information for each of the six scenarios in their packet, and work together to answer the free-response questions for each scenario. Each question aligns with at least one learning objective for the lesson.

The following slides (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slides 101-106) describe the scenarios for each of the six case studies of potential speciation in the student worksheet (Supporting File S3. Speciation and Conservation – Student Worksheet Packet). By the end of the activity, students will discuss six scenarios: evolution of snail chirality in Japan (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 101), speciation of tuco-tucos (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 102), conservation of helmeted honeyeaters (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 103), debate regarding the conservation of tiger salamanders (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 104), breeding outcomes of cruciferous vegetables (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 105), and media coverage of the "cat-fox" (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 106). The instructor can also select a subset of these scenarios based on which organisms they would like to highlight and which learning objectives they would like to reinforce (e.g., different ways species are defined, which mechanisms of evolution are more important in a particular scenario).

10. Debrief after application worksheet activity, summary, and additional resources

The lesson concludes with a debrief for students to discuss their answers to the application worksheet activity (Supporting File S2. Speciation and Conservation – Discussion After Application Worksheet Activity; Supporting File S3. Speciation and Conservation – Student Worksheet Packet). This debrief additionally provides an opportunity for the instructor to assess students' understanding of speciation in the context of conservation, but if time is running short, this part of the lesson may be condensed or eliminated. The questions on the following slides (Supporting File S2. Speciation and Conservation – Discussion After Application Worksheet Activity, Slides 2-26) are a subset of those presented in the application worksheet (Supporting File S3. Speciation and Conservation – Student Worksheet Packet), with the addition of answer choices that students can individually select and discuss (CQ15-20, Supporting File S4. Speciation and Conservation – Clicker Questions). Many of the questions were converted into clicker questions, but we have structured certain questions (GDQ6-9) as free-response group discussions to encourage students to share their thoughts.

Students revisit Scenario 1 (chiral snails in Japan) from the application worksheet activity (Supporting File S3. Speciation and Conservation – Student Worksheet Packet). Students answer and discuss a clicker question regarding the species concepts that most likely classify left- and right-twisted snails as different species (CQ15, Supporting File S2. Speciation and Conservation – Discussion After Application Worksheet Activity, Slide 2; Supporting File S4. Speciation and Conservation – Clicker Questions). The correct answer and corresponding explanation is revealed on the following slide (Supporting File S2. Speciation and Conservation – Discussion After Application Worksheet Activity, Slide 3); this same format applies to all clicker questions for the application worksheet debrief.

Discussion of Scenario 2 (tuco-tucos) focuses on students identifying difficulties of the morphological species concept in distinguishing species of tuco-tucos (CQ16, Supporting File S2. Speciation and Conservation – Discussion After Application Worksheet Activity, Slides 4-5; Supporting File S4. Speciation and Conservation – Clicker Questions).

For Scenario 3 (helmeted honeyeaters), students answer and discuss a clicker question on reproductive outcomes associated with introducing captive-bred helmeted honeyeaters to the existing wild population for conservation purposes (CQ17, Supporting File S2. Speciation and Conservation – Discussion After Application Worksheet Activity, Slides 6-7; Supporting File S4. Speciation and Conservation – Clicker Questions). Students next discuss a scenario in which captive-bred helmeted honeyeaters have a different mating call from the wild population and are asked to brainstorm consequences this may have on the conservation project (GDQ6, Supporting File S2. Speciation and

Conservation – Discussion After Application Worksheet Activity, Slide 8). Students use species concepts to justify their answers.

Review of Scenario 4 (tiger salamanders) begins with students answering and discussing a clicker question regarding the species concepts that most likely classify the barred and California tiger salamanders as different species (CQ18, Supporting File S2. Speciation and Conservation – Discussion After Application Worksheet Activity, Slides 9-10; Supporting File S4. Speciation and Conservation – Clicker Questions). Next, students have a brief debate on whether they agree with the current treatment of barred and California tiger salamanders as separate species (GDQ7, Supporting File S2. Speciation and Conservation – Discussion After Application Worksheet Activity, Slide 11; Supporting File S3. Speciation and Conservation – Student Worksheet Packet). Students use species concepts to justify their answers.

For Scenario 5 (*Brassica oleracea*), students answer and discuss a clicker question regarding the species concepts that most likely classify the many cultivated varieties of *Brassica oleracea* as belonging to the same species (CQ19, Supporting File S2. Speciation and Conservation – Discussion After Application Worksheet Activity, Slides 12-13; Supporting File S4. Speciation and Conservation – Clicker Questions). Students next discuss (GDQ8) the likelihood of the following mechanisms of evolutionary change in forming these different variants: limited gene flow (Supporting File S2. Speciation and Conservation – Discussion After Application Worksheet Activity, Slides 14-15), mutation (Supporting File S2. Speciation and Conservation – Discussion After Application Worksheet Activity, Slides 16-17), natural selection (Supporting File S2. Speciation and Conservation – Discussion After Application Worksheet Activity, Slides 18-19), artificial selection (Supporting File S2. Speciation and Conservation – Discussion After Application Worksheet Activity, Slides 20-21), and genetic drift (Supporting File S2. Speciation and Conservation – Discussion After Application Worksheet Activity, Slides 22-23).

Students begin their review of Scenario 6 by identifying the speciation concepts classifying the “cat-fox” as a different species from other cats based on a quote from *People* magazine (CQ20, Supporting File S2. Speciation and Conservation – Discussion After Application Worksheet Activity, Slides 24-25; Supporting File S4. Speciation and Conservation – Clicker Questions). Students then hypothesize how the remote habitat location of the “cat-fox” may influence gene flow between the “cat-fox” and other species of cats (GDQ9, Supporting File S2. Speciation and Conservation – Discussion After Application Worksheet Activity, Slide 26).

Finally, the instructor briefly highlights the main points of the lesson (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 107) and offers optional supplementary readings (Supporting File S1. Speciation and Conservation – Presentation Slides with Instructor Notes, Slide 108).

Post-lesson Follow-up

The instructor administers a series of post-test questions on speciation (Supporting File S5. Speciation and Conservation – Pre/Post-Test Questions; also refer to the Assessment section). These questions are identical to those from the pre-test so that the instructor can assess students’ progress. If the instructor identifies any conceptual difficulties remaining after the post-test, the instructor should revisit these concepts in class before any subsequent assessments. We also encourage the instructor to include questions on speciation and conservation on a future exam. Eight possible exam questions and answers are provided (Supporting File S6. Speciation and Conservation – Exam Questions).

TEACHING DISCUSSION

A variety of methods were used to assess the effectiveness of this lesson, including formative clicker and group discussion questions throughout the lesson, as well as summative pre/post-test and exam questions (Table 2). Formative questions (abbreviated CQ for Clicker Questions and GDQ for Group Discussion Questions) are presented in the *Progressing through the lesson section*, and student answers to the clicker questions (before and after peer discussion) are discussed in Supporting File S4. Speciation and Conservation – Clicker Questions. Here we discuss the summative questions.

Pre/Post-Test Questions

Students completed five identical, multiple-choice pre- and post-test questions (abbreviated PPTQ for Pre/Post-Test Questions) on speciation and conservation (Supporting File S5. Speciation and Conservation – Pre/Post-Test Questions). Each question focuses on potential student conceptual difficulties and targets at least one of the four learning objectives of the lesson (Table 2). Here we report the results for the students who completed both the pre- and post-test and agreed to the informed consent (n=46). The overall average pre-test score was 66.5 ± 2.8% (SEM), and the overall average post-test score was 85.2 ± 2.6% (SEM). Improvement was observed on all individual pre/post-test questions (Figure 2).

The first question (PPTQ1) asks students to quantify the relative amount of gene flow involved in the speciation process (Supporting File S5. Speciation and Conservation – Pre/Post-Test Questions). This question targets LO-3 (Table 2). While just over

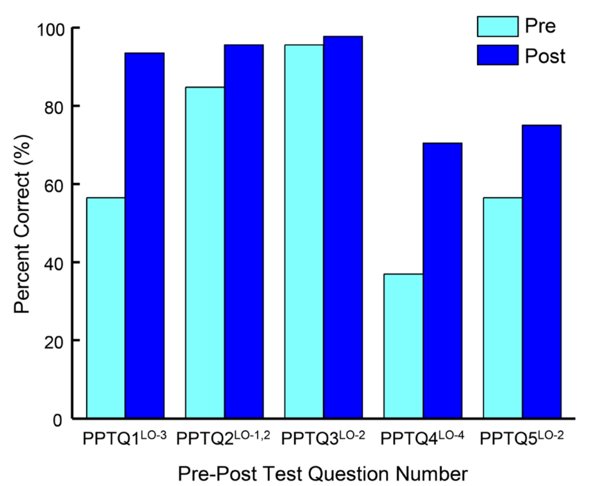

Figure 2. Percent of students answering correctly before and after instruction on pre/post-test questions (PPTQ). Light blue bars represent class average scores for each pre-test question. Dark blue bars represent class average scores for each post-test question. Pre-test questions 1-5 were answered by 46 students. Post-test questions 1-3 were answered by 46 students, and post-test questions 4-5 were answered by 44 students. Targeted learning objectives are indicated for each question (LOs are listed in the Learning Objective section of the paper).

half (56.5%) of the students correctly identified "lack of gene flow" on the pre-test, nearly all (93.5%) students selected the correct answer after participating in the lesson (Figure 2).

PPTQ2 tests students' understanding of the modern species concepts and is aligned with LO-1 and LO-2 (Supporting File S5. Speciation and Conservation – Pre/Post-Test Questions) (Table 2). Here, students are asked to identify which of the following does not define a species: A) The ability to interbreed and produce fertile offspring; B) Overlapping location (e.g., inhabiting the same area); C) Genotype (e.g., DNA); or D) Phenotype (e.g., outer physical appearance, internal anatomy). On the post-test, 95.7% of students correctly answered that overlapping location is not a valid criterion for defining a species (Figure 2).

PPTQ3 and PPTQ5 both focus on LO-2 (Supporting File S5. Speciation and Conservation – Pre/Post-Test Questions; Table 2). PPTQ3 features a hypothetical phylogenetic tree of four different species of fish; here, students must correctly identify the node that represents the most recent common ancestor between two highlighted species. Most students correctly answered this question both before and after participating in the lesson (Figure 2). PPTQ5 includes two photos of different-looking butterflies. These butterflies are members of the same species (*Araschnia levana*) with variation in wing color and pattern due to their exposure to different temperature and light conditions (33); this information is not provided to the students. Here, students are asked to use the phylogenetic species concept to determine whether these species are the same, different, or whether students need additional information to provide a response. This question is designed to test students' ability to evaluate evidence in the context of a specific species concept. On the pre-test, 56.5% and on the post-test 75% of students correctly identified that they would need more information to determine whether these two butterflies are the same species according to the phylogenetic species concept (Figure 2). The most common incorrect answer was to use the morphological species concept to classify these two butterflies with distinct physical appearances as different species.

PPTQ4 asks students to identify reproductive outcomes that could arise after introducing a newly-discovered population of critically-endangered woolly monkeys to an existing northern population (Supporting File S5. Speciation and Conservation – Pre/Post-Test Questions). This question aligns with LO-4. While this question showed an increase in performance from pre-test to post-test (Figure 2), only 70.5% of students selected the correct response on the post-test. These data suggest that many students do not consider that these two populations of woolly monkeys could be different species. When we asked students more about this question, they said it was unlikely that enough time had passed for these populations to be two different species, suggesting that students were making inferences about time that we had not asked about in the question.

Exam Questions

Approximately one month after the lesson, students answered eight multiple-choice exam questions (abbreviated EQ for Exam Questions) (Supporting File S6. Speciation and Conservation – Exam Questions) in two rounds using a two-stage exam format (49,50): first individually, and again in small groups of 5-6 students, which were randomly determined. The individual exam questions were identical to the group exam questions. Each exam question focused on potential student conceptual difficulties and targeted at least one of the four learning objectives of the lesson (Table 2). Here we report the results for the students who completed both the individual and group exam questions (n=49). The overall average individual exam score was 87.0 ± 2.2% (SEM), and the overall average group exam score (n=9 groups) was 97.2 ± 2.6% (SEM).

EQ1 explains that western and eastern meadowlarks look alike and have overlapping ranges, but they have different songs that prevent them from interbreeding. Here, students are asked to classify the western and eastern meadowlarks as the same or different species based on the biological and morphological species concepts (Supporting File S6. Speciation and Conservation – Exam Questions). This question aligns with LO-1 and LO-2 (Table 2). On both the individual and group portions of this exam, 100% of students correctly answered this question by classifying these birds as different species according to the biological species concept and the same species via the morphological species concept (Figure 3).

EQ2 asks students to identify which mechanism of evolutionary change is most likely to prevent the speciation process from proceeding between two populations (Supporting File S6. Speciation and Conservation – Exam Questions). This question targets LO-3 (Table 2). Here, 95.9% of students (individually) and 100% of students (group) selected "high gene flow between populations" as the correct response (Figure 3).

EQ3 presents a hypothetical scenario detailing a possible new species of tropical treefrog that has 1) a distinct skeletal structure from any other known species, 2) genetic work showing it has been evolving as an independent lineage for ~1 million years, and 3) the ability to breed and produce fertile offspring with other treefrogs found in the region (Supporting File S6. Speciation and Conservation – Exam Questions). Students are asked to identify which species concepts would classify this newly-discovered population of treefrogs as a new species. This question targets LO-1, LO-2, and LO-4. Here, 95.9% of students (individually) and 100% of students (group) correctly identified that this newly-discovered population would be classified as a new species based on the phylogenetic and morphological species concepts, but would not be classified as a new species based on the biological species concept (Figure 3).

EQ4-6 align with LO-2 (Table 2). For EQ4 and EQ5, students are provided with a phylogenetic tree of individuals from different populations. In EQ4, students are told that all individuals from a select population can interbreed and produce fertile offspring and are asked to identify which species concept(s) would consider these individuals as the same species (Supporting File S6. Speciation and Conservation – Exam Questions). Here, 63.5% of students (individual) and 88.9% of students (group) correctly answered this question by selecting both the biological species concept and the phylogenetic species concept (Figure 3). The most common incorrect response resulted from students choosing only the biological species concept (selected by 30.6% of students individually), suggesting students did not fully consider all of the data presented to them in this prompt. These same trends (63.5% correct responses on the individual portion, 88.9% correct responses on the group portion) were observed in EQ5, where students are given new information about a different sub-population in which all individuals look alike and are again asked to select which species concept(s) would identify these individuals as the same species (Supporting File

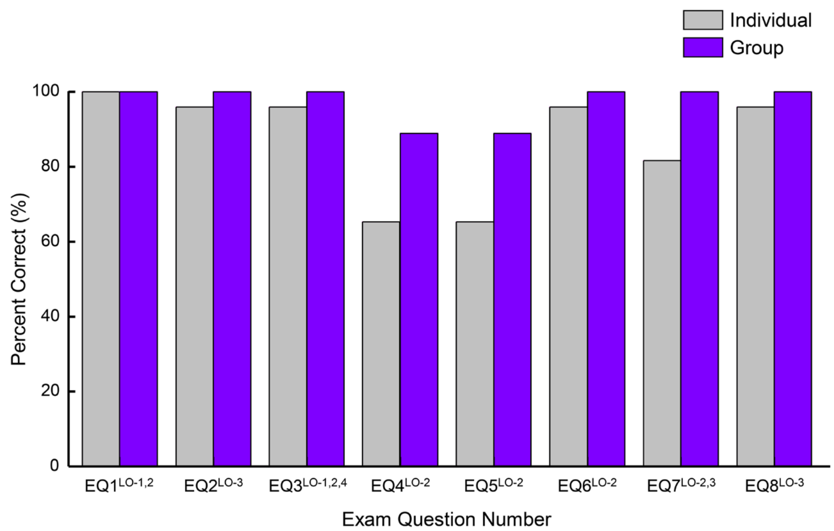

Figure 3. Percent of students answering correctly on individual and group exam questions (EQ). Gray bars represent class average scores for each exam question. Purple bars represent average group exam scores for each exam question. Each exam question was answered by 49 students. Targeted learning objectives are indicated for each question (LOs are listed in the Learning Objective section of the paper).

S6. Speciation and Conservation – Exam Questions, Figure 3). Here, the most common incorrect answer resulted from students choosing only the morphological species concept (selected by 34.7% of students on the individual portion).

EQ6 asks students to use the biological species concept to determine the evidence needed to consider a population of subway mosquitoes as a new species (Supporting File S6. Speciation and Conservation – Exam Questions). Here, 95.9% of students (individually) and 100% of students (group) selected "inability to breed and produce viable offspring" as the correct response (Figure 3).

In EQ7, students are asked to identify the circumstance (different environment or similar environment) in which speciation is more likely to occur, according to the biological species concept (Supporting File S6. Speciation and Conservation – Exam Questions). This question aligns with LO-2 and LO-3 (Table 2). While the majority (81.6%) of students individually selected a different environment as the correct response, 100% of students selected the correct answer on the group portion of the exam.

Finally, EQ8 asks students to identify the circumstance in which natural selection is more likely to play a significant role in speciation over the same time period (Supporting File S6:. Speciation and Conservation – Exam Questions). This question targets LO-3 (Table 2). Here, 95.9% of students (individually) and 100% of students (group) selected that natural selection is more likely to occur in a new environment.

Conclusions

In this lesson, students explore speciation and its real-world implications for conservation. A variety of inclusive instructional practices are used throughout the lesson, including clicker questions, collaborative group work, worksheets, and instructor-facilitated discussions. Socially relevant examples featuring different systems across the tree of life are used to help students connect their learning to contemporary global issues (14,15,54). The lesson also highlights the work of international researchers from diverse backgrounds. After participating in this lesson, students showed increases in performance in the shorter term, as evident by their performance on clicker questions (Supporting File S4. Speciation and Conservation – Clicker Questions) and pre/post tests (Figure 2), and in the longer term, as revealed by students' high exam scores (Figure 3). Making these changes is important because speciation is a fundamental concept in evolutionary biology (55,56) and a solid understanding of speciation - and its relation to conservation - is important for understanding issues that have an impact on both local and global scales.

SUPPORTING MATERIALS

- S1. Speciation and Conservation – Presentation Slides with Instructor Notes
- S2. Speciation and Conservation – Discussion After Application Worksheet Activity
- S3. Speciation and Conservation – Student Worksheet Packet
- S4. Speciation and Conservation – Clicker Questions
- S5. Speciation and Conservation – Pre/Post-Test Questions
- S6. Speciation and Conservation – Exam Questions
- S7. Speciation and Conservation – References Table

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Table 1. Speciation and Conservation: Teaching Timeline. Progression through the lesson with approximate timestamps. Clicker questions are labeled as "CQ," and group discussion questions are labeled as "GDQ."

Activity	Description	Estimated Time
Preparation for Class		
Instructor preparation	1. Review lecture notes and learning goals and objectives. 2. Prepare pre- and post-tests. 3. Create an online prompt for students to identify their symbolically significant animal. Compile photos of these animals to display on Slide 13 of the lecture slides (Supporting File S1). 4. Make one copy of the Student Worksheet Packet for each student (Supporting File S3). 5. Read literature referenced in the lesson.	60-120 minutes
Student preparation	1. Complete pre-test before the start of class (questions and answers are provided in Supporting File S5). 2. Respond to the online prompt by sending the instructor a photo of an animal that is symbolically significant to their own cultures or identities.	10-15 minutes
Class Session I (75 minutes)		
1. Introduction and assessing prior knowledge. *Slides 1-10* in Supporting File S1	1. CQ1-4: Students individually answer a series of four rapid-fire clicker questions that ask them to identify whether two organisms belong to the same species. • Clicker questions and answers are provided in Supporting File S4. 2. Orient student thinking towards what defines a species.	10 minutes
2. Learning outcomes and symbolically significant animals *Slides 11-14* in Supporting File S1	1. Provide students with the learning goals and objectives for this lesson. 2. Show photos of animals that students have identified as symbolically significant to their cultures or identities. 3. Ask students to imagine the impact of these animals becoming extinct.	5 minutes
3. Why are giraffes important, and can we save them from extinction? *Slides 15-25* in Supporting File S1	1. Introduce the giraffe as the focus animal for this lesson. 2. GDQ1: Students share their initial thoughts on the ecological, economic, and cultural significance of giraffes. 3. GDQ2: Students brainstorm factors that may have contributed to the population decline of giraffes in recent years. 4. Describe conservation efforts to protect species from extinction. Introduce the example of giraffe translocation in Uganda. 5. CQ5: Students answer and discuss a clicker question identifying possible challenges that could arise upon translocating giraffes. 6. Emphasize the importance of distinguishing species in maintaining biodiversity and healthy populations as conservation goals.	20 minutes
4. How do we understand speciation in giraffes? *Slides 26-51* in Supporting File S1	1. CQ6: Students individually answer a poll asking them to predict the number of giraffe species. 2. Introduce the speciation process by walking students through the flow chart and providing examples of giraffe speciation at each step of the process. 3. GDQ3: Students discuss and share factors that may cause a population to separate. 4. CQ7: Students answer and discuss a clicker question regarding which mechanisms of change can contribute to fixed differences between isolated populations. 5. CQ8: Students answer and discuss a clicker question asking them to identify possible mechanisms of change that may have contributed to the evolution of coat patterns in giraffes. 6. Explain how different mechanisms of change can influence speciation, using giraffes as examples.	15 minutes

Activity	Description	Estimated Time
5. How do we define a species? *Slides 52-80* in Supporting File S1	1. Introduce students to the morphological, biological, and phylogenetic species concepts. Convey both appropriate applications of each concept in defining a species (using giraffes as examples) and explain the limitations. 2. CQ9: Students answer and discuss a clicker question asking them to determine whether two populations of giraffes belong to the same species based on the morphological species concept. 3. CQ10: Students revisit CQ9 but now must use the biological species concept to determine whether these two giraffe populations belong to the same species. After discussing answers, emphasize that it is not always straightforward to determine whether two populations belong to the same species. 4. CQ11: Students draw on prior knowledge of tree reading to answer a clicker question asking them to predict which phylogenetic tree best depicts populations of giraffes which belong to different species. 5. CQ12: Students revisit CQ11 but now must determine the node in the tree in which the speciation event occurred between these two different giraffe populations.	25 minutes
Class Session II (75 minutes)		
6. Data exploration *Slides 81-89* in Supporting File S1	1. Begin by revisiting CQ6 and explaining that students are now equipped with the knowledge of how speciation works, as well as what defines a species. 2. Introduce the data exploration activity by explaining that the students will now be applying this knowledge to three data sets on giraffes. 3. Students form groups of three. All 1's are experts in the morphological species concept; all 2's are experts in the biological species concept; all 3's are experts in the phylogenetic species concept. 4. Students discuss the number of giraffe species within each data set based on the definitions of their assigned species concept: morphological, biological, or phylogenetic. 5. CQ13: Students use a clicker to vote on whether their group members agree on the same number of giraffe species across all three data sets. 6. CQ14: Students use a clicker to select the number of giraffe species that has the most support among their group.	10 minutes
7. Literature debate on number of giraffe species and the Giraffe Genome Project *Slides 90-97* in Supporting File S1	1. Emphasize that scientists have still not reached a consensus on the number of giraffe species. Highlight (22-25) to demonstrate that this topic is still an ongoing debate in the literature. 2. GDQ4: Students identify the species concept involved in the argument between Bercovitch et al. and Fennessy et al. regarding breeding behaviors as a way to classify species. 3. GDQ5: Students identify the species concept involved in the argument between Bercovitch et al. and Fennessy et al. regarding fur patterns as a way to classify species. 4. Wrap up this segment by emphasizing that defining a species is not straightforward. Conclude with the Giraffe Genome Project to show that scientists are still actively trying to resolve this disagreement over the number of giraffe species.	12 minutes
8. The bigger picture *Slides 98-99* in Supporting File S1	1. Broaden students' views of speciation by showing that distinguishing and identifying species is important for everyone, not just scientists. 2. Students reflect on the impacts of classifying species on conservation efforts.	3 minutes
9. Application worksheet activity *Slides 100-106* in Supporting File S1	1. Distribute one Student Worksheet Packet (Supporting File S3) to each student. 2. Students work in small groups to answer the questions for each scenario.	35 minutes
10. Debrief after application worksheet activity, summary, and additional resources *Slides 1-26* in Supporting File S2; *Slides 107-108* in Supporting File S1	1. Regroup the class to have an open discussion regarding the answers to the questions for each scenario. 2. CQ15-20: These clicker questions are identical to the questions in the Student Worksheet Packet, with the addition of answer choices that students can individually select and discuss. 3. GDQ6-9: Certain questions lend themselves better to group discussions because this format is effective in capturing multiple student voices. 4. Briefly highlight the main points of the lesson and offer optional supplementary readings for enrichment.	15 minutes

Activity	Description	Estimated Time
Follow-up		
Instructor completion	1. Administer post-test after Class Session II. Compare pre-test and post-test data. • Post-test questions and answers, which are identical to those of the pre-test, are provided in Supporting File S5. 2. Include questions about speciation and conservation on the next exam, EQ1-8. • Exam questions and answers are provided in Supporting File S6.	10 minutes
Student completion	1. Complete post-test. 2. Complete exam questions on speciation and conservation.	10-20 minutes

Table 2. Speciation and Conservation: Assessment Questions. Assessments used to examine student understanding of speciation arranged by learning objective. Question types are abbreviated as PPTQ (Pre/Post-Test Question), CQ (Clicker Question), GDQ (Group Discussion Question), and EQ (Exam Question).

	Learning Objective #1: Difficulties in defining species	**Learning Objective #2:** Common ways in which species are defined (morphological, biological, phylogenetic) and use of these definitions to evaluate scientific evidence	**Learning Objective #3:** Likelihood of speciation to occur in different scenarios and how this is influenced by mechanisms of evolutionary change	**Learning Objective #4:** Global issues (e.g., conservation) for which it is important to know whether similar organisms are one or more species
Pre/Post-Test Questions	PPTQ2	PPTQ2-3 PPTQ5	PPTQ1	PPTQ4
Clicker Questions	CQ1-4 CQ13 CQ16	CQ9-12 CQ15 CQ16 CQ18-20	CQ7-8	CQ5 CQ17
Group Discussion Questions	GDQ7	GDQ4-5 GDQ6-7	GDQ3 GDQ8-9	GDQ6-7
Exam Questions	EQ1 EQ3	EQ1 EQ3-7	EQ2 EQ7-8	EQ3