Using camera-trap photographs to identify individual fox squirrels (Sciurus Niger) in the Southeastern United States

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Tools and Technology

Using Camera-Trap Photographs to Identify Individual Fox Squirrels (*Sciurus niger*) in the Southeastern United States

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ABSTRACT Fox squirrels (*Sciurus niger*) in the southeastern United States have been difficult to survey because of their sparse distribution, large home ranges, and low capture success. Remote cameras have proven to be an effective tool for surveying larger mammals, including identifying individual animals using their unique markings. To test whether cameras can be used to identify individual fox squirrels in the southeastern United States, we 1) inspected digital photographs of fox squirrels; 2) identified variable pelage features for individual identification; 3) tested the ability of wildlife students and professionals to identify individuals; and 4) evaluated whether training improved participants' ability to identify individuals. We found that fox squirrels could be individually identified using a combination of sex and 10 variable pelage features, including the color morph, presence of an eye ring, distribution of white on the rostrum, and facial markings. In total, we developed $\geq$25,920 possible combinations of features to identify southeastern fox squirrels. We found survey participants were capable of identifying fox squirrels without training, but their average ability to do so increased significantly from 73.6% to 80.3% after training ($t[df = 106] = 5.068$, $P < 0.001$). The results from this study demonstrate that camera-trapping, particularly when combined with training that focuses on which features vary between individuals, provides an alternative method to live-trapping for population-level studies of southeastern fox squirrels.

KEY WORDS camera-trapping, coloration, Florida, photo-identification, *Sciurus niger*, Sherman's fox squirrel.

Fox squirrels (*Sciurus niger*) occur naturally over much of the United States east of the Rocky Mountains and are considered an important game species throughout most of their range (Hall 1981, Koprowski 1994). In the midwestern United States, fox squirrel populations appear to be increasing and expanding their distribution (Swihart and Nupp 1998), while those in the southeastern United States appear to be declining as a result of habitat loss and altered disturbance regimes (Loeb and Lennartz 1989, Weigl et al. 1989, Loeb and Moncrief 1993). There are 10 recognized subspecies of fox squirrel (Moncrief et al. 2010), 3 of which have a conservation status. In the mid-Atlantic region of the United States, the Delmarva fox squirrel (*S. n. cinereus*) is listed as federally endangered. In Florida, the Sherman's fox squirrel (*S. n. shermani*) is a species of special concern and the Big Cypress fox squirrel (*S. n. avicennia*) is listed as threatened (Humphrey and Jodice 1992, Kantola 1992, Loeb and Moncrief 1993).

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Six of the fox squirrel subspecies which range from Virginia to Mississippi are collectively known as "southeastern fox squirrels" (Loeb and Moncrief 1993). Southeastern fox squirrels are typically characterized by their high variability in pelage color, and occupy environments substantially different from those in the Midwest (Weigl et al. 1989, Loeb and Moncrief 1993). Although fox squirrels in the Southeast inhabit many different habitat types, they often are sparsely distributed, occur in low densities, and have large home ranges (Weigl et al. 1989, Kantola and Humphrey 1990, Wooding 1997). Surveying and generating population estimates for southeastern fox squirrels has been challenging because they are difficult to live-trap (Weigl et al. 1989, Kantola and Humphrey 1990), with capture success often <1% (Steele and Koprowski 2001). Other methods, such as point counts and transect surveys have also proven ineffective for surveying southeastern fox squirrels (D. U. Greene, unpublished data), and signs of occurrence (dreys and chewed pinecones) are of limited use because of the difficulty in differentiating signs of eastern gray squirrels (*S. carolinensis*) from fox squirrels (Wood 2001).

Not only is live-trapping of southeastern fox squirrels hampered by low capture success, but there is always a risk of injury associated with capturing an animal (Mendoza et al. 2011, Chesser 2012). Moreover, live-trapping has been shown to alter some animal behaviors and bias population estimates (Rodda et al. 1988). Alternatively, passive methods, such as camera-trapping, can be used to detect species without these risks. Additionally, cameras have been an effective tool for identifying individuals when populations possess natural variation of pelage color or patterns, which can be used to estimate abundance through a mark–recapture analysis (e.g., Karanth 1995, Heilbrun et al. 2006). Most published studies estimating population size from camera traps have been conducted on larger mammals, especially felids (Foster and Harmsen 2012). However, southeastern fox squirrels have unique pelage colors and patterns that differ among individuals (Weigl et al. 1989, Kiltie 1992), making them a candidate for passive individual identification.

The ability to use natural variation to recognize individuals of a cryptic species can be a valuable tool for collecting information regarding population dynamics and distribution (Kelly 2001, Heilbrun et al. 2003, Trolle and Kéry 2003, Jackson et al. 2006). For southeastern fox squirrels, there is a need to develop a reliable method to survey their populations. Therefore, our goals for this project were to 1) determine whether southeastern fox squirrels can be individually identified from camera-trap photographs; 2) determine which pelage features were most useful for individual identification; 3) test the ability of wildlife students and professionals to identify individual fox squirrels; and 4) determine whether training improves the participants' ability to identify individual fox squirrels.

STUDY AREA

Our study was conducted at Camp Blanding Wildlife Management Area on the Camp Blanding Joint Training Center in Clay County, Florida, and Ordway–Swisher Biological Station in Putnam County, Florida. Within our study area, the habitat at Camp Blanding was classified as a mixture of sandhill and mesic flatwoods, and Ordway was sandhill (Florida Natural Areas Inventory 2010). At Camp Blanding, longleaf pine (*Pinus palustris*), slash pine

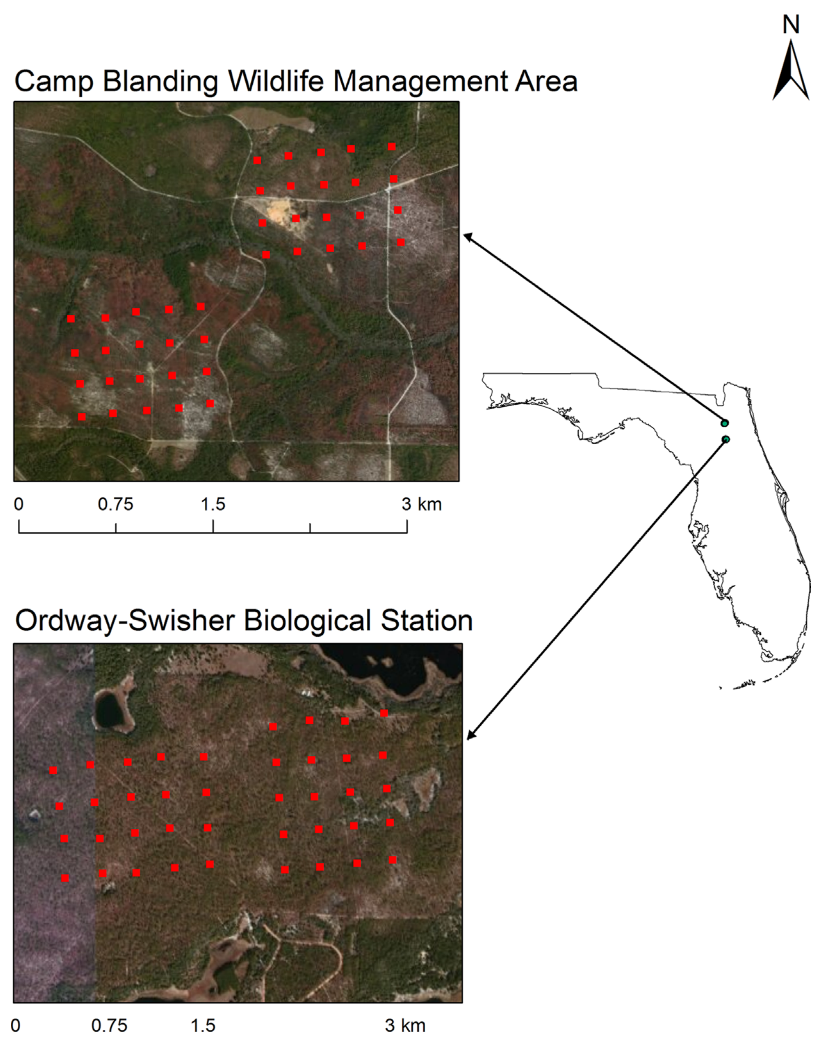

Figure 1. Distribution of the 4 grids used in a camera-trapping study of Sherman's fox squirrels to test whether individuals can be identified from photographs. Grids were located at Camp Blanding Wildlife Management Area in Starke, Florida, and the Ordway–Swisher Biological Station, in Melrose, Florida (USA) from 2011 to 2012.

(*P. elliottii*), and turkey oak (*Quercus laevis*) were the dominant tree species. The understory was primarily saw-palmetto (*Serenoa repens*), gallberry (*Ilex glabra*), and swamp bay (*Persea palustris*). At Ordway, longleaf pine, turkey oak, and live oak (*Q. virginiana*) were the dominant trees, and the ground cover was primarily wiregrass (*Aristida stricta*).

METHODS

Camera-Trapping

At each study site we randomly established 2, 75-ha grids in areas known to have fox squirrels (Fig. 1). Each grid was a 4×5 arrangement, with survey points placed 250 m apart. We separated grids by 645 m at Camp Blanding and 510 m at Ordway to minimize movements between grids. We placed a camera (Bushnell Trophy Cam model 119436c; Bushnell Outdoor Products, Overland Park, KS) 70 cm above the ground and angled it toward a bait pile (pecans and cracked corn) placed at the base of a tree 1.5 m from the camera. We surveyed each point for 6 consecutive days during each season (autumn = Oct–Nov 2011, winter = Feb–Mar 2012, spring = Apr–May 2012, summer = Aug–Sep 2012), adding bait on the first and fourth days. We equipped cameras with a 2-GB SanDisk memory card (SanDisk Corporation, Milpitas, CA) and set them to take 3 photographs every 10 seconds on the normal sensitivity setting.

Individual Identification

To develop an effective protocol for identifying southeastern fox squirrel individuals, we visually inspected museum specimens at the University of Florida; these specimens represented multiple subspecies, 2,976 camera-trap photographs, and approximately 1,000 photographs from a concurrent mark–recapture study using live-trapping to determine unique color patterns and features useful for identification. Our goal was to develop a suite of ≥7 variable morphological features useful for individually identifying southeastern fox squirrels. When examining fox squirrel photographs, we selected morphological features that were stable over time and uniquely variable among individuals (Rodda et al. 1988). In an effort to reduce subjectivity and observer bias, we used a database in Microsoft Access to catalog photographs based on features that varied among individuals. We re-evaluated all possible individuals each season when incorporating new data. We limited our ability to identify individuals using only the variable morphological features, and did not use visible ear tags on marked fox squirrels as an additional feature.

Identification Test

We developed a 16-question online test to determine the ability of wildlife students and professionals to correctly identify individual fox squirrels from their unique features. The test consisted of 16 multiple-choice photo-matching

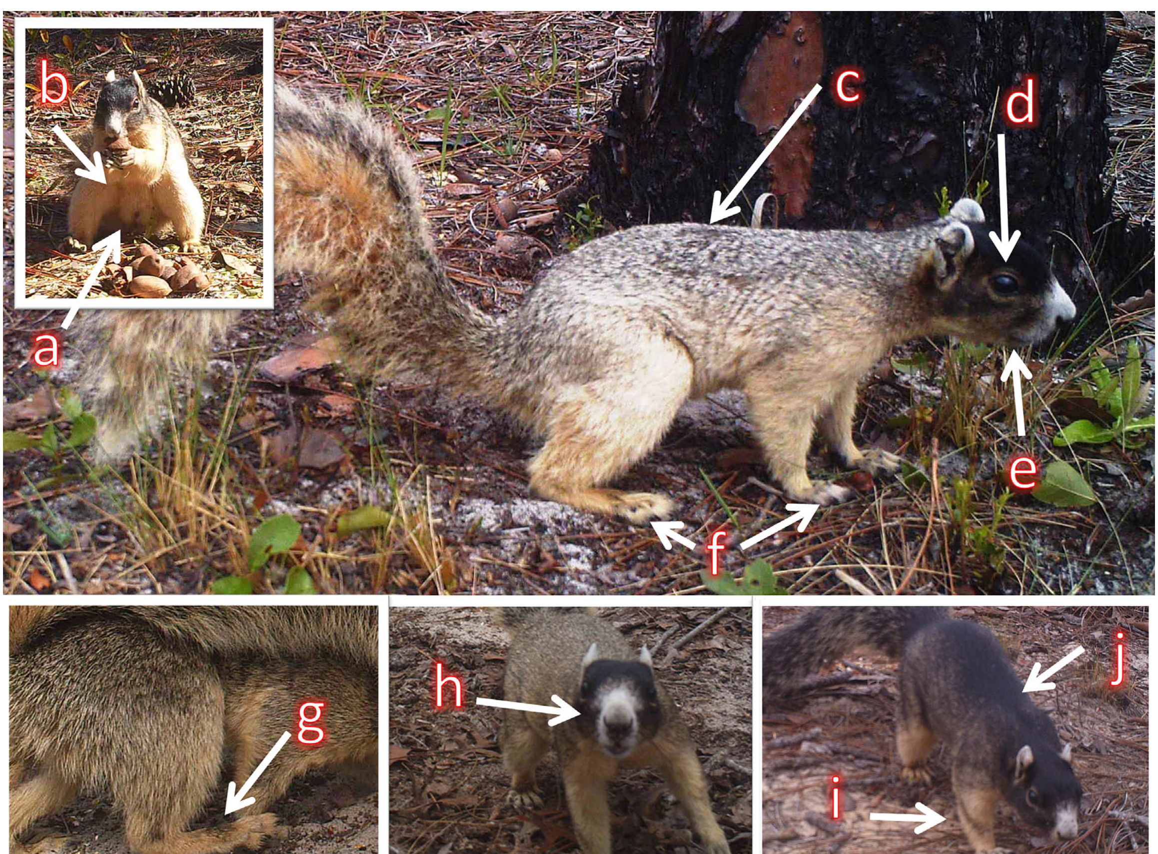

Figure 2. Ten of the highly variable features used to identify individual southeastern fox squirrels from camera-trapping photographs taken at Camp Blanding Wildlife Management Area, Starke, Florida, and the Ordway–Swisher Biological Station, Melrose, Florida (USA) from 2011 to 2012. Features included are a) sex, b) ventral color, c) dorsal color, d) eye ring, e) facial pattern, f) toe color, g) dark spot on foot, h) distribution of white on the rostrum, i) forearm patterns, and j) melanistic dorsal stripe.

questions, with 4 matching options and ≥ 1 correct answer for each question. The test contained 5 questions to detect changes in pelage color, 2 questions each to detect variation in the color around the eye, forearm, toe, rostrum, and face. We also had one question to detect a difference in the sex of the squirrel. Participants did not know which feature was being tested by a given question.

We developed and administered the tests using Qualtrics (http://www.qualtrics.com) software and solicited participants from 2 main groups: Florida Fish and Wildlife Conservation Commission (FFWCC) biologists; and University of Florida (UF) biology, ecology, wildlife ecology, and zoology students. To determine whether training improved participants' ability to identify individual fox squirrels, we compared results from 2 randomly assigned groups. Group 1 (hereafter, Training group) consisted of 9 UF students and 43 FFWCC employees, totaling 52 participants. This group reviewed training materials (Appendix S1; available online at www.onlinelibrary.wiley.com) with multiple photographs of each pelage feature and associated explanatory text on how to identify each feature. After completing the training session, the participants took a test. Group 2 (hereafter, No training group) consisted of 8 UF students, 1 U.S. Fish and Wildlife Service employee, and 50 FFWCC employees, totaling 59 participants. Participants in the No training group took the same test without training.

Data Analysis

To assess the participants' ability to distinguish between fox squirrel individuals, we scored each potential match on the test as 1 point each, where the 16 questions with 4 possible answers yielded 64 total points. For the data analysis, we first tested for homogeneity of variance between test groups using a Levene's test in the Car Package (Fox and Weisberg 2011) for Program R (R Development Core Team 2012). We assessed significance $\alpha = 0.05$, with a null hypothesis that the test group variances were equal. As this test failed ($P = 0.033$), we used a series of Welch's t-tests for possible unequal variances (Welch 1947) at $\alpha = 0.05$. We tested whether there was a significant difference in mean total scores between groups, and mean scores for each color feature.

RESULTS

Individual Identification

Based on manual inspection of museum specimens and all digital photographs, we found the distribution and amount of white on the rostrum to be highly variable between individuals. This feature, along with sex and 9 other color features were useful in identifying fox squirrels to an individual level (Fig. 2; Table 1). Specifically, we used variation in dorsal coloration, ventral coloration, presence of a dorsal melanistic stripe, presence and/or completeness of an eye ring, presence of a dark spot on a foot, forearm patterns, hind-leg patterns, toe coloration, and facial pattern to differentiate individuals (Table 1).

In total, we developed $\geq 25{,}920$ possible combinations of features to identify southeastern fox squirrels. We successfully identified 53 individuals on our study areas using the camera traps. The number of photographs needed to identify individuals depended on the variability and uniqueness of features an individual possessed, and on the lighting, angle, and quality of the photographs. If a squirrel possessed an abnormal pelage feature or coloration (e.g., an orange patch of hair against brown or black hair, or dorsally melanistic with an orange ventral coloration), then 1–5 photos were sufficient to match the individual. However, if a squirrel was a typical color morph for Central Florida (e.g., agouti coloration, full eye ring, and tan ventral coloration), 5–20 photos were often needed for identification. Fewer features were available for identification in melanistic squirrels, but their low proportion in the populations (14.5%) and the variation between individuals (white on the rostrum, toe coloration, nonblack hairs) still allowed for their identification. We also found ephemeral features such as scars, molt lines, and ear tears to be reliable when comparing squirrels within a trapping session, but less reliable between sessions.

Table 1. Nine variable pelage features and their percentages observed within the populations of Sherman's fox squirrels surveyed at Camp Blanding Wildlife Management Area, Starke, Florida, and the Ordway–Swisher Biological Station, Melrose, Florida (USA) from 2011 to 2012.

Pelage feature and subclass	% observed
Dorsal color	
Melanistic	14
Agouti	83
Silver	3
Ventral color	
Tan	84
Black	10
White	2
White–tan	2
Tan–black	2
Dorsal melanistic stripe	
Absent	48
Along back	33
Behind head only	19
Eye ring	
Present	70
Absent	30
Eye ring complete	
Yes	67
No	33
Forearm patterns	
Absent	51
Present	36
Fully melanistic	13
Hind leg patterns	
Absent	71
Present	27
Fully melanistic	2
Toe color	
Tan	68
White	12
Black	11
White–black	5
White–tan	2
Tan–black	2
Dark spot on foot	
Absent	52
Present	48

Data Analysis

Participants who received training scored significantly higher (80.3% [95% CI = 78.7–81.9]) than those who did not (73.6% [71.5–75.6], t[df = 106] = 5.068, $P < 0.001$).

Table 2. Comparison of 7 features from the Training group and No training group used to assess the effectiveness of training participants to identify individual southeastern fox squirrels surveyed at Camp Blanding Wildlife Management Area, Starke, Florida, and the Ordway–Swisher Biological Station, Melrose, Florida (USA) from 2011 to 2012. Estimates include the degrees of freedom (df), corresponding *t*-statistic, 95% lower (L) and upper (U) confidence intervals (CI), mean score for each color feature for the Training group ($\bar{x}$) and the No training group ($\bar{y}$), and the *P*-value from the respective *t*-test.

Feature	df	*t*-statistic	L CI	U CI	$\bar{x}$	$\bar{y}$	*P*-value
Color morph	106	2.278	0.108	1.555	15.865	15.034	0.025*
Sex	109	2.290	0.036	0.505	3.423	3.153	0.024*
Facial pattern	109	2.234	0.050	0.835	6.731	6.288	0.028*
Forearm	99	0.818	−0.266	0.639	6.000	5.814	0.416
Nose	105	4.403	0.594	1.569	7.115	6.034	<0.001*
Eye ring	109	2.013	0.008	1.008	5.423	4.915	0.047*
Toe color	109	3.469	0.420	1.539	6.827	5.847	0.001*

*Indicates significant at $\alpha = 0.05$.

Similarly, training improved participants' ability to identify fox squirrels for all color features except forearm patterns (t[df = 99] = 0.818, $P = 0.416$; Table 2). Overall, training appeared to be most effective for increasing the average score for white on the rostrum (No training = 75.4% [70.9–80.0], Training = 88.9% [85.1–92.8]) and toe color (No training = 73.1% [68.5–77.7], Training = 85.3% [81.2–89.4]; Fig. 3).

DISCUSSION

We found that the variability of the color features among southeastern fox squirrels allowed for individual identification from photographs. To our knowledge, this is the first time this novel approach has been used for a rodent species.

Although many participants were able to distinguish among individual fox squirrels without training, their scores increased significantly after training. Participants in both groups were most successful at identifying the more obvious pelage patterns, such as color morph and the facial patterns. Training was most useful when identifying squirrels with the subtle features, such as toe color, distribution of white on the rostrum, and eye ring. Forearm patterns and the presence of an eye ring were more often overlooked. We recommend that future training should put greater emphasis on these problematic, but essential features.

One drawback to this methodology is that shadows, low lighting, and pictures of squirrels >10 m from the camera make visibility of features difficult. These issues can often be addressed by altering the brightness and adjusting the zoom on a photograph. An additional constraint was that specific angles were needed to capture certain features, such as the distribution of white on the rostrum or sex of the squirrel. Single photographs displaying only one flank of an individual can impede identification because of asymmetrical features (McClintock et al. 2013). Some of these constraints may be removed with increasing the sensitivity and speed of the camera to take more photographs, and including a second camera at each station to provide photographs of both flanks and to minimize lighting issues (Negrões et al. 2012).

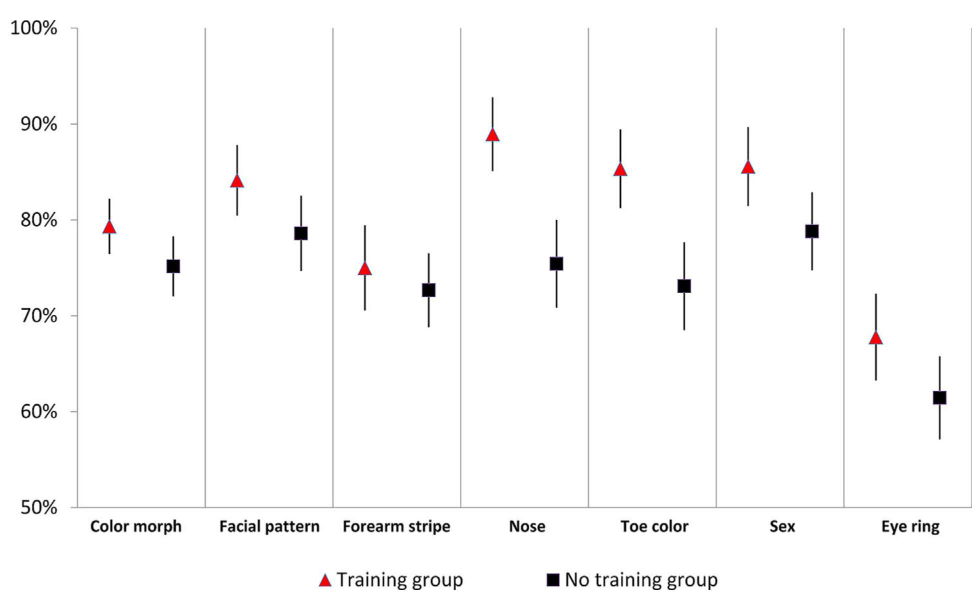

Figure 3. Comparison of mean test scores with 95% confidence intervals from 2 groups of participants that either received training (Training group) or no training (No training group) for identifying individual southeastern fox squirrels from photographs. Test questions were based on 7 variable features we observed between individual fox squirrels at Camp Blanding Wildlife Management Area, Starke, Florida, and the Ordway–Swisher Biological Station, Melrose, Florida (USA) from 2011 to 2012.

The results from this study demonstrate that with training on certain variable features seen in camera-trap photographs, researchers can more accurately identify individual southeastern fox squirrels. Having the ability to identify individual fox squirrels using photographs provides an opportunity to replace live-trapping for many studies, eliminating the risk associated with direct capture and handling of individuals, and minimizing the behavioral effects encountered with live-trapping. This unique method to study southeastern fox squirrels will improve our understanding of their ecology, including activity patterns, habitat use, distribution, and will aid in evaluating population trends.

Although our study was limited to diurnal fox squirrels, we believe this approach could be applied to other diurnal and nocturnal species that possess distinct external characteristics. If our methods are replicated on southeastern fox squirrels or applied to other species, the accuracy of the individual identification could be further evaluated on individuals that are known to researchers (e.g., already marked, or in a captive setting), but that are unknown to the observers.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's website.

Appendix S1: Identification training document for individual identification of southeastern fox squirrels.