

A COMPARISON OF BEHAVIOR AND MORPHOLOGY BETWEEN TWO CRYPTIC SKINK SPECIES ON MOOREA, FRENCH POLYNESIA

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Abstract. Cryptic Species Complexes are recognized with increasing frequency as genetic tools allow scientists to discern between nearly identical looking organisms. On the island of Moorea, French Polynesia, there are two sympatric, cryptic skinks, *Emoia cyanura* and *Emoia impar*, that are difficult to discern from one another using morphological cues alone. This study aims to qualify the morphological traits that are used for identification of these skinks, and determine which traits are most reliable for species identification in the field. Additionally, an ethogram of both species behavior was created, and field observations were conducted to determine if there were distinct differences in behavior or substrate preference between the two species. Behavioral differences might have been useful for field identification, as well as provided an explanation for a reproductive barrier between the species. After collection events and field observations, fourteen distinct behaviors were described on seven different substrates. No prominent differences in behavior or substrate use were recorded.

Key words: skinks; *Emoia cyanura*; *Emoia impar*; color; behavior; morphology; ethogram; Moorea

INTRODUCTION

With the increased use of DNA barcoding, cryptic species have been described with increasing frequency (Herbert et al. 2004). The Cryptic Species Complex (Bickford 2007), or crypsis, refers to the phenomenon in biology where two or more distinct species have been historically classified as one species due to morphological similarities (Mayr 1963). With the help of genetic analyses and observations about reproductive habits, complexes containing two or more distinct species arise from what was previously thought of as one species. The recognition of cryptic species indicates that the biological diversity on earth is much more complex than previously thought. Understanding the processes that lead to crypsis, as well as the ecological significance of cryptic species, is one important goal in understanding diversity.

The skinks of Moorea, French Polynesia, provide an ideal model to study crypsis in an island setting. Two skink species, *Emoia cyanura* (the "white-bellied copper-striped skink") and *Emoia impar* (the "dark-bellied copper striped skink"), are cryptic and widespread on Moorea (Zug 2013).

Biochemical evidence has proven that these two species are not as genetically similar as their morphologies would suggest (Bruna et al. 1995). Surprisingly, each skink is more closely related to other skinks in the genus than they are to each other (Bruna et al. 1995). Additionally, there is an alternative, copper color morph found of both of the species (Bruna et al. 1996). The common morph has a triple dorsal stripe lengthwise over a darker body, while the alternative morph has a bronze dorsal coloration (Crombie 1986). Selection pressures may have created similar looking organisms that at one time had ancestors that were more morphologically different.

A few key ecological traits are already known about the two skink species. They are sympatric (Vickers 2002) and their geographic ranges additionally overlap in other Pacific Islands (Zug 2013). It has also been shown that *E. impar* and *E. cyanura* have different body temperature ranges, and that each species might inhabit different microhabitats depending on access to sun exposure (McElroy 2007). *E. cyanura* is more of a thermal generalist, meaning it prefers to live in a wider range of temperatures compared to *E. impar* (McElroy 2007). It is not known if

there are any other behavioral differences between the two species. In fact, the general behaviors of either species have not been cataloged in their natural habitats. The mechanism of habitat separation is poorly understood

It is also still poorly understood if selective pressures that lead to similar physical appearances can additionally shape nonphysical traits, like behavior. Behavior, compared to morphology, is rarely used as a tool for species identification in the field.

One goal of this study is to document and understand the two skinks' behavior in the wild, and to discern whether behavioral tendencies differ between the two cryptic skink species. A supplemental objective of this study is to determine whether tail and belly coloration are effective traits to discern between species in the field.

MATERIALS AND METHODS

Study site and study organisms

All research was conducted on the island of Moorea, French Polynesia (17.5333° S, 149.8333° W). All skinks were collected from one of four locations on the island: The UC Berkeley Gump Station (-17° 29.454', -149° 49.668' and -17° 29.506', -149° 49.611'), at the Pao Pao River crossing (-17° 31.548', -149° 50.175'), Marae Titiroa (-17° 32.185', -149° 49.797'), and in Mari Mari Kellum's property (-17° 30.430', -149° 51.103').



Fig. 1. Map of study locations on Moorea. Red and Blue markers represent observation sites, while red markers alone represent collection sites. Base map courtesy of the Geospatial Innovation Facility, University of California, Berkeley.

Observations were conducted at all of these sites and two additional sites: The Pao

Pao pineapple farms (-17° 31.239', -149° 49.276') and along the 'Āfareaitu trail (-17° 32.852', -149° 48.516') (Fig.1).

Study organisms consisted of *Emoia cyanura* and *Emoia impar* (Appendix 1), which are two reptiles in the infraorder Scincomorpha and family Eugongylidae (Zug 2013). This work conformed to the guidelines of UC Berkeley Animal Use Protocol T042-0814.

Behavioral surveys

Sites with minimal human disturbance and with adequate access to sunlight were located, and observations were collected from an area with a view of the observation area. A listing of behaviors for each skink observed was initially created to make observations consistent. Once a skink was spotted, 7X binoculars were used to follow and dictate the skink's appearance and behavior until it disappeared from sight. Pictures of each skink were taken if possible, in order to identify to species later. A consistent set of features were recorded for each observation, including time of observation, the duration of the behaviors, location and substrate behaviors were performed on, any physical characteristics of the skinks, and time of pictures. After twenty minutes in an area, another location was selected in order to assure observations of the same skink were not made more than once. Observations were later compiled to understand behavioral trends that might vary between species.

Color variation

In order to determine whether tail and belly color are effective traits for distinguishing between species, skinks were captured by hand or net at the Pao Pao River Crossing, the Marae, Mari Mari's property, and the Gump Station. Measurements of snout to vent length were taken, and sex was determined if possible by hemipene inversion. Additionally, pictures of the tail, the belly, the head, and the dorsal side of the skink were taken along with a white standard for subsequent color correction, using a 16 megapixel Nikon Coolpix AW110 camera. Non-toxic, washable paint bought from the supermarket was applied to the skinks' dorsal side to prevent recapture.

Statistical analyses

After measurements were made in the field, Adobe Photoshop CS2 was used to obtain red, green, and blue (RGB) values for tails and bellies (Adobe Systems Incorporated 2013). Methods in Photoshop followed the guidelines of Stosh Ozog in 2009. The Ruler Tool was used to find the midpoint of either the belly or the tail, and the Adjustments Tool was used to find RGB values. All statistical analyses were conducted in R (R Development Core Team 2013). A Principle Components Analysis (PCA) was used to find the first two principle components of skink trait matrices that included Snout-Vent-Length, RGB values for tail and belly, and presence of parietal eye and scale fusion. Wilcoxon–Mann–Whitney two-sample rank-sum tests or paired t-tests were used to compare red, green, or blue values between species. To test the variables of species and substrate on the proportion of time spent on each substrate, a two-way analysis of variance (ANOVA) was conducted in R. TukeyHSD post-hoc comparisons between substrates were used to compare proportion of time spent on different substrates.

RESULTS

Ethogram of behaviors

After observations in the field, a total of fourteen distinct behaviors were established on six different substrates. The behaviors include resting, sunning, walking, eating, flicking of tongue, climbing, chasing, jumping, hiding, running, face rubbing, confrontations, mating, rapid opening and closing of the mouth, and moving in place. Four general categories of behavior were identified: stationary behaviors, locomotion, body and facial movements, and interactions.

(a) **Stationary**

Resting: Resting consisted of any instance where a skink would reside on a substrate with open eyes and no other movement. Resting could be performed in shade or sunlight, but as opposed to sunning, skink does not tuck in arms or seek out sunlight for the resting behavior.

Sunning consists of a skink positioning itself in a sun patch and remaining relatively motionless while tucking its limbs into the body.

(b) **Locomotion**

Walking was the continuous, unhurried movement of a skink across a relatively flat substrate.

Climbing was characterized as a skink moving in a predominantly vertical direction up a steep substrate like a fern or tree. Sometimes climbing was performed to obtain food, but sometimes skinks climbed up and down a tree for no apparent reason.

Running was when a skink moved in a rapid manner across a relatively flat substrate, without chasing or being chased by another skink. Running was often only a few seconds long, or lead to a skink leaving the observation area.

Jumping only lasted one to two seconds, and was characterized as a skink moving from one area to another lower area by pushing off the substrate and becoming airborne. Jumps were never higher than one or two feet.

Moving in place was characterized as a skink turning in circles, moving back and forth in a contained area, or otherwise moving its body without traveling any distance.

Hiding was only observed in coconuts or under leaves. In coconuts, it was impossible to observe what was happening inside, so hiding meant that a skink disappeared from view into a coconut. Hiding in leaf litter consisted of a skink moving under a leaf and then remaining motionless.

(c) **Body and facial movements**

Rapid opening and closing of the mouth was only observed in one skink. It happened between a bout of tongue flicking, and was characterized as the skink opening and closing its mouth repeatedly for a few seconds.

Eating includes biting and chewing of some food item. The skinks were observed consuming mashed up beetles, an unidentifiable white substance, and a nursery of aphids on a hibiscus leaf.

Face rubbing would occur immediately after eating, but not always after an eating event. A skink would rub its face on the substrate, and face rubbing was observed on a rock and a tree branch. This behavior was often a very jerky movement of the head against the substrate.

Flicking of Tongue consisted of the extension and contraction of the tongue. These movements were fast, each lasting less than a half of a second and repeated about once every other second. Tongue flicking accompanied other behaviors, such as head movements, eating, or moving in place. This behavior frequently precluded a confrontation or chase between two skinks.

(d) Interactions

Chasing occurred when one skink either ran after another or ran away from another skink. Chases were observed in every environment, and usually occurred between two skinks of the same species. Chases were usually brief, only a few seconds long. However, a few were observed to last for about eight seconds, and some chases were intermittent and occurred consecutively between two or more skinks within the span of a minute or less.

Confrontations consisted of two or more skinks physically interacting or fighting in an aggressive manner. Confrontations were very brief, lasting only a second or two, and were always preceded or followed by chasing. Confrontations were also almost always preceded by tongue flicking.

Mating was only observed once between two *E. cyanura* for 72 seconds. The male was positioned on the dorsal side of the female, and the male skink's jaws were locked onto the side of the neck of the female for the entire encounter. There was some moving in place, but much of the encounter was motionless. After the mating event, the male was observed to have prominently everted hemipenes.

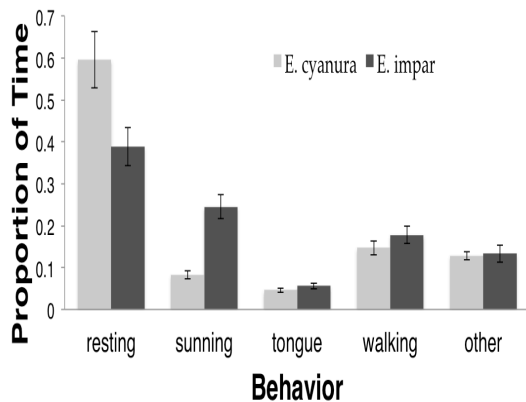


Fig.2 Proportion of Common Behaviors of *Emoia cyanura* and *Emoia impar*.

Substrate categories

Substrates were recorded as well as behavior for most observations. Substrate prevalence depended on location, but included trees, coconuts, leaf litter, palm fronds, sticks, ferns, and rocks.

General behavioral differences

After observations of forty-nine different skinks in various habitats, including 21 *Emoia impar* and 28 *Emoia cyanura* observations, the four most common behaviors observed (running, walking, sunning, and tongue flicking) were plotted against proportion of time spent performing each behavior (Fig.2). The other eleven behaviors were included in the "other" category; the rest of the behaviors were relatively rare for both species.

Substrate residence times

The substrates that behaviors were conducted on were recorded, and average proportion of time spent on each substrate was calculated for both species (Fig. 3). A two-factor analysis of variance (ANOVA) was used to test if the proportion of average skink substrate residence time differences between seven different substrates. Results showed no significant difference between species, $F(1,6)=1.47$, $p=.23$; no significant interaction between species and proportion of substrate residence time $F(1,5)=1.60$, $p=.18$; but the difference in combined proportions of time spent on different substrates was significant, $F(1,6)=2.58$, $p=.029$. After species was excluded from the model, another two-way ANOVA was run to test if proportion of time spent on the seven substrates was significantly different. Results showed that there was a significant difference, $F(1,6)=2.42$, $p=.037$. Tukey post-hoc comparisons were run on the simplified model after species was excluded. Results showed comparisons of the combined proportion of time spent on substrates, and no significant difference was exposed at $p<.05$.

Morphological differences

Thirty-five *Emoia impar* and twenty *Emoia cyanura* were captured over a one-month period, and various pictures were taken and measurements recorded to determine which traits are most reliable for species identification. A summary of quantitative traits is found in Figure 4.

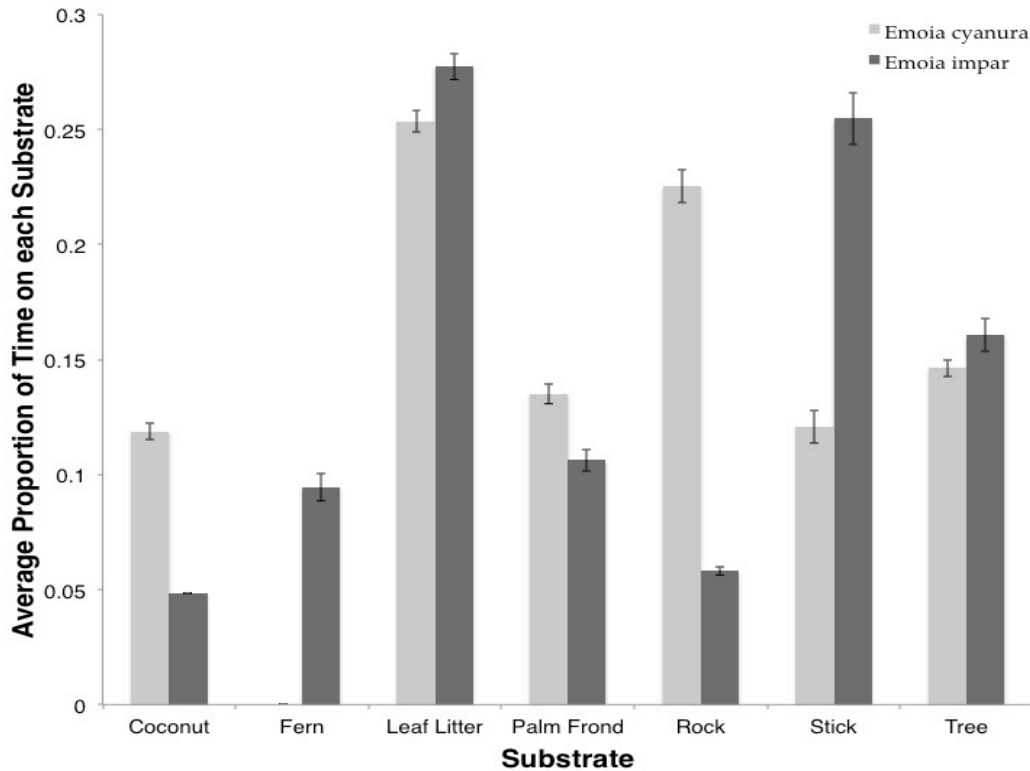


Fig.3. Average proportion of time spent on each substrate by *Emoia cyanura* (grey) and *Emoia impar* (black).

To test the reliability of certain diagnostic traits, snout-to-vent length, status of scale fusion, presence of parietal eye, tail coloration, belly coloration, and central stripe coloration were used as variables in a Principle Component Analysis (Fig.5). Additionally, PCA's were constructed using either RGB values for tail (Fig.6) or belly (Fig.7) alone. An independent-samples t-test was conducted to compare tail red, green, or blue values in *E. cyanura* and *E. impar*. There was a significant difference in the red value scores for *E. cyanura* (M=97.17, SD=4.5) and *E. impar* (M=63.27, SD=3.8); $t(35)=5.03$, $p = .0017$. Additionally blue values were significantly different between *E. cyanura* (M=46.17, SD=4.1) and *E. impar* (M=99.73, SD=5.2); $t(35)=4.95$, $p=.00097$. However, green values were not significantly different between *E. cyanura* (M=70.66, SD=3.2) and *E. impar* (M=88.33, SD= 3.9); $t(35)=2.01$, $p=.088$.

Species	Parietal Eye present	Scale Fusion present	Average Snout-Vent-Length
<i>E.cyanura</i>	10/10	0/10	4.37 ± .15
<i>E.impar</i>	0/37	31/37	4.29 ± .08
<i>E.impar, copper</i>	0/5	3/5	4.3 ± .08

Fig.4. Summary of quantitative traits in captured skinks.

RGB values for belly color were also compared between species. *E. cyanura* (M=157.83, SD=6.3) *E. impar* (M=129.47, SD=6.0) The blue ($t(14.58)=3.8$, $p=.0017$) and green ($W(35)=148.5$, $p= .014$) components of belly color were significantly different between the two species; however, the red value was not ($W(35)=126.5$, $Z=$, $p= .13$).

Snout-to-vent length of the two species was compared using the Wilcoxon-Mann-Whitney two-sample rank-sum test. The two species differed significantly in SVL with $W(35) = 134.5$, $Z = 4.21$, $p = .0593$.

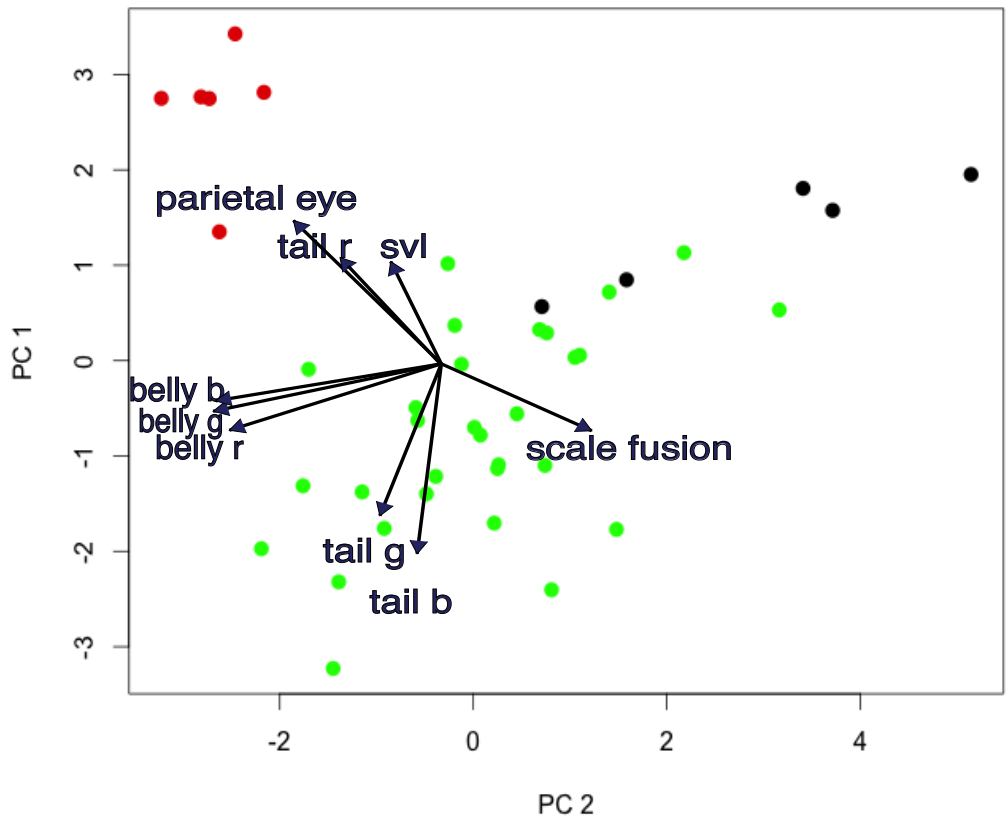


Fig.5. Principle Components Analysis of all combined traits. Green markers are *E.impar*, red markers are *E.cyanura*, and black markers are the copper morph of *E. impar*.

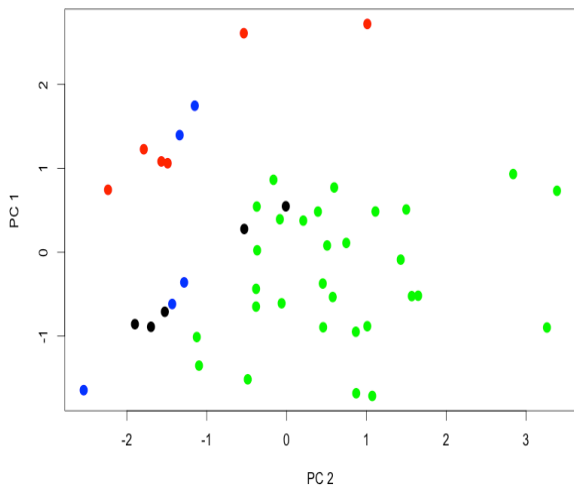


Fig.6. PCA using tail RGS values. Green markers are *E.impar*, red markers are *E.cyanura*, blue markers are *E.impar* with regrown tails, and black markers are the copper morph of *E. impar*.

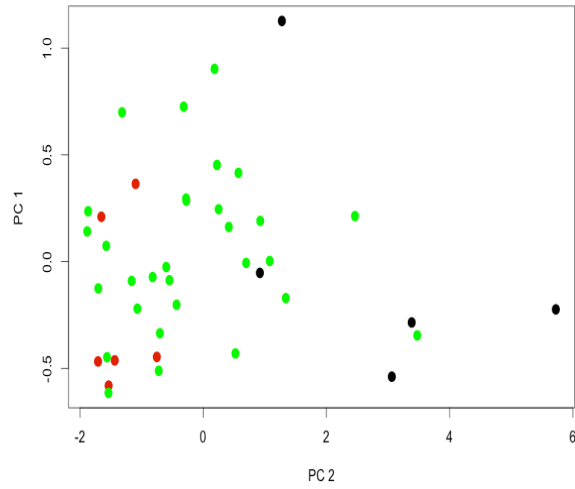


Fig.7. PCA using belly RGB values. Green markers are *E.impar*, red markers are *E.cyanura*, and black markers are the copper morph of *E. impar*.

DISCUSSION

Ethogram of behaviors

This study explores an array of common skink behaviors, as well as some rarer behaviors perhaps characteristic of island life. For example, skinks of both species were seen entering coconuts for periods of time, and their purposes for entering and activities inside are still unknown. In preliminary studies in the lab, two *E. impar* and one *E. cyanura* were observed entering the same coconut at the same time. Zug (2013) reported that in one instance, an old coconut shell contained over 400 *E. impar* eggs. It is possible that observations of skinks "hiding" in coconuts were in fact unobservable reproduction events.

General behavioral differences

Results showed that there was no statistical difference in proportion of time spent performing identifiable behaviors between the two species, when both species were observed performing the behavior. In summary, behavior is not an ideal "trait" to use for species identification. Some behaviors, such as face rubbing, hiding, opening and closing mouth, mating, and chasing, were only observed in one species. However, these were all rarely observed behaviors, and likely exist in both species if more observations were made.

Interspecific interactions were rare, and skink "patches" tended to be all of one species. In preliminary lab studies, individuals of both species were placed in the same terrarium on three separate occasions for ten minutes total, and no aggressive behaviors were recorded. Only two interspecific interactions were recorded in the field, and neither interaction was aggressive. These observations suggest that skink species likely don't use aggression as the primary method of segregation. Differences in population densities might explain why aggressive tendencies appeared to be more prevalent in *E. impar*. As a proportion of total time, *E. impar* was observed tongue flicking more frequently (13.2%) than *E. cyanura* (1.2%).

If there is a mechanism of habitat segregation or reproductive isolation between the two species, that mechanism remains unknown. Past studies on invertebrates have suggested that behavioral differences between cryptic species exist in the form of different

male mating calls (Mendelson 2005). It is possible that the two skink species on Moorea coexist and behave nearly identically, but have different mating cues and reproductive barriers.

Pre-zygotic or post-zygotic barriers to reproduction could explain how species of nearly identical morphology and behavior can coexist within the same habitats without hybridization. It has been discovered that water skinks use chemical cues to detect female receptivity (Head 2005). The females of *E. cyanura* and *E. impar* might release different chemicals that allow for the males of their respective species to identify them.

Substrate preferences

Substrate was recorded to explore an alternative approach to understanding the thermal preferences of *Emoia*. Past studies have used lab trials to suggest that the skinks may be partitioning habitats based on the thermal qualities of their preferred substrates (McElroy 2007). By examining what substrates the skinks reside on and finding the proportion of time spent on each substrate, substrate preference can be inferred. Considering that different substrates have different heat capacities, it might have been possible to correlate the results of the McElroy study with this one. However, no major differences in substrate use were discovered in this study.

Additionally, substrate abundance within each habitat likely has a large effect proportion of time spent on each substrate. For example, *E. cyanura* spent a much greater proportion of their time on coconuts likely because they either exist in many habitats where coconuts exist, or the sampled habitats were uneven in their substrate dispersions.

Morphological differences

Certain traits were found to be more reliable for species identification than others. Past studies on the *Emoia* genus have shown that the two species are genetically distinct but morphologically similar (Bruna et al. 1995), and that species identification can be successful with the use of five key morphological traits (Zug 2013). One of those traits, belly color, was found to be relatively variable across species. Tail color was more reliable, except for when *E. impar* had regrown tails. *E. impar* regrown tails are dark copper in color, and using tail color to identify species in

the field may be unreliable because of this fact. However, all skinks with distinctly *blue* tails were found to be *E. impar*. Copper *E. impar* morphs had tail colors that had similar RGB values to *E. impar*, although the copper morphs had qualitatively darker tails.

The Principle Component Analysis of tail and belly colors found principle components to determine which values, red, green, or blue, were most indicative of species. Blue values were the most significant with $p < .001$, meaning that the blue aspect is most variable in tail color differences. For belly color, both green ($p < .02$) and blue ($p < .002$) values were significantly different between species. However, when plotted in a principle component analysis, there was a lot of overlap in the placements of both species. This result supported field observations where ventral coloration seemed to be inconsistent and subjective based on the observer and the lighting.

The most recent, comprehensive field guide to Pacific Island reptiles (Zug 2013) included many potential traits for identification. However, most of these traits were very minute and observable only with practice. Color traits were quantified to meet this purpose and reach a conclusion about taxonomic uncertainties. One successful target of this study was to identify other possible traits for field identification, and to see if behavior can be used as a supplemental tool for this purpose.

Conclusion

Obtaining a basic understanding of reptilian behavior is important for many reasons. Behavioral knowledge can help explain species decline, predict future habitat invasions, study social structure, and support reasons for habitat preference. Understanding the subtle morphological and behavioral distinctions between cryptic species contributes to a better understanding of the evolutionary processes that might lead to the existence of these similar species. Future research could go into understanding reproductive behavior of the *Emoia* genus, especially regarding any behavioral or chemical cues preceding reproductive events. Additional future research may include analyzing the use of coconuts in reproduction.

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APPENDIX A
Photographic comparisons between *E. impar* and *E. cyanura*.

