# Morphological Discrimination of Gray Bats and Southeastern Bats

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Abstract - *Myotis grisescens* (Gray Bat) and *Myotis austroriparius* (Southeastern Bat) generally do not utilize similar habitats; however, in areas of range overlap where they both may be captured foraging in riparian areas or observed roosting in caves, it may be difficult to discriminate between them due to contradictory information found in mammal identification guides. In order to find characteristics that can reliably be used to identify these species, we examined museum specimens and live-captured individuals to obtain data on length of toe hairs, point of attachment of the plagiopatagium to the foot or ankle, forearm length, and the presence or absence of notches on the claws of feet and thumbs. The presence or absence of a notch in the claws and forearm length were found to be the most objective methods of identifying these species.

## Introduction

*Myotis grisescens* (Howell) (Gray Bat) and *Myotis austroriparius* (Rhoads) (Southeastern Bat) are closely related insectivorous bats that are each found in areas of the southeastern United States (Brown 1997, Stadelmann et al. 2007). Gray Bats generally roost in caves of upland areas, often near rivers, whereas Southeastern Bats occupy bottomland hardwood forests where they primarily roost in tree cavities, though they are known to roost in bridges and culverts, both of which sometimes are utilized by Gray Bats (Powers et al. 2016, Rice 1957, Sasse 2019). However, in some parts of their range, Southeastern Bats inhabit caves and have been found roosting in the same caves, and sometimes the same cluster, as Gray Bats (Bole 1943, Hall 1961, LaVal 1967, Rice 1955). Because Gray Bats are federally listed as an endangered species and Southeastern Bats are considered of conservation concern by 17 states, it is critical that surveyors can distinguish between them in areas where they are sympatric (Fig. 1; Decher and Choate 1995, Jones and Manning 1989, O'Shea et al. 2018).

Teeth and skull characteristics are useful in differentiating *Myotis* species but are not practical as the basis of an identification technique in the field (Jansky 2013, Menzel et al. 2005). Descriptions of these bats found in state, regional, and national guides to bats and mammals primarily discriminate between these species based on dorsal hair color, the posterior attachment site of the plagiopatagium, forearm length, or some combination of these characteristics. However, there is a risk of misidentification because there is not universal agreement among publications on the characteristics that delineate these 2 species.

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Most guides indicate that the plagiopatagium attaches at the tarsus (ankle) in Gray Bats versus at the posterior margin of the metatarsuls (base of the toes) in Southeastern Bats (Appendix 1). Several authors explicitly state that the attachment site observed in Gray Bats distinguishes them from all other *Myotis* species (e.g., Brown 1997, Lyon 1936, Whitaker 1998); Barbour and Davis (1969) suggested that this characteristic is diagnostic for distinguishing Gray Bats from Southeastern Bats. However, Caire et al. (1989) and Mengak (2002) indicated that the plagiopatagium attached at the ankle in Southeastern Bats. Although Glass and Ward (1959) agreed with wing attachment at the ankle, they said it was much more variable in Southeastern Bats than Gray Bats. Miller and Allen (1928) indicated the plagiopatagium usually attached at the base of the toes in Southeastern Bats, but some individuals showed attachment at or near the ankle; they suggested these cases might have been a result of study skin preparation.

Dorsal hair coloration of Southeastern Bats ranges from bright orange brown to dull brown or gray, can vary both geographically and seasonally, and may be affected by environmental conditions in maternity caves (Laval 1970). Similarly, the generally slate gray dorsal hair of Gray Bats can be faded by ammonia fumes in summer colony sites to a color described as cinnamon brown, dusky brown, or russet (Decher and Choate 1995). One of the authors (T.S. Risch) has captured Gray Bats with hair that was best described as orange. Regardless of overall color, the dorsal hair of Gray Bats is reported to be uniform in color while that of the



Figure 1. Approximate ranges of Gray Bats and Southeastern Bats in the United States, and areas of potential range overlap between the two species. Range distributions were generated from county occurrences across the Southeast provided by NatureServe.org and https:// vafwis.dgif.virginia.gov.

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Southeastern Bat is said to have a darker base and a lighter tip. However, a number of authors indicate that the degree to which the dorsal hair of Southeastern Bats is bicolored is slight and there is little contrast between the upper and lower parts of the hair (Appendix 1). In the first description of Southeastern Bats based on prepared specimens, Rhoads (1897:227) suggested this bicoloration was "only to be distinguished by close scrutiny", and detailed analysis of the morphology of hair of North American bats by Nason (1948) noted that the tip of the dorsal hair was only slightly lighter in color than the base. Although there is some indication that individuals working in caves under artificial lighting can conduct many normal activities without loss of visual capabilities, the ability to identify small differences in color contrast under low light conditions may be impaired; therefore, this characteristic may not be suitable for identifying these species in the field (Johnson and Casson 1995, Lanca et al. 2016).

Forearm length is often used as a characteristic for bat identification, with several keys indicating that the forearm is  $\geq$ 40 mm in Gray Bats and <40 mm in Southeastern Bats (Choate et al. 1994, Sealander and Heidt 1990). However, Marks and Marks (2006) and Whitaker and Mumford (2009) indicated that the forearm length of Southeastern Bats was <42 mm and that Gray Bats exceeded this measurement. Other guides provided a span of measurements for the 2 species that indicated overlap between 40 and 41 mm (Golley 1962, Kays and Wilson 2009, Morgan et al. 2019, Webster et al. 1985). Only Menzel et al. (2002) suggested that adults of both species had forearm lengths  $\leq$ 40 mm. The largest analysis of forearm length in Southeastern Bats (n = 427) found average forearm length was 37.43 mm in males and 38.46 mm in females; some female forearms were up to 41.3 mm, and forearm length was found to occasionally exceed 40 mm in areas across their range (LaVal 1970). Sherman (1930) observed 5/30 (17%) of adult females and 0/11 adult males with forearm lengths  $\geq$ 40 mm.

The length of the hair on the toes of Southeastern Bats is said to be "long" or to extend beyond the toes (Barbour and Davis 1969, 1974; Best and Dusi 2014; Choate et al. 1994; Hoffmeister 1989; Jones and Manning 1989; Kays and Wilson 2009; Marks and Marks 2006; Morgan et al. 2019; Schwartz and Schwartz 2016; Sealander and Heidt 1990; Webster et al. 1985; Whitaker 1998; Whitaker and Hamilton 1998). The length of toe hair of Gray Bats is rarely mentioned but has been called "short" (Kays and Wilson 2009, Morgan et al. 2019, Schwartz and Schwartz 2016).

Whitaker and Hamilton (1998) indicated that Southeastern Bats had larger hind feet than other *Myotis* in the United States, and Hamilton (1963) agreed that they had large feet; however, reported measurements often show overlap between Southeastern and Gray Bats in foot length (Best and Dusi 2014, Schwartz and Schwartz 2016, Whitaker and Hamilton 1998, Whitaker and Mumford 2009).

Southeastern Bats are often reported to have pink or flesh-colored noses (e.g., Golley 1962, Mumford and Whitaker 1982, Pagels et al. 2003). Nose color of Gray Bats has not been described, although Mumford and Whitaker (1982) implied that it was not flesh-colored when they suggested the Southeastern Bat was the only *Myotis* in Indiana with a pink nose.

One characteristic that appears to be unique to Gray Bats is the presence of a notch in the claws of the thumb and the hind feet (Fig.2; Best and Dusi 2014, Decher and Choate 1995, Schwartz and Schwartz 2016). Only Schwartz and Schwartz (2016) reported claw characteristics in Southeastern Bats and indicated claws of Southeastern Bats were not notched.

Because of the conflicting information on important characteristics distinguishing these 2 species, we used museum specimens and live captures to determine which characteristics best differentiate them.

## Methods

We used museum study skins and specimens preserved in alcohol to visually examine length of toe hair and the presence of notches on the thumb and toe claws in both species. We recorded these data from live captures of Gray Bats made in 2018; however, we conducted no new field work with Southeastern Bats after the start of this project in 2018 and thus have no live-capture data for these characteristics for Southeastern Bats. We used only specimens preserved in alcohol to examine plagiopatagium attachment on both species because this characteristic was not reliably observable on study skins due to shrinkage of skin membranes (Miller and Allen 1928) and was not recorded for all live captures of both species (Appendix 2). We recorded plagiopatagium attachment as being at the base of the toes if the membrane attached on a place perpendicular to the base of the toes, as being the base of the foot if the membrane attached distal to the point the foot



Figure 2. Claws of (A) Gray Bats and (B) Southeastern Bats.

began to narrow, and as at the ankle when the plagiopatagium and uropatagium were attached across from each other in such a way as to clearly show a separation of the foot and these wing membranes.

We measured left forearm length of volant bats of adults and juveniles of both species obtained during live capture in the field from parts of Arkansas where the 2 species are not sympatric. We captured Southeastern Bats by hand, harp trap, and mist net at 13 sites, including roost trees, bridges, and forest openings on and near the Cache River National Wildlife Refuge in Woodruff County Arkansas during 2014–2017. Gray Bats were captured at 25 sites in 11 counties in caves, at cave entrances, in mist nets, and in bridges throughout northern Arkansas from 2005 to 2018. Uniquely numbered bands (2.9 mm, Porzana, Ltd., Icklesham, East Sussex, UK) were placed on forearms of captured bats. We excluded from analysis the data from 834 Gray Bats and 34 Southeastern Bats that were captured during field work in Arkansas due to incomplete sex, age, or forearm information. We did not include forearm measurements from recaptured bats or measurements outside previously reported values for this characteristic. We used a student's *t*-test to compare mean forearm length.

Capture and handling procedures for Southeastern Bats were approved by Arkansas State University IACUC (approval no. 451729-1) and Arkansas Game and Fish Commission Scientific Collecting Permits 050620151, 051020161, 051620181, and 080120191. Capture and handling of Gray Bats was done by an Arkansas Game and Fish Commission biologist (DBS) in accordance with an agreement with the US Fish and Wildlife Service made under terms of Section 6 of the Endangered Species Act.

## Results

Although no (0/79) Gray Bats and only 5 of 80 (6.3%) Southeastern Bats demonstrated attachment of the wing at the base of the toe, both species exhibited wing attachment points at the base of the foot (Gray Bats = 12.7%, Southeastern Bats = 77.5%) and at the ankle (Gray Bats = 87.3%, Southeastern Bats = 16.3%) (Table 1). Notches were observed in the claws in nearly all (99.0%) Gray Bats and no Southeastern Bats (Table 1). Hairs on toes usually (Gray Bats = 64.0%, Southeastern Bats = 79.7%) extended past the tip of the toe claws on both species (Table 1).

Left forearm measurements of adults and volant juveniles were taken from 148 Gray Bats and 388 Southeastern Bats. Forearm length averaged 43.0 mm (SD = 1.3, min-max = 40.0-46.0 mm) for Gray Bats and 37.3 mm (SD = 1.6, min-max = 30.5-41.2 mm) for Southeastern Bats. Forearm lengths were <40.0 mm for almost all Southeastern Bats (382/388, 98.5%) and ≥40.0 mm for all Gray Bats. Forearm lengths of Gray Bats were significantly greater than those of Southeastern Bats (*t* = -45.394, df = 268, *P* < 0.001).

Using these results, we constructed a dichotomous key based on these characteristics in decreasing order of importance: presence of notch in claw, forearm length, plagiopatagium attachment point, and dorsal hair coloration (Table 2).

## Discussion

Due to difficulty of discerning certain characteristics under field conditions, varying descriptions in the literature (Appendix 1), and lack of an objective method of measurement, we recommend that dorsal hair coloration not be used as a primary method to distinguish Gray Bats from Southeastern Bats. While the wings of both species almost always attach proximal to the base of the toe, this is too inconsistent to be used on its own to distinguish one from the other, but may be helpful in differentiating these 2 species from other North American *Myotis*. As the length of hair on the toes usually extends past the claw of both species (Table 1), this characteristic should not be used in describing these species.

Rather, the presence of notches in the claws of Gray Bats serves as a more reliable method for identifying this species and for separating them from Southeastern Bats. Although Gray Bats have significantly larger forearms than Southeastern Bats, some Southeastern Bats exceeded the 40 mm length that was the minimum observed in Gray Bats; thus, there is enough overlap that forearm length alone should generally not be used to separate the 2 species. These 2 characteristics (forearm length and claw notch) in combination are adequate for differentiating these species except for

Characteristic		(	Gray Ba	t	Sou	theaster	n Bat
Wing attachment	Ankle		69			13	
	Base of foot		10			62	
	Base of toes		0			5	
Thumb claw notch	Present		158			0	
	Absent	2			120		
Toe claw notch	Present		161			0	
	Absent		1			121	
Toe hair length		Alcohol	Skin	Total	Alcohol	Skin	Total
	Longer than toes	68	5	73	68	22	90
	Shorter than toes	8	33	41	3	20	23

Table 1. Characteristics of Gray and Southeastern Bats recorded from live captures in Arkansas and museum specimens from throughout the range of these species.

Table 2. Dichotomous key for differentiating adult Gray Bats and Southeastern Bats.

1a 1b	Claws with visible notch	Gray Bat
2a 2b	Forearm length $\geq$ 42 mm Forearm length <42 mm	Gray Bat
3a 3b	Forearm length <40 mm Forearm length 40–41 mm.	Southeastern Bat
4a 4b	Plagiopatagium attached at base of toe Plagiopatagium attached at ankle or base of foot	.Southeastern Bat
5a 5b	Dorsal hair uniform in color Dorsal hair bicolored, though often not strongly	Gray Bat Southeastern Bat

those rare occasions when a notch is not observed and forearm lengths are 40–41 mm; in these situations the plagiopatagium attachment point and dorsal hair coloration may be the most useful identification characteristics (Table 2).

Though our forearm length data were similar to that of other authors (e.g., Glass and Ward 1959, LaVal 1970, Stevens and Platt 2015), a limitation of this study is that these measurements were restricted only to bats caught in Arkansas, and a range-wide examination of this characteristic may be warranted to confirm our results. The minimum forearm length observed in Southeastern Bats in this study (30.5 mm) was lower than that seen in other populations, but only 6/388 (1.6%) Southeastern Bats measured had forearms lengths less than the minimum length (34 mm) reported by LaVal (1970).

Unfortunately, observation of claw notch presence and forearm length require bat capture and could, due to cave configuration and a desire to keep disturbance to a minimum, be problematic in caves where both species are present. In these situations, overall dorsal hair coloration and visual estimation of bat size may be the only available, although not definitive, ways to differentiate the species. However, nose color (e.g., Golley 1962, Mumford and Whitaker 1982, Pagels et al. 2003), if validated by field observational data, may be a practical method of distinguishing these species in caves.

The variation in discriminating characteristics utilized for Gray Bats and Southeastern Bats is worrisome if indicative of similar issues for other species. Mammal identification guides may not always be used while conducting fieldwork, but they, along with guidance from experienced biologists, are an important part of the process used to train students, and their accuracy is critical. Guidebooks should be consistent with each other unless justified by variance in these characteristics across a species range. Authors of future mammal identification guides should consider validating characteristics used in keys and species descriptions using data obtained at the state, regional, or national scale as appropriate to the publication.

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Characteristic	Gray Bat	Southeastern Bat		
Wing attachment				
Ankle	Barbour and Davis (1969, 1974), Bee et al. 1981, Best and Dusi 2014, Brown 1997, Caire et al. 1989, Choate et al. 1994, Decher and Choate 1995, Gol- ley 1962, Hall 1981, Hamilton 1963, Hoffmeister 1989, Lyon 1936, Marks and Marks 2006, Menzel et al. 2002, Miller and Allen 1928, Morgan et al. 2019, Mumford and Whitaker 1982, Schwartz and Schwartz 2016, Sealander and Heidt 1990, Webster et al. 1985, Whitaker and Hamilton 1998, Whitaker and Mumford 2009, Wilson and Ruff 1999	Caire et al. 1989, Glass and Ward 1959, Mengak 2002		
Base of toe or "foot"		Barbour and Davis (1969, 1974), Choate et al. 1994, Hall 1981, Hoffmeister 1989, Lyon 1936, Marks and Marks 2006, Menzel et al. 2002, Miller and Allen 1928, Morgan et al. 2019, Schwartz and Schwartz 2016, Sealander and Heidt 1990, Webster et al. 1985		
Dorsal hair				
Uniform	Barbour and Davis (1969, 1974), Bee et al. 1981, Best and Dusi 2014, Brown 1997, Caire et al. 1989, Decher and Cho- ate 1995, Golley 1962, Hall 1981, Ham- ilton 1963, Hoffmeister 1989, Lyon Jr. 1936, Marks and Marks 2006, Menzel et al. 2002, Miller and Allen 1928, Morgan et al. 2019, Mumford and Whitaker 1982, Schwartz and Schwartz 2016, Sealander and Heidt 1990, Webster et al. 1985, Whitaker and Hamilton 1998, Whitaker and Mumford 2009, Wilson and Ruff 1999	Schwartz and Schwartz 2016		

Appendix 1. Characteristics of Gray Bats and Southeastern Bats mentioned in mammal identification guides.

Bicolored	Hall 1981, Marks and
	Marks 2006, Menzel et
	al. 2002, Mumford and
	Whitaker 1982, Schmidly
	2004, Sealander and Heidt
	1990, Whitaker and Mum-
	ford 2009
Bicolored but	Barbour and Davis (1969,
little contrast	1974), Best and Dusi 2014,
	Brown 1997, Caire et al.
	1989, Hamilton 1963,
	Jones and Manning 1989,
	Lyon 1936, Miller and
	Allen 1928, Nason 1948,
	Webster et al. 1985

**Appendix 2.** Museum specimens examined for claw notch presence, plagiopatagium attachment, and toe hair length.

Arkansas State University: 211, 416, 491, 645, 675, 714, 730, 877, 882, 883, 887, 953, 955, 958, 965-967, 1304, 1312, 1334, 1488, 1561, 2147, 2149, 6401, 28124, 28125, 28130–28132

University of Kansas: 10447, 10451, 10458, 10463, 10467, 12894, 12897, 12969, 128405–128406, 128408, 128410, 128413, 128698-128709, 128726, 128728, 128730–128732, 128733–128738, 128760, 128928–128943, 129387, 129390, 129393-129395, 129396, 129400, 129401, 129490, 129492, 129497, 129509, 129514, 129593, 129600, 129666, 129668, 129669–129671, 129775, 129827, 129828, 129830-129838, 129854, 129855, 129892, 129893, 129895, 129896, 129898, 129918, 129919, 129921-129926, 129930, 129934, 129939–129941, 129943, 130024–130045, 130048-130054, 130058, 130060, 130061, 130063, 130064, 130067

Louisiana State University: 2243, 6152, 10516, 10518, 10520, 10534, 11276-11285, 11295, 11300-11304, 11306-11308, 11311, 11312, 11314, 11318-11321, 11331-11333, 11335, 11350-11356, 11358, 11359, 11365-11367, 11428, 11429, 11547, 11552, 15178, 17354, 19482, 19483, 19484, 19487-19489, 21075, 21077, 21078, 21082, 21085, 21091, 21095, 21098, 21101, 21104, 21190