

## USE OF VOCALIZATIONS TO ESTABLISH SPECIES LIMITS IN ANT BIRDS (PASSERIFORMES: THAMNOPHILIDAE)

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**ABSTRACT.**—We introduce an empirically derived methodology for the definition, measurement, and application of vocal characters in assessing species limits in the numerically important Neotropical family *Thamnophilidae*. On the basis of available evidence, we assume that vocalizations are innate and that all vocal characters have a role in maintenance of species integrity in *thamnophilids*. Vocalizations of eight syntopic pairs, the members of which resemble one another in both plumage and voice, were analyzed to identify diagnosable vocal characters. To be diagnosable, characters had to distinguish members of a pair unambiguously. Three of the eight pairs were diagnosable by three vocal characters, four pairs by four characters, and one pair by seven characters. Vocalizations differed most consistently by characters reflecting loudsong note structure, loudsong pace, and note structure of calls. In establishing species limits for allopatric antbird populations, we suggest that three vocal characters, the minimum number distinguishing the syntopic pairs, be used as a point of reference. Keeping this point of reference in mind, we recommend that multiple diagnosable vocal characters be present when vocalizations are a major factor in determining species limits in *thamnophilid* antbirds. To the extent that populations differ strongly in other characters (e.g. morphological, molecular, other behavioral), fewer vocal characters may suffice. The identification of multiple vocal characters as a point of reference, as opposed to individual characters (e.g. note shape) or vocalization types (e.g. the loudsong), allows for greater latitude in the diagnosis. A focus on the number of characters is appropriate given the possibility that the role and importance of vocalization types in species' repertoires may differ across groups of taxa. The methodology and results may also have application in the study of other avian groups in which vocalizations are innate, especially the *suboscines*. Received 16 September 1997, accepted 2 February 1998.

THE CONSISTENT DEFINITION OF SPECIES is a necessary foundation for systematic, zoogeographic, ecological, and conservation research. The Neotropics are home to more species of birds than any other region and support the most diverse ecosystems on earth (see Stotz et al. 1996). Among Neotropical birds, recent studies (Robbins and Ridgely 1992, Willis 1992, Prum 1994, Whitney et al. 1995, Bierregaard et al. 1997, Isler et al. 1997, Krabbe and Schulenberg 1997, Zimmer 1997) exemplify a growing concern that many taxa presently considered subspecies are more appropriately recognized at the species level. Additionally, undescribed but diagnosable populations are known to exist (Fjelds  and Krabbe 1990, Whitney 1994, Brumfield and Remsen 1996). One of the numerically most important families of Neotropical birds is the *Thamnophilidae* (*sensu* AOU 1997), repre-

ented by 35 to 50 sympatric species in Amazonian forest localities. As with other Neotropical birds, their current ranking into species and subspecies was established by Hellmayr (Cory and Hellmayr 1924), Zimmer (1931 and subsequent papers), and more recent authors solely on assumptions of the significance of differences and similarities in plumage and external measurements. Furthermore, as Mayr (1982) noted, "every isolated 'species' was . . . scrutinized for the possibility that it was simply a geographic representative of some other species, in which case it was reduced to the rank of subspecies." The resulting judgments were unavoidably inconsistent and sometimes highly arbitrary.

Since these decisions were made, and especially over the past 20 years, knowledge of Neotropical birds has been augmented greatly. New specimens clarify our understanding of morphological variation and distribution. Molecular evidence regarding genetic variation

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among thamnophilid populations also is beginning to emerge (Capparella 1987, Hackett and Rosenberg 1990, Hackett 1993, Bates 1995, Brumfield and Capparella 1996). Thanks to a growing body of new information from the field, we now have the opportunity to consider vocalizations, behavior, and ecology together with morphology and patterns of distribution in assessing taxonomic rank and systematic relationships. Of all the newly emerging data sets complementing the study of specimens, that of vocalizations is the most complete. We now have in hand an inventory of nearly 9,000 recordings of thamnophilid antbirds, contributed by many field workers.

The use of vocalizations in avian systematics studies has been reviewed by Becker (1982) and Payne (1986). One potential problem with the use of vocal characters as a guide to species relationships is the possible effect of song learning. In this respect, it is important to distinguish between oscines, among which several elements of song learning have been documented, and suboscines, among which there is no evidence of song learning (Kroodsma and Konishi 1991). Experimental evidence that vocalizations are innate in one family of suboscines, the Tyrannidae, is substantial and convincing (Kroodsma 1984, 1985, 1989). In addition, neurological evidence indicates that a motor pathway in the anterior brain is essential for song learning (Brenowitz and Kroodsma 1996), and that the cell clusters that control song production in oscine forebrains are absent in at least one tyrannid studied (Kroodsma and Konishi 1991). Evidence for the lack of vocal learning in suboscines is supplemented by a finding of geographic congruence between genetic and vocal differentiation of a tyrannid complex (Johnson 1980, Johnson and Marten 1988) and by field observations, prominent among which are studies demonstrating the lack of geographic variation in tyrannid songs (e.g. Lanyon 1978).

The lack of within-taxon geographic variation in vocalizations is as evident in the Thamnophilidae (Whitney et al. pers. obs.) as in the Tyrannidae. E. S. Morton (*in* Kroodsma 1984) hand-reared a Barred Antshrike (*Thamnophilus doliatus*) from seven days of age whose fledgling calls were similar and adult song identical by ear to those of birds in nature. Furthermore, vocal differences among antbirds appear to ac-

company findings of large genetic distances. For example, large molecular distances found in the Black-faced Antbird (*Myrmoborus myotherinus*) across the Amazon River just east of the Rio Napo (Capparella 1987) are being corroborated by vocalizations (Isler et al. unpubl. data). In a related suboscine family, the Rhinocryptidae, genetically differentiated populations in the genus *Scytalopus* have distinctive songs even though the taxa are barely distinguishable morphologically (Arctander and Fjeldså 1994).

The greater diversity of syringeal morphology in suboscines, as compared with oscines, has been suggested to result from natural or sexual selection on suboscine vocalizations (Prum 1992). Our principal understanding of the particular vocalizations that serve in the process of antbird pair formation comes from a study by Morton (1996), who experimentally removed mates of Dusky Antbirds (*Cercomacra tyrannina*). Morton and his colleagues found that the survivors delivered what Morton has termed a "courtship song." In the case of females, the songs differed substantially from those delivered by paired females, and they incorporated elements of the male song. However, vocal characters employed in this paper (e.g. note shape, pace) do not appear to differ appreciably between the courtship songs and the loudsongs of paired individuals (Morton 1996: figure 14.1).

Thus, based on available evidence, we assume that vocalizations in thamnophilid antbirds are innate, have an important role in the maintenance of species integrity, and can serve as characters by which populations can be diagnosed. To date, however, taxonomic studies have lacked a consistent methodology for employing vocal characters in assessing species limits in antbirds and other suboscines, especially for allopatric populations. Here, we present such a methodology through analysis of songs and calls of eight syntopic pairs of congeneric species of antbirds.

## METHODS

### SELECTION OF SUBJECTS

*Criteria for selecting species and study areas.*—We reviewed all thamnophilid antbirds systematically to identify the congeneric pairs of syntopic species that looked and sounded most alike. To be considered

syntopic, pairs had to live in sufficiently close proximity to hear each other's vocalizations and thus potentially interbreed, regardless of whether their microhabitats differed. We sought pairs whose plumage was not highly differentiated on the premise that if congeners look at least superficially alike, their vocalizations are likely to be important in species recognition (Stein 1963, Lanyon 1978). Of these morphologically similar pairs, we gave priority to those that resembled one another vocally, seeking pairs that were likely to be closely related. Eight pairs (including the same pair from two regions) of antbirds were selected that best met these criteria and for which we had sufficient recordings. Recordings were selected from as small a region of sympatry as possible to obtain approximately 10 recordings of each species. Confining our sample to a restricted region allowed us to minimize anomalies introduced by unstudied geographic variation. We employed all available recordings within the designated regions. Recording locations are identified in the Appendix. Unless stated otherwise, observations of syntopy were derived from personal experience. The eight pairs and regions are listed below.

**Thamnophilus doliatus/T. palliatus.**—Although they mostly replace one another in a checkerboard pattern, T. A. Parker III (pers. comm.) noted *T. doliatus* and *T. palliatus* in syntopy at Perseverancia (northern Santa Cruz, Bolivia), and S. Herzog (pers. comm.) found both species in the Rio Masicuri valley (southwestern Santa Cruz). Their habitat preferences differed, but Parker found the two species within hearing distance, and Herzog felt that *T. doliatus* and *T. palliatus* undoubtedly come within hearing distance. Recordings were from Santa Cruz and La Paz, Bolivia, and Rondônia, Brazil.

**Thamnophilus schistaceus/T. murinus.**—These species are sympatric over a large region of western Amazonia. Despite possible habitat differences, voices of the two species are often heard together. Recordings used were from the region of the Rio Napo and Rio Tigre in northern Peru and eastern Ecuador.

**Dysithamnus mentalis/D. stictothorax.**—These species overlap in southeastern Brazil and are often observed in the same mixed-species flocks. Recordings were taken from the entire region of sympatry.

**Myrmotherula brachyura/M. obscura.**—These species are sympatric throughout the range of *M. obscura* in western Amazonia. Vocalizations of the two species are frequently heard together, and habitat differences between them are unclear. All recordings came from the vicinity of Quebrada Sucusari on the left bank of the Rio Napo in Loreto, Peru.

**Myrmotherula surinamensis/M. cherriei.**—These species are syntopic in Amazonas, Venezuela (Zimmer and Hilty 1997), and northern Peru (Alvarez 1994) where both species have been found in the same location although not at the same time of year

(B. Whitney pers. obs.). Two named *M. surinamensis* subspecies (*surinamensis* and *multostriata*) are involved. Consequently, *M. cherriei* vocalizations were compared separately with those of both subspecies. A paucity of recordings from known regions of syntopy required that we use all available recordings of *M. s. surinamensis* and *M. cherriei* and a geographically balanced sample from the entire range of *M. s. multostriata*.

**Myrmoborus leucophrys/M. myotherinus.**—These species are sympatric over a large region. In general, *M. leucophrys* inhabits river-edge and seasonally flooded forest, and *M. myotherinus* terra firme forest, but the two are often found together at ecotones. The recordings in our sample were from southeastern Peru, northwestern Bolivia, and Acre, Brazil.

**Hypocnemis cantator/H. hypoxantha.**—These species often occur in syntopy in western Amazonia and to the east in the region between the Rio Tapajós and Rio Xingu in central Brazil. We examined recordings from both regions. The sample from Region 1 was confined to the area around the mouth of the Rio Napo, north of the Rio Amazonas, Loreto, Peru (*H. c. saturata* and *H. h. hypoxantha*). In Region 2, the *H. hypoxantha* (*H. h. ochraceiventris*) sample was drawn from the area east of the Rio Tapajós from Santarém to the vicinity of Rurópolis, Pará, Brazil. To obtain a sufficient sample of *H. cantator* (*H. c. striata*), this area was extended (east of the Rio Tapajós) south to the Rio Cristalino, extreme northern Mato Grosso.

#### VOCALIZATION ANALYSIS PROCEDURES

We assumed that all vocalization types are relevant, that birds hear sounds in the same frequency range as do humans, and that birds have an ability to discriminate among differences in frequency and time intervals, the principal variables expressed in spectrograms (Dooling 1982). Series of notes delivered loudly by thamnophilid antbirds, usually throughout the year in the equatorial region, were termed loudsongs (following Willis 1967) to distinguish them from other vocalizations that fit the general definition of songs. Recordings were obtained from archives and individuals listed in the Appendix. We initially viewed every recording in its entirety as a real-time spectrogram on a Uniscan II (Multigon Industries) to identify the number and sex of individuals vocalizing and to label each vocalization as to type. At the initial viewing, we screened recordings for the following: (1) recordings that were unmeasurable because of "over-recording" (gain set too high) or because the signal was too faint; (2) re-recording of an individual (which would have distorted the sample size); (3) erroneous documentation (e.g. identification of sex appeared to be incorrect); and (4) premature termination of a multinote vocalization, as can happen when a bird is startled.

Following the screening procedure, we used Canary 1.2 (Bioacoustics Research Program, Cornell Laboratory of Ornithology) to obtain measurements and descriptive data from each recording. The default settings of Canary were maintained except that the display style employed was smooth rather than boxy, and overlap was set at 96.88% in preparing spectrograms for figures. Brightness and contrast settings were adjusted for each recording to match recording levels. Most recordings included repetitions of the same vocalization type by the same individual. We typically measured the first three repetitions and every third one thereafter. Measurements for each repetition were entered as one record in an Access (Microsoft Corporation) database application. Ranges, means, and standard deviations were calculated in the database for each individual and for species. Spectrograms chosen for figures reflected central tendencies in key vocal characteristics. Information entered for individual records included sex and whether the recording was made after playback. Because of possible differences between sexes (Ratcliffe and Otter 1996), measurements were initially sorted for each type of vocalization by sex, and records were aggregated only if no significant sexual differences were identified. Some antbirds are rarely identifiable to sex, in which case it was necessary to use all recordings regardless of sex. Finally, we analyzed at least one well-documented recording (if available) of an individual vocalizing naturally and after playback to determine whether vocalizations differed between these two circumstances.

#### VOCAL CHARACTERS STUDIED

We studied a standard set of vocal characters and measures. Characters were defined to reflect independent features of vocalizations. Typically, multiple measures were taken that reflected the same character. If two or more overlapping measures were diagnosable, we employed the one that appeared to be most reflective of the character.

*Number of notes.*—A note was defined as an unbroken trace on a spectrogram, including associated overtones.

*Duration of vocalization.*—Duration was measured in seconds, expressed to the nearest hundredth, from the beginning of the first note to the end of the last note.

*Pace.*—Pace was defined as the number of notes per second. Overall pace was computed by counting the number of notes and measuring the duration from the beginning of the first note to the beginning of the last note. We also divided lengthier vocalizations into three or five (depending on complexity) approximately time-equivalent sections and computed the pace for each section.

*Change in pace.*—To obtain measures of how the pace of a lengthy vocalization changed (e.g. speeded

up or slowed down), we calculated ratios between the pace of sections as defined above. We also compared the duration of representative notes and inter-note intervals that followed them. For example, we divided the duration of the second note and the following interval by the duration of the second-from-last note and interval.

*Frequency.*—To provide a measure of the overall frequency, we used the peak frequency measurement of Canary, which is the frequency at the point of highest amplitude (Charif et al. 1995).

*Change in frequency.*—Using sections defined for pace measurements, we obtained peak frequencies within each section of long vocalizations. These were examined for consistent differences in pattern, such as increase in the frequency of the vocalization of one species and decrease in frequency of the other. If possible, given the structure of the notes, we also manipulated the Canary screen cursors to ascertain change of frequency of individual notes (obtaining the frequency of each, for example, at the peak of the note).

*Amplitude.*—The point in time of highest amplitude ("peak time," as defined by Canary) was expressed as a percentage of total duration of the vocalization. Raw amplitude was not compared because there is no way to calibrate the recording levels set by recordists nor the attenuating effects of the recording environment.

*Change in amplitude.*—Using sections defined for pace measurements, we obtained "Average Intensity" (as calculated by Canary) for each section of lengthy vocalizations. We compared these to ascertain whether there were consistent differences in pattern, such as when one species consistently became louder and the other's vocalization declined in amplitude.

*Note structure and tonality.*—Individual notes were measured for duration and examined visually for distinctions in note shape (e.g. upslurred or hill-shaped) and tonality. Measures of tonality (Baptista 1996) included the presence or absence of: (1) "noise," (2) overtones, and (3) minute frequency modulations. "Noise" was defined as a random mixture of audio frequencies that made a note sound dissonant. Overtones appeared as separate and simultaneous traces on a spectrogram, one above the other. Because overtones may be generated by recording equipment, consistency in the entire set of spectrograms was required. Frequency modulated notes produced a spectrogram trace looking like a "zig-zag" sewing machine stitch and sounded warbled or harsh. To be considered frequency-modulated, a note had to exhibit a clear pattern of regularly spaced modulation at an analysis resolution setting of 349.70 Hz filter bandwidth in Canary. This filter bandwidth permitted obvious frequency modulations to be observed clearly, while rejecting spurious modulations.





















