USING DNA TO EXPLORE LIZARD PHYLOGENY

OVERVIEW

This lesson serves as a supplement to the film *Lizards in an Evolutionary Tree*. The film focuses on a closely related group of lizards called anoles (genus *Anolis*) that live in the Caribbean islands. They have evolved to occupy different niches in the environment, and studying them is giving researchers surprising insights into evolutionary processes, such as adaptation by natural selection, convergent evolution, and the formation of new species.

In this activity, students explore the evolutionary relationships of the Caribbean anole lizards. In Part 1, students examine the diverse appearance of the lizards and group them into categories according to different body types. Differences between the species illustrate adaptive radiation; students can generate hypotheses about possible reasons why different adaptations have evolved and infer evolutionary relationships among lizards based on their adaptations. In Part 2, mitochondrial DNA sequence data from these same species of lizards are used to generate a phylogenetic tree to reveal their true evolutionary relationships. Students need to reconcile why the evolutionary tree obtained in Part 2 is different from the original grouping proposed in Part 1. Students watch the film in stages at different points in the activity.

KEY CONCEPTS AND LEARNING OBJECTIVES

- An adaptation is a structure or function that confers greater ability to survive and reproduce in a particular environment.
- Adaptive radiation occurs when an ancestral species diversifies into many descendant species that occupy different environmental niches.
- Different groups of organisms can independently evolve similar traits by adapting to similar environments or ecological niches in a phenomenon known as convergent evolution.
- DNA sequence comparisons among different populations and species allow scientists to determine the degree of relatedness of different species.

After completing this activity, students should be able to

- explain how the ecomorphs of anole lizards in the Caribbean are examples of both adaptive radiation and convergent evolution,
- use sequence alignment and phylogeny-generating software to compare mitochondrial DNA sequences, and
- analyze data from a phylogenetic tree and determine whether the data support a hypothesis.

CURRICULUM CONNECTIONS

Curriculum	Standards
NGSS (April 2013)	MS-LS1-5, MS-LS4-2, MS-LS4-4, MS-LS4.A, MS-LS4.B, MS-LS4.C HS-LS4-1, HS-LS4-3, HS-LS4-4, HS-LS4-5, HS-LS4.A, HS-LS4.B, HS-LS4.C
AP Biology (2012–13)	1.A.1, 1.A.2, 1.A.4, 1.B.2, 1.C.1, 1.C.2, 1.C.3
IB Biology (2015)	4.1, 5.1, 5.2, 5.4, C.1

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TIME REQUIREMENTS

Allowing time for discussion and viewing the film, this activity will take two 50-minute periods for an AP Biology class. Allow for more time if you need to cover basics of DNA alignment in greater detail.

PRIOR KNOWLEDGE

Before doing this lesson, students should have a basic understanding of natural selection, evolution, and adaptation; familiarity with the concept that organisms fill specific niches in their environments; an understanding that DNA sequence changes during evolution and that closely related species have similar DNA sequences; and a basic understanding of phylogenetic trees, especially the concept that closely related species are located close together on adjacent or nearby branches of a phylogenetic tree.

MATERIALS

- A set of lizard cards that accompanies this document
- Access to a computer that is connected to the Internet
- HHMI Short Film *Lizards in an Evolutionary Tree*
- (Optional) A computer with the freely available ClustalX program installed

BACKGROUND SUMMARY

This section provides a brief summary. For more information about the anole lizards and the film, please refer to the In-Depth Guide http://media.hhmi.org/biointeractive/activities/lizard/IDG_Lizards.pdf).

Different Morphologies of Anole Lizards

Figure 1. Caribbean islands are home to the anoles. The anole species featured in the film and this activity live on the islands of Cuba, Jamaica, Hispaniola (which comprises Haiti and the Dominican Republic), and Puerto Rico. (Reproduced with permission from Losos, J. *Lizards in an Evolutionary Tree*. UC Press, 2009.)

Species of Caribbean anoles can be categorized into six groups according to their body characteristics (morphology) and the ecological niches they occupy. The groups are referred to as ecological morphotypes, or ecomorphs. Table 1 lists the six anole ecomorphs found in the Caribbean islands and their body features. The film discusses only four ecomorphs: the crown-giant, trunk-ground, twig, and grass-bush anoles. In this activity, representatives from the trunk-crown, trunk-ground, twig, and grass-bush anoles are shaded in the table.

Table 1. Six Ecomorphs of Anole Lizards Found in the Caribbean Islands

Ecomorph	Body length	Limb length	Toe pad size	Tail length	Color	Habitat
Crown-giant	130–191 mm	Short	Large	Long	Usually green	High trunks and branches
Trunk-crown	44–84 mm	Short	Very large	Long	Green	Trunks, branches, and leaves
Trunk	40–58 mm	Intermediate	Intermediate	Short	Gray	Trunks
Twig	41–80 mm	Very short	Small	Short	Gray	Narrow twigs
Trunk-ground	55–79 mm	Long	Intermediate	Long	Brown	Lower trunk and ground
Grass-bush	33–51 mm	Long	Intermediate	Very long	Brown	Bush and grasses

The body features of the ecomorphs are adaptations to their particular niches. (See Figure 2 for an illustration of the habitats of different ecomorphs.) In the film, the long-legged trunk-ground anoles are shown to move faster on the ground than the short-legged twig anoles. The long-legged adaptation helps them not only catch prey on the ground but also avoid predators. However, on twigs, the twig anoles can move easily with their short legs, while the trunk-ground anoles are clumsy. The grass-bush anoles are small and have long legs and strikingly long tails that help them balance on thin branches and blades of grass, and the trunk-crown anoles, with their large toe pads, are adapted to living on leaf surfaces in the canopy.

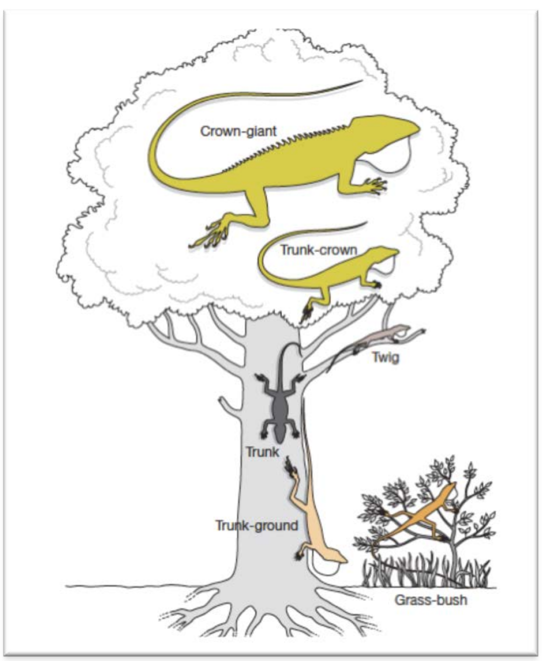

Figure 2. Anoles occupy a variety of ecological niches. Different types of anole lizards have evolved adaptations that enable them to be successful in different ecological niches—different parts of trees, grasses, and bushes. The figure shows the six ecomorphs of anole lizards found in the Caribbean islands in their habitats. (Reproduced with permission from Losos, J. *Lizards in an Evolutionary Tree*. UC Press, 2009.)

Convergent Evolution Among Anole Lizards

There are different explanations for why similar ecomorph classes can be found on each of the different islands.

One hypothesis is that each ecomorph evolved once, and the species belonging to the same ecomorph are closely related to one another. Under this scenario, one possibility is that the islands were contiguous in the past when the ecomorphs evolved, and subsequent plate tectonics separated the islands. Another possibility is that the ecomorphs all evolved on the same island, then they all migrated over the narrow channels and established themselves in each of the islands. In these scenarios, you would predict that DNA analysis would reveal, for example, that the twig anole species on all of the islands are more closely related to one another than they are to the grass-bush, trunk-ground, or trunk-crown anoles on the same island.

An alternative hypothesis is that the ecomorphs evolved repeatedly and independently on each of the islands. A prediction based on this hypothesis is that the anoles on each island would be more closely related to one another than they would be to species on other islands belonging to their ecomorph.

When scientists examined DNA sequences from dozens of species of Caribbean anoles, they found that, in general, species on the same island tend to be more closely related to one another than to species with similar body types found on different islands. This suggests that the same adaptations evolved independently in different anole populations on each of the islands, an example of convergent evolution.

SUGGESTED PROCEDURE

Students can work individually or in small groups of perhaps between two and four, depending on your classroom. A student worksheet is provided to guide the activity, but, as explained below, there are points at which you may wish to stop and conduct a whole group discussion, show the film to the whole class, or explain a concept. Alternatively, you can interact with each individual student group, depending on the dynamics of your classroom.

PART 1: Identifying the Ecomorphs and Exploring Anole Adaptive Radiation

At this point, students have not seen the film *Lizards in an Evolutionary Tree* yet.

1. Hand out the student worksheet for Part 1 and a set of lizard cards to each group. Let teams of students examine photographs of 16 species of anole lizards and sort them into as many groups as they want.

Students can use any clues in the photographs to group the lizards as they see fit. They may use features of the ecomorphs, including color, thickness or length of legs, body shape, tail length, or size. They may even group them by the habitat pictured, such as branches, tree trunks, or grass.

2. Allow students time to answer Question 1, then ask the teams to explain how they grouped the lizards and their rationale for the various groupings. If they pick a body feature, ask them to speculate about the advantages or disadvantages of such a body feature in the environment the species occupy.

3. Watch the first part of the film (up until 09:48, the end of the experiment on a small rocky island).

4. Allow students time to answer Question 2 and revise their groupings if they wish.

5. Reveal the four ecomorphs. The 16 species represent the following four ecomorphs. Refer to Table 1 for more descriptions of ecomorphs. Also, the first page of the lizard picture file can be used as a master reference.

Four ecomorph classes:

- Trunk-crown: green, foot pads
- Twig: short legs, pencil-like morphology
- Grass-bush: Slender limbs, body, and long thin tail
- Trunk-ground: Robust and chunky appearance, with long legs

6. Allow students time to answer Questions 3–6. Discuss the body features of the ecomorphs, how they are adapted to the environment they live in, and the concept of adaptive radiation. For example, the large toe pads of the trunk-crown anoles allow them to adhere to leaves and move about freely among the leaves of the tree canopy. The short legs of the twig lizards are adaptations to living on thin twigs. Refer to the “Different Morphologies of Anole Lizards” section in the background. Discuss Question 6, as it leads into Part 2.

PART 2: Generating a Phylogeny from DNA Sequences and Revealing Evolutionary Relationships of Anoles

1. (Optional) If your students have never been exposed to generating and interpreting phylogenies from DNA sequences, you may want to use the following resources from the BioInteractive website:

   a. Follow the procedure outlined in *Sequence Alignment Introduction Using ClustalX*: http://media.hhmi.org/biointeractive/activities/lizard/Sequence-Alignment-Introduction.pdf

   b. Have your students explore the Click and Learn *Creating Phylogenetic Trees from DNA Sequences*: http://www.hhmi.org/biointeractive/creating-phylogenetic-trees-dna-sequences

2. Hand out the first two pages of Part 2 of the student worksheet and address any questions students might have. Have students follow the directions to download the *Anolis* sequences text file and then use www.phylogeny.fr to align the sequences and create a phylogenetic tree.

3. The website will generate a phylogenetic tree that can be saved in a variety of formats. Because of the nature of the phylogenetic analysis that involves random resampling, each student may get a slightly different tree, but the overall pattern should be mostly similar.

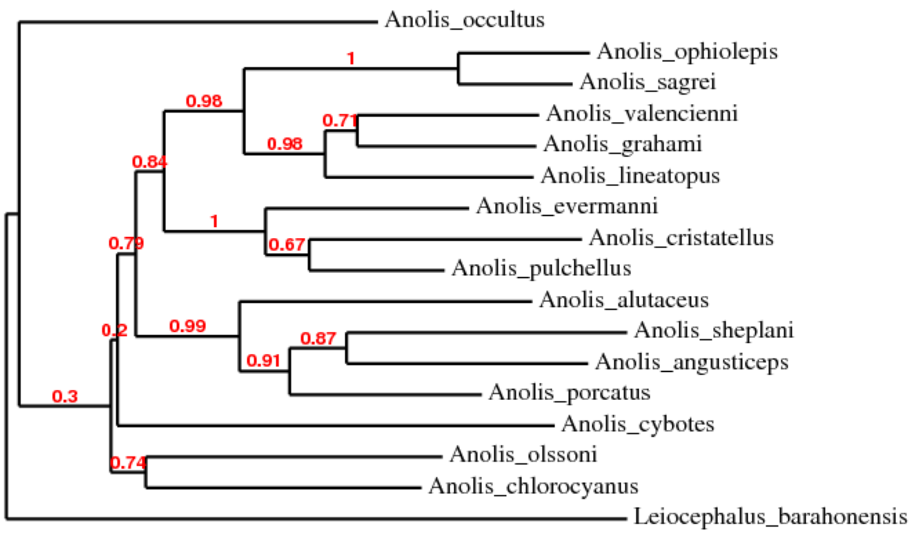

Figure 3. Example phylogeny from www.phylogeny.fr. Students’ phylogeny should be similar, but there may be small differences.

4. The red numbers in the phylogeny result are bootstrapping values and denote how confident the program is about the specific branch pattern. A value of 1 indicates high confidence; 0 indicates no confidence. Values less than 0.5 are generally regarded as uncertain. However, because the purpose of this activity is to illustrate the principle of DNA sequence comparison, students should not worry about it too much.

5. Print out two copies of the students' phylogenetic tree per group of students and hand out the rest of the student handout.

6. Have them use the list of lizard species to color the trees in the following ways:

a. On one copy, color each branch according to the island of origin (Cuba, Hispaniola, Jamaica, or Puerto Rico). If both branches at a node are the same color, color the root branch and continue left to the next node.

b. On the other copy, color each branch similarly according to the ecomorph.

7. Allow students time to examine the phylogenetic trees and answer Questions 1–6. Discuss as a class if you wish.

8. Watch the rest of the film and allow students time to answer Question 7. Discuss as a class.

9. Students may ask whether convergent evolution occurs in groups other than the anoles. Perhaps students can be enticed to volunteer if they know anything that looks like a shark. They could list tuna, dolphins, manatees, and ichthyosaurs. Wings in vertebrates are another classic example: birds, bats, pterosaurs. Reduced or absent limbs is another: snakes, caecilians, sirens, slow worms, worm lizards.

10. (Optional) Refer to the reference-phylogeny figure at the end of this lesson. This is one of the phylogenies published in a scientific journal. Find the 16 species used in this activity and see if their phylogeny differs from the tree generated by the student groups. Some reasons for differences: The paper uses many more species, which can generate different patterns; the paper also uses different calculations to align the sequences and generate phylogenies.

List of Lizard Species

Species name	Island	Ecomorph
Anolis alutaceus	Cuba	Grass-bush
Anolis angusticeps	Cuba	Twig
Anolis chlorocyanus	Hispaniola	Trunk-crown
Anolis cristatellus	Puerto Rico	Trunk-ground
Anolis cybotes	Hispaniola	Trunk-ground
Anolis evermanni	Puerto Rico	Trunk-crown
Anolis grahami	Jamaica	Trunk-crown
Anolis lineatopus	Jamaica	Trunk-ground
Anolis occultus	Puerto Rico	Twig
Anolis olssoni	Hispaniola	Grass-bush
Anolis ophiolepis	Cuba	Grass-bush
Anolis porcatus	Cuba	Trunk-crown
Anolis pulchellus	Puerto Rico	Grass-bush
Anolis sagrei	Cuba	Trunk-ground
Anolis sheplani	Hispaniola	Twig
Anolis valencienni	Jamaica	Twig
Leiocephalus barahonensis		Outgroup

ANSWERS

PART 1: Identifying Ecomorphs and Exploring Anole Adaptive Radiation

For Questions 1–4, student answers will vary. It's important that they can explain their rationale.

5. Explain how the different ecomorphs are an example of adaptive radiation.

Adaptive radiation occurs when an ancestral species diversifies into many descendant species that occupy different environmental niches. Each of the different ecomorphs displays physical characteristics that make the organism suited to the particular niche in which it lives.

6. Develop a hypothesis about why similar ecomorph classes can be found on many of the different islands in the Caribbean.

Student answers will vary. This answer can be referenced again in Part 2 if you wish.

PART 2: Generating a Phylogeny from DNA Sequences and Revealing Evolutionary Relationships of Anoles

1. What general patterns do you see in the tree?

a. Colored by island:

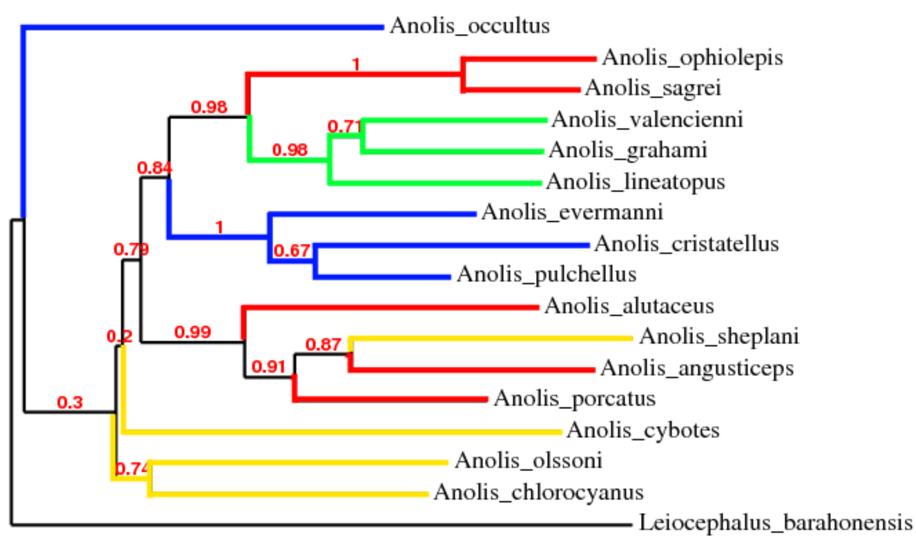

Figure 4. Phylogeny colored in according to geographical distribution.
Red = Cuba, Green = Jamaica, Blue = Puerto Rico, Yellow = Hispaniola. All species from Jamaica cluster together, and many species from Puerto Rico and Hispaniola cluster together. Species from Cuba are more divided but still show some clustering.

b. Colored by ecomorph:

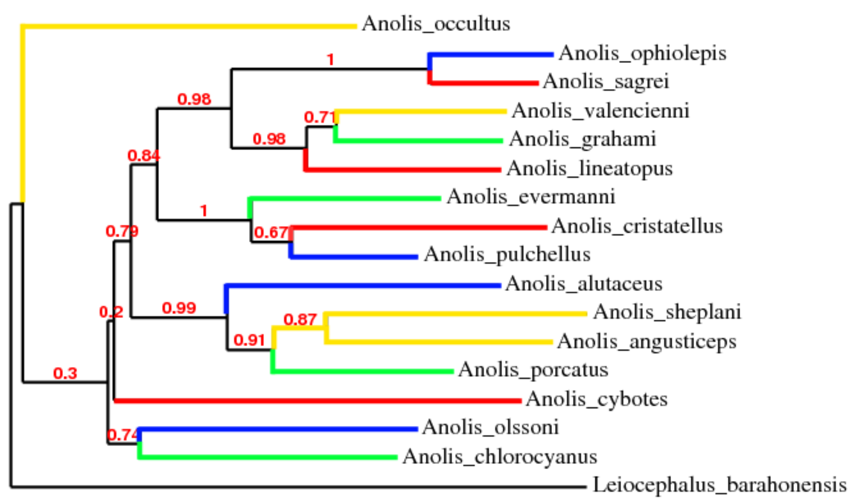

Figure 5. Phylogeny colored in according to ecomorphs.
Red = Trunk-ground, Green = Trunk-crown, Blue = Grass-bush, Yellow = Twig. Except for two twig species, none of the ecomorphs cluster together, meaning they are not closely related.

In general, when you look at species colored by islands, they seem to cluster together. For example, the three Jamaican species cluster together, as do three of the four Puerto Rican species. The Cuban and Hispaniola species also cluster, but the pattern is more complicated. When you look at the species colored by ecomorphs, there are no clear patterns.

2. Do species from the same ecomorph group together on the tree? Provide evidence to support your answer.

Different ecomorphs are grouped together on branches. Except for two twig species, none of the ecomorphs cluster together.

3. Do species from the same island group together on the tree? Provide evidence to support your answer.

All species from Jamaica cluster together, and many species from Puerto Rico cluster together. Species from Cuba and Hispaniola also cluster, but the pattern is more complicated.

4. Based on the trees you colored, develop a claim about whether the species that belong to the same ecomorphs or the species that live on the same islands are more closely related to each other. Justify your claim with several pieces of evidence from the colored phylogenetic trees.

The species that live in the same island are more closely related to each other than are species of the same ecomorph group but living on different islands.

5. Let's review the two hypotheses discussed earlier. One hypothesis is that each ecomorph evolved once, and the species belonging to the same ecomorph are closely related to one another. An alternative hypothesis is that the ecomorphs evolved repeatedly and independently on each of the islands. Which hypothesis is supported by the DNA analysis shown in the tree? Justify your answer and explain your reasoning.

The DNA evidence suggests that species from the same island are more closely related than are species from the same ecomorph group. This finding supports the hypothesis that most of the lizards on each island evolved independently, in a process of convergent evolution, although there are exceptions.

6. Examine the relationship between *Anolis sheplani* and *Anolis angusticeps*. Does it obey the hypothesis you provided in the question above? Explain your answer. Develop a possible hypothesis that could explain this relationship.

***Anolis sheplani* is a Hispaniola twig species that is closely related to another twig species from Cuba, *Anolis angusticep*s, in a branch that is otherwise all Cuban. Student answers will vary, but one could hypothesize that *A. sheplani* evolved after Hispaniola was colonized by a twig ecomorph species from Cuba.**

7. Watch the rest of the film. Identify and explain any similarities and differences between the phylogeny shown in the film and the phylogeny you made in this activity.

The results should agree in general with what is shown in the film. The film explains that species on an island are more closely related to one another than to species belonging to the same ecomorph on other islands. Students will likely note that the data set they were working with presented a more complex set of relationships (not as clean as in the film). The general picture is the same, but some cases (see Question 6) don't fit. Furthermore, the film discusses four ecomorphs (the crown-giant, trunk-ground, twig, and grass-bush anoles), one of which is different from the species in this activity.

RELATED CLASSROOM RESOURCES

Other Resources for the Film (http://www.hhmi.org/biointeractive/origin-species-lizards-evolutionary-tree)

Check this page to see other resources developed for the film.

Sorting Seashells (http://www.hhmi.org/biointeractive/sorting-seashells)

In this Click and Learn, students explore principles of taxonomy by sorting seashells according to their morphological characteristics and constructing an evolutionary tree.

Classroom Activities: Biodiversity and Evolutionary Trees (http://www.hhmi.org/biointeractive/biodiversity-and-evolutionary-trees)

This is a companion classroom resource to accompany the Click and Learn above. It also includes a DNA sequence comparison activity of the seashells. In the seashell activity, phylogeny generated by examining morphology closely aligns with that generated from DNA, so it is a good contrast to the case of the anole lizards, in which the two do not agree.

Creating Phylogenetic Trees from DNA Sequences (http://www.hhmi.org/biointeractive/creating-phylogenetic-trees-dna-sequences)

This Click and Learn explains how DNA sequences can be used to generate such trees, and how to interpret them.

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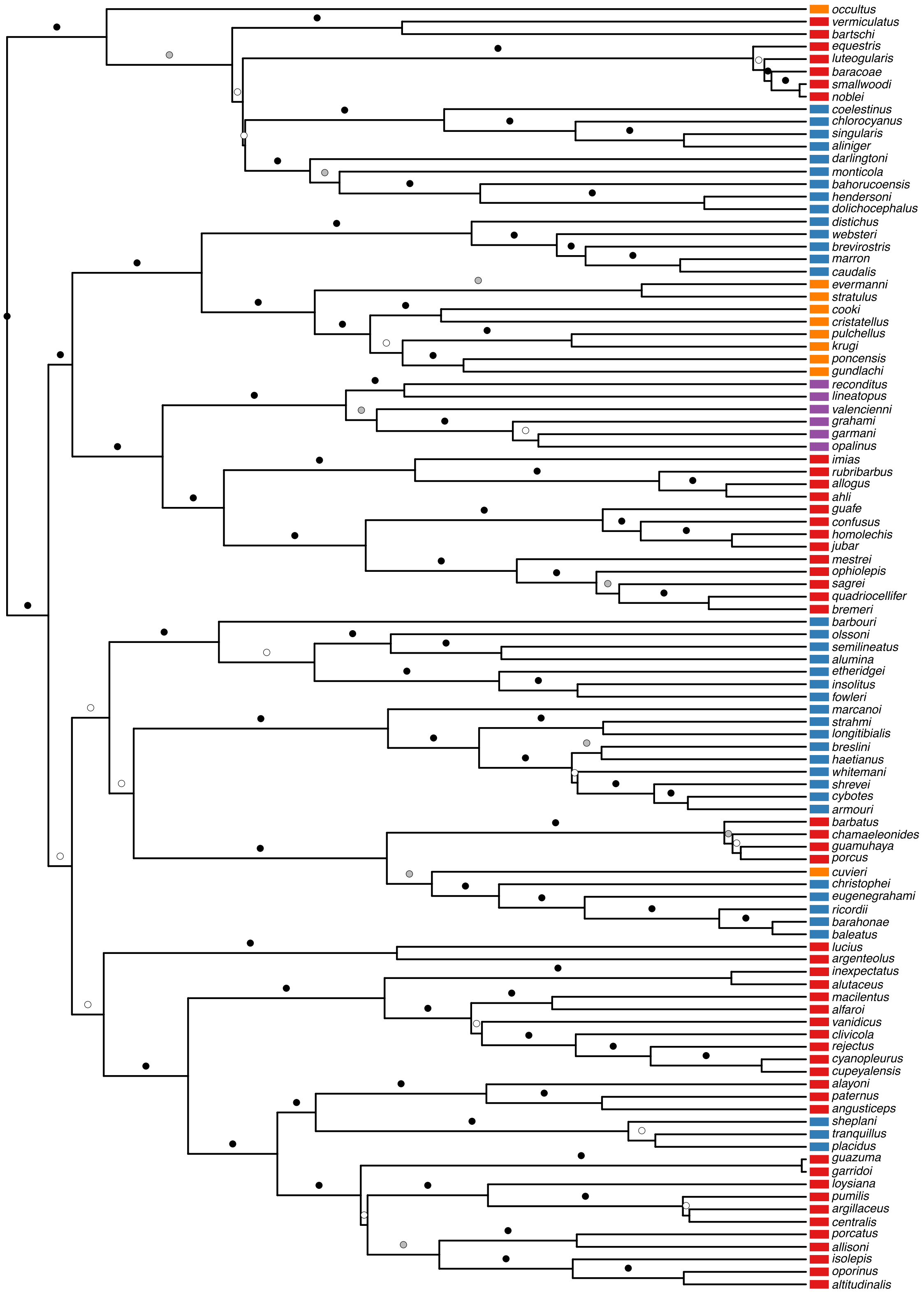

Fig. S1. Maximum clade credibility (MCC) tree resulting from BEAST analyses. Posterior probability (pp) values from the MrBayes analyses used to generate the starting tree for BEAST are indicated by circles above branches (black, pp > 0.95; gray, 0.95 > pp < 0.70; white, pp < 0.70). Island occupancy is indicated across the tips of the tree (red, Cuba; blue, Hispaniola; orange, Puerto Rico; purple, Jamaica).

NOTE: Color key descriptions in the figure legend have been modified from the original publication to match the actual colors in the figure.