Acta Chiropterologica, 12(1): 217–229, 2010
PL ISSN 1508-1109 © Museum and Institute of Zoology PAS
doi: 10.3161/150811010X504716

Echolocation calls of the bats of Trinidad, West Indies: is guild membership reflected in echolocation signal design?

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Time-expanded echolocation calls were recorded from 29 species of Neotropical bats in lowland moist tropical forest in Trinidad, West Indies with three aims: (1) to describe the echolocation calls of the members of a diverse Neotropical bat community, especially members of the family Phyllostomidae, whose calls are not well documented (2) to investigate whether multivariate analysis of calls allows species and foraging guilds to be identified and (3) to evaluate the use of bat detectors in surveying the phyllostomids of Neotropical forests. The calls of 12 species of the family Phyllostomidae are described here for the first time and a total of 29 species, belonging to five families (Emballonuridae, Mormoopidae, Phyllostomidae, Molossidae and Vespertilionidae) were recorded. Quadratic discriminant function analysis (DFA) was used to obtain classification rates for each one of 11 individual species and for six guilds (based on diet, foraging mode and habitat) comprising 26 species. Overall classification rates were low compared to similar studies conducted in the Palaeotropics. We suggest that this may be due to a combination of ecological plasticity for certain species and a loose relationship between echolocation call shape, fine-grained resource partitioning and resource acquisition in phyllostomids.

Key words: bats, Chiroptera, echolocation, guilds, Phyllostomidae, Trinidad

INTRODUCTION

Echolocation in bats is characterised by variation in call intensity, frequency, shape, and patterns of pulse emission (Fenton *et al.*, 1998). These differences are sufficiently large in some bat species to facilitate species identification among sympatric species (Rydell *et al.*, 2002). Recently more and more studies of bat echolocation are attempting to assess how accurately species in different bat communities can be identified by their echolocation calls (MacDonald *et al.*, 1994; Fenton *et al.*, 2001; Preatoni *et al.*, 2005; Murray *et al*., 2009). This is linked to the wider question of whether bat detectors provide a reliable means of sampling a bat community without capturing individuals, or whether they can be used as an adjunct method to inventory the community more completely (Fenton and Griffin, 1997; Vaughan *et al.*, 1997; Ahlen and Baagøe, 1999; Barclay, 1999; Rydell *et al.*, 2002). Capturing bats can be difficult, time consuming and relatively costly in terms of manpower. Moreover, many bat species in tropical forests are undersampled by mist nets and harp traps as they rely almost exclusively on echolocation for orientation in space and when foraging are able to avoid nets and traps (Kalko, 1998; Simmons and Voss, 1998).

Echolocation studies are also used to investigate resource partitioning and guild assemblies (Schnitzler and Kalko, 1998; Siemers and Schnitzler, 2004), as echolocation call characteristics reflect habitat and dietary partitioning (Aldridge and Rautenbach, 1987; Jones *et al.*, 1992; Vaughan *et al.*, 1997; Fenton and Ratcliffe, 2004). Bats can be divided into guilds according to their wing morphology, preferred habitat, diet and foraging behaviour, which are thought to coincide with distinct adaptations in signal structure (Schnitzler and Kalko, 1998). Although coarse partitioning of niche space is generally accepted, it is not clear how niches differ within guilds, or whether fine-grained niche differentiation is reflected in echolocation signal structure (Siemers and Schnitzler, 2004).

Bat assemblages in the forests of the Old World are typically characterised by many species with strong and distinct calls of high intensity. In the families

Hipposideridae, Rhinolophidae and Myzopodidae, bats echolocate at a specific frequency making species identification relatively straightforward. Thus bat detectors are useful tools in Rapid Biodiversity Assessments in the Palaeotropics (RBAs) (Russ *et al*., 2003). Within the same genus, species can be distinguished by variations in emitted call frequency (Barclay, 1999), which can be related to age, body size and sex (Neuweiler *et al.*, 1987; Jones and Rayner, 1989; Jones *et al.*, 1992; Russo *et al.*, 2001).

Our knowledge of Neotropical bat echolocation is largely confined to aerial insectivores (e.g., Molossidae) or insectivores specialised in feeding at forest edges or in gaps in forest cover (*Saccopteryx bilineata*, *S. leptura*, *Rhogeessa io* — O'Farrell and Miller, 1997; Kalko, 1995; Rydell *et al*., 2002) as these groups produce strong signals, which are relatively easy to record using bat detectors. However, the Phyllostomidae, the most speciose and ecologically diverse family of New World bats, have seldom been the subject of detailed echolocation studies (Murray *et al*., 2009). Members of this family generally produce low intensity calls and are thought to be difficult to detect with bat detectors. Many phyllostomids are found in highly cluttered habitats, making visual observations and identification problematic. Many are gleaners, taking their food from surfaces and using several different methods to detect food, including vision, prey-generated sound, olfaction and echolocation (Grant, 1991; Kalko and Condon, 1998; Thies *et al.*, 1998). While evidence for resource partitioning in the Phyllostomidae is abundant, it is based mostly on differences in diet, habitat preference and flight morphology (Giannini and Kalko, 2004; Weinbeer and Kalko, 2004), and not on echoocation signal characteristics.

Jennings *et al.* (2004) described the echolocation calls of nine species of phyllostomid bats from the Caribbean, although no attempt was made to investigate whether echolocation design reflects fine-scale partitioning. As part of a large bat community study in the Victoria-Mayaro Forest Reserve (VMFR), Trinidad, West Indies, bats were sampled with harp traps and mist nets set at ground level and with mist nets set in the canopy, allowing bats to be identified to species level and their echolocation calls recorded on release (Clarke *et al.*, 2005*a*, 2005*b*). By means of a time-expansion detector, echolocation calls were recorded from an ecologically diverse community of Neotropical bats within the VMFR.

We aimed: (1) To describe the echolocation calls of a Neotropical bat community, especially the members of the family Phyllostomidae; (2) To investigate whether multivariate analysis of 'hand release' calls allows species and foraging guilds to be identified; (3) To assess the use of bat detectors as a tool to survey phyllostomid bat communities.

Materials and Methods

Study site

All sound recordings were made within the VMFR, in the southern-eastern part of Trinidad (10º04'–10º18'N, 61º01'–61º18'W). The reserve covers an area of approximately 52,000 ha of lowland tropical moist forest, in which the canopy is dominated by*Mora excelsa.* The next most dominant canopy species are *Carapa guianensis*, *Terminalia dichotoma*, *Pterocarpus rohrii* and *Spondias mombin*.

Capture methods

Mist nets of 2.6 × 6 m were employed at ground level at each sampling site. Nets were positioned to sample all microhabitats at the sites: ridge tops, valley bottoms and streams, flat well-drained ground, swampy areas, under closed canopy, or in tree-fall gaps. One 3 × 12 m mist net was set in the forest subcanopy at four sampling points. All nets were 50-denier weight, 2-ply nylon, with a 38-mm mesh size (Avinet, Dryden, New York, USA) and were deployed between 5 pm and midnight. A two-frame harp trap, with a catching surface of 4.2 m^2, was erected at each sampling point (AUSTBAT Research Equipment, Victoria, Australia). A complete list of all captured species, as well as a detailed description of capture rates is published in Clarke *et al.* (2005*a*, 2005*b*).

Recording conditions and equipment

Bats were recorded when released from the hand after capture and identification (Parsons and Jones 2000, Fukui *et al.*, 2004). Bat taxonomy follows Simmons (2005). All bats were released 5 m from the detector on forest trails. For some species recordings were made inside a custom made flight chamber (3x3x3m), made from nylon mosquito netting. Recordings obtained in this way were not used in the analysis presented here, but some sonograms of calls recorded in the flight chamber are represented in Fig 1. Ultrasound was converted to audible signals via a time expansion bat detector (D-980, Petersson Elektronik AB, Uppsala, Sweden). The detector digitally stores three seconds of 'real' time, and slows it down by a factor of ten. Time expanded ultrasound was stored on metal tapes (type IV) via a WM-D6C Sony Professional Walkman (Sony Cooperation, Tokyo, Japan).

Measurements of call parameters

Echolocation calls were analysed using the software package BatSoundPro, Ver. 3.0 (Pettersson Elektronik AB, Uppsala, Sweden). Only one call per bat was measured except for *Rhynchonycteris naso* where two calls per bat (one for a higher call and one for a lower call) were measured, though in the analysis only the lower call was used as a larger sample size for this call was available. For *Saccopteryx bilineata* and *S. leptura*, the alternating pulse often reported by other authors (Jung *et al.*, 2007) was observed only occasionally, therefore a formal distinction between higher and lower pulse was not made. Typically the middle call of each call sequence was selected in

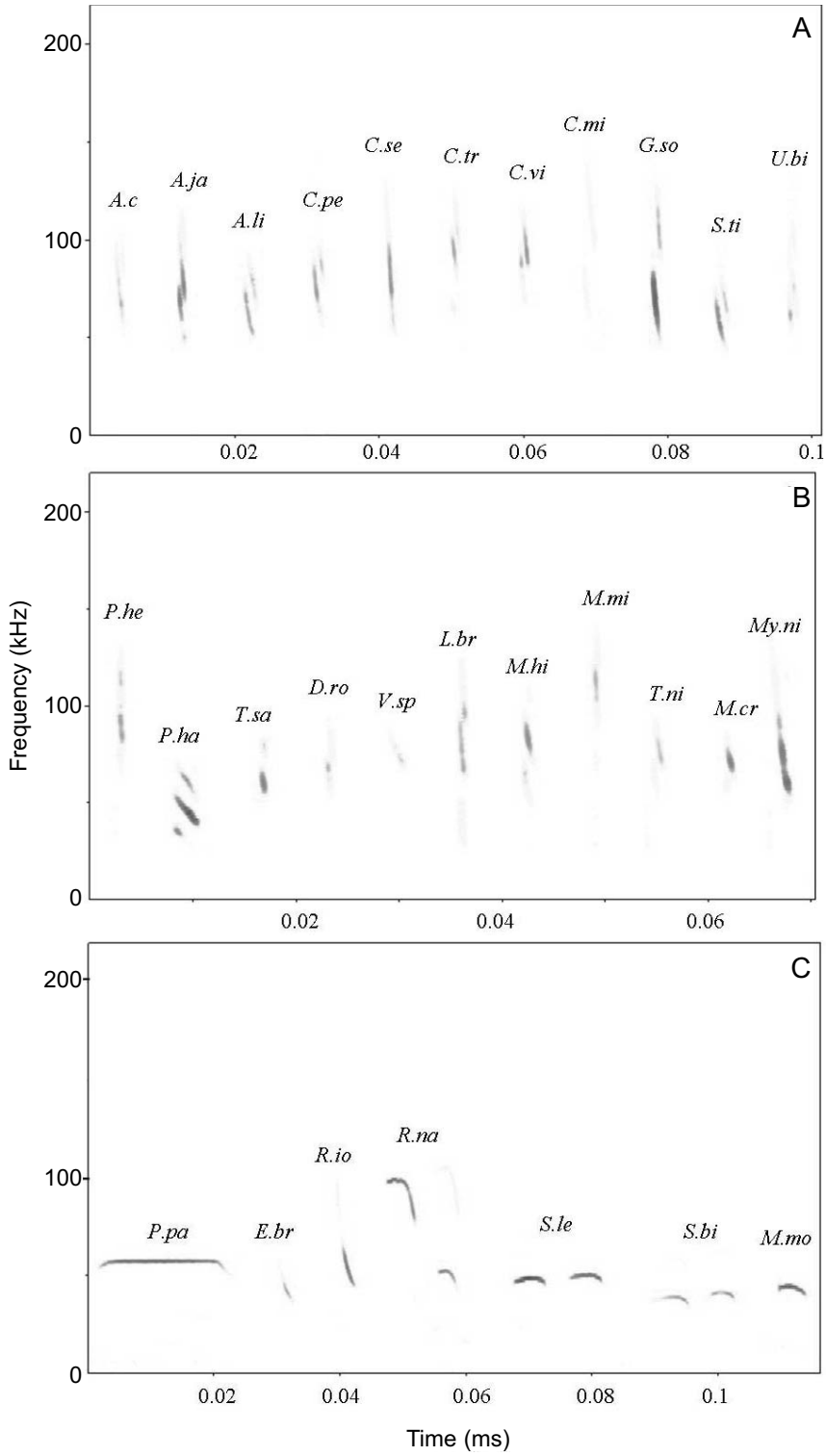

FIG. 1. Sonograms of one echolocation call for each of 29 bat species recorded from hand released bats (unless otherwise stated): 21 phyllostomids: *A.ci*. — *Artibeus cinereus*, *A.ja* — *Artibeus jamaicensis*, *A.li* — *Artibeus lituratus*, *C.pe* — *Carollia perspicillata*, *C.se* — *Centurio senex* (in flight chamber), *C.tr* — *Chiroderma trinitatum*, *C.vi* — *Chiroderma villosum*, *C.mi* — *Choeriniscus minor*, *G.so* — *Glossophaga soricina*, *S.ti* — *Sturnira tildae*, *U.bi* — *Uroderma bilobatum*, *P.he* — *Platyrrhinus helleri*, *P.ha* — *Phyllostomus hastatus*, *T.sa* — *Tonatia saurophila*, *D.ro* — *Desmodus rotundus*, *V.sp* — *Vampyrum spectrum*, *L.br* — *Lampronycteris brachyotis* (in flight chamber), *M.hi* — *Micronycteris hirsuta*, *M.mi* — *Micronycteris minuta* (in flight chamber), *T.ni* — *Trinycteris nicefori*, *M.cr* — *Mimon crenulatum* (in flight chamber); three vespertilionids: *My.ni* — *Myotis nigricans* (in flight chamber), *E.br* — *Eptesicus brasiliensis*, *R.io* — *Rhogeessa io*; three emballonurids: n *R.na* — *Rhynchonycteris naso* (two calls shown to illustrate variation in harmonic usage), *S.le* — *Saccopteryx leptura* (high and low call shown), *S.bi* — *Saccopteryx bilineata* (highand low call shown); one mormoopid: *P.pa* — *Pteronotus parnellii* and one molossid: *M.mo* — *Molossus molossus*

an effort to exclude both shorter 'take-off' calls at the start of a sequence and very attenuated calls at the end of a sequence. Care was taken to avoid calls that showed signs of interference, background noise or overloading. Most calls analysed here comprised of two or more harmonics. The harmonic containing the most energy was usually diagnostic for the species, though occasionally the switching of energy from one harmonic to another was observed for some species. For each call the harmonic containing most energy was identified from the power spectrum and measurements were taken from it. The following call characteristics were measured from the sonogram and power spectrum: Frequency containing maximum energy (or peak frequency, FMAXE), minimum frequency (FMIN), maximum frequency (FMAX), duration of the call (DUR) and time between calls (TBC). DUR and TBC (ms) were measured from oscillograms, FMAXE (kHz) from power spectra, and all other spectral parameters (kHz) from spectrograms.

Data analysis

Quadratic multivariate discriminant analysis (quadratic DFA) has recently been shown to give an objective measure of confidence in species indetification and to correctly classify more cases than other methods (Jones *et al.*, 2000; Preatoni *et al.*, 2005). DFA was applied with cross validation to call parameters of 11 species, as sample sizes for the remaining 18 were insufficient for statistical analysis. In the species classification quadratic DFA, FMAXE was the only normally distributed parameter. All other parameters were subjected to logarithmic transformations, but only TBC was normalised. To illustrate the effect of reduced sample sizes on quadratic DFA outcome, sample sizes of five phyllostomids were reduced to match the remaining six species. In this case all but FMAX were either normal or became so following logarithmic transformations. In guild classifications only TBC and FMAXE resulted in normal distributions following logarithmic transformations. However, DFA is robust to the violation of this assumption.

Correlation analysis was used to explore the strength of the relationship between echolocation parameters. All except TBC were found to show strong correlations. The lack of multicolinearity is not a specified assumption of the discriminant model, but it may have important consequences for the interpretations of the canonical functions (McGarigal *et al*., 2000; Quinn and Keough, 2002). Therefore all variables involved in high pairwise correlations ($r > 0.7$) were subject to a univariate, one-way ANOVA with the grouping variable as the main effect. For each pair of highly correlated variables with significant among-group differences, the variable retained was the one with the greatest among-group variance (or largest *F*-value). The others were eliminated. The removal of one offending variable did not eliminate other pairwise correlations, as sometimes is the case (McGarical *et al.*, 2000); therefore none was omitted from the analysis. Box's M test showed that covariances were not homogeneous ($P < 0.001$), therefore quadratic analyses were used. Wilk's values were calculated to produce a measure of the discrimination power of each parameter. In all tests, values of $P < 0.05$ were considered statistically significant. All tests were performed in MINITAB version 14 (Minitab Inc., USA) and SPSS for Windows version 12.

Results

Out of 468 attempted recordings, 79 were empty (missed recordings may be due to low intensity calls or bats using visual cues for orientation), 78 were discarded due to poor sound quality (low signal to noise ratio or interference), 24 belonged to species with too small a sample size to be included and 287 were used successfully in analysis. Sequences were obtained from 29 species, though data from 25 were used in guild classification and from 11 for species classification analyses, again due to sample size considerations (Table 1).

New Call Descriptions

The calls of 12 phyllostomid bat species are described in this study for the first time (Fig. 1A–B, Table 1 and Appendix).

Subfamily Stenodermatinae

1. Gervais's fruit-eating bat (*A. cinereus*) emits short (1.5 ± 0.5 ms) multiharmonic, steep frequency modulated (FM) calls of relatively high frequency peak frequency (70.5 ± 15.6 kHz), broad bandwidth and high variability;
2. The great fruit-eating bat (*A. lituratus*) emits multiharmonic, FM calls of longer duration than *A.cinereus* (2.3 ± 0.6 ms), and of lower peak frequency (63.0 ± 8.8 kHz). The calls have a broad bandwidth and are very variable;
3. The little big-eyed bat (*C. trinitatum*), a fruit-eating species, emits a short (1.4 ± 0.3 ms), FM, multiharmonic call, with a peak frequency of 96.9 ± 4.6 kHz;
4. The hairy big-eyed bat (*C. villosum*), a fruit-eating species, emits calls of similar shape (FM), duration (1.4 ± 0.3 ms) to those produced by *C. trinitatum* but with a slightly lower peak frequency (91.8 ± 5.8 kHz);
5. The tent-making bat (*U. bilobatum*), a fruit-eating species, emits a multiharmonic FM call of short duration (1.6 ± 0.4 ms) and high frequency (74.7 ± 10.6 kHz);
6. Heller's broad-nosed bat (*P. helleri*), a fruit-eating species, also produces short (1.3 ± 0.1 ms), high frequency (99.0 ± 6.4 kHz) multiharmonic FM calls.

Subfamily Glossophaginae

A nectarivorous species, *C. minor*, emits short (1.5 ± 0.4 ms), highly variable, high frequency (97.9 ± 23.3 kHz) FM sweeps.

Subfamily Phyllostominae

The yellow-throated bat (*L. brachyotis*), the hairy big-eared bat (*M. hirsuta*), the little big-eared

TABLE 1. Mean values ± SDs are displayed above ranges for 5 temporal and frequency echolocation call variables measured from echolocation calls produced by 29 species of Neotropical bats: time between calls or interpulse interval (TBC (ms)), duration (DUR (ms)), frequency at maximum energy (FMAXE(kHz)), minimum frequency (FMIN(kHz)) and maximum frequency (FMAX(kHz)). Sample size (*n*) and guild classification are shown for each species. Guilds represented (following Schnitzler and Kalko 1998) are: aerial insectivores feeding in open spaces (AIOS), aerial insectivores feeding in intermediate clutter (AIIC), aerial insectivores feeding in high clutter (AIHC), gleaning insectivores feeding in high clutter (GIHC), gleaning frugivores feeding in high clutter (GFHC) and gleaning nectarivores feeding in high clutter (GNHC), gleaning sanguivores feeding in high clutter (GSHC) and gleaning carnivore feeding in high clutter (GCHC). *Saccopteryx bilineata* and *S. leptura* are often reported to have a higher call and a lower call. In our recordings, the difference between these calls was rarely unequivocal, therefore no distinction is made. *Rhynchonycteris naso* calls can display more energy in a higher harmonic or in a lower harmonic. Both call types are reported here

Taxon	IPI (ms)		DUR (ms)		FMAXE(kHz)		FMIN (kHz)		FMAX (Hz)		*n*	Guild
	$\bar{x}$ ± SD	min–max	$\bar{x}$ ± SD	min–max	$\bar{x}$ ± SD	min–max	$\bar{x}$ ± SD	min–max	$\bar{x}$ ± SD	min–max		
EMBALLONURIDAE												
Saccopteryx leptura	66.7 ± 30.2	18.8–129.7	4.9 ± 1.1	3.1–7.3	51.1 ± 1.2	48.9–52.2	42.9 ± 1.3	41.0–45.0	53.3 ± 1.4	51.0–55.0	15	AIIC
S. bilineata	56.2 ± 24.2	41.3–111.8	5.2 ± 1.1	3.6–6.5	42.0 ± 1.6	39.4–44.3	32.8 ± 3.2	28.0–38.0	43.9 ± 1.4	42.0–46.0	9	AIIC
Rhynchonycteris naso (call a)	29.3 ± 13.7	15.4–58.3	5.3 ± 1.0	4.3–7.2	51.3 ± 0.8	49.7–52.0	42.2 ± 1.4	40.3–44.7	52.2 ± 0.9	50.6–53.4	7	AIIC
R. naso (call b)	25.9 ± 6.7	19.8–34.4	5.9 ± 1.2	4.9–7.7	100.0 ± 2.0	97.2–102.4	74.8 ± 7.6	74.6–83.6	101.3 ± 1.6	99.9–103.5	4	AIIC
MOLOSSIDAE												
*Molossus molossus**	40.7 ± 16.5	17.8–64.7	4.8 ± 1.2	1.8–6.5	47.0 ± 6.0	29.6–53.2	22.7 ± 2.6	21.0–30.0	50.3 ± 5.1	37.0–55.0	12	AIOS
MORMOOPIDAE												
Pteronotus parnellii	25.0 ± 15.6	4.9–45.5	21 ± 5.5	11.1–25.7	58.2 ± 0.7	57.7–59.6	46.3 ± 1.9	44.0–51.1	60.2 ± 0.8	59.0–61.0	5	AIHC
PHYLLOSTOMIDAE												
Carollinae												
Carollia perspicillata	47.2 ± 39.7	15.2–159.4	1.8 ± 0.6	1.1–3.5	74.9 ± 11.8	60.1–100.3	60.5 ± 11.6	48.0–85.8	92.4 ± 10.8	80.1–116.0	19	GFHC
Desmodontinae												
Desmodus rotundus	31.5 ± 26.1	15.1–61.6	1.8 ± 0.5	1.3–2.3	74.3 ± 7.4	67.6–82.3	55.7 ± 8.5	47.0–64.0	81.7 ± 10.8	74.0–94.0	3	GSHC
Glossophaginae												
Glossophaga soricina	37.2 ± 30.1	9.3–117.0	2.0 ± 0.8	1.1–3.5	94.5 ± 26.6	43.0–122.6	72.4 ± 27.0	21.0–105.4	119.3 ± 31.0	66.0–161.5	27	GNHC
Choeriniscus minor	24.9 ± 8.4	14.9–37.4	1.5 ± 0.4	1.0–2.3	97.9 ± 23.3	80.3–137.6	77.8 ± 24.3	53.0–118.0	124.0 ± 30.2	87.0–158.0	5	GNHC
Phyllostominae												
Mimon crenulatum	25.7 ± 5.2	20.3–30.7	1.5 ± 0.2	1.2–1.7	66.1 ± 3.8	63.6–70.5	58.0 ± 2.0	56.0–60.0	83.0 ± 4.4	80.0–88.0	3	GIHC
Trinycteris nicefori	52.1 ± 27	32.9–72.2	1.6 ± 0.1	1.4–1.6	75.3 ± 1.8	74.0–76.6	65.0 ± 1.4	64.0–66.0	99.5 ± 6.4	95.0–104.0	2	GIHC
Micronycteris minuta	13.7 ± 7.6	6.8–12.8	1.6 ± 0.2	1.3–1.7	61.2 ± 26.0	32.5–83.3	48.0 ± 22.5	22.0–62.0	82.0 ± 30.8	47.0–105.0	3	GIHC
Lampronycteris brachyotis	23.1 ± 7.2	16.2–36.2	1.3 ± 0.1	1.1–1.4	74.6 ± 7.8	66.2–85.5	63.2 ± 5.3	57.0–69.0	97.8 ± 3.3	94.0–102.0	6	GIHC
Micronycteris hirsuta	32.0 ± 24.1	10.0–60.7	1.4 ± 0.7	1.0–1.7	80.8 ± 14.2	60.6–106.1	69.1 ± 19.1	45.0–109.0	97.9 ± 17.4	74.0–129.0	5	GIHC
M. megalotis	38.4 ± 16.1	20.6–73.2	1.5 ± 0.4	1.0–2.1	98.1 ± 15.6	90.4–120.9	81.2 ± 9.2	62.0–89.3	116.0 ± 8.1	98.0–119.8	6	GIHC
Phyllostomus hastatus	50.8 ± 9.7	44.1–62.0	2.7 ± 0.2	2.5–2.9	47.1 ± 0.7	46.3–47.6	38.0 ± 1.7	36.0–39.0	58.3 ± 1.5	57.0–60.0	3	GIHC
Tonatia saurophila	43.6 ± 17.9	28.2–77.0	1.4 ± 0.2	1.0–1.5	56.5 ± 2.3	54.8–60.4	51.5 ± 2.1	49.0–55.0	71.0 ± 2.8	66.0–77.0	6	GIHC
Vampyrum spectrum	29.9		2.8		79.4		64.0		97.0		1	GIHC
Sternodermatinae												
Artibeus cinereus	49.0 ± 39.4	10.6–174.5	1.5 ± 0.5	0.9–2.1	70.5 ± 15.6	56.4–95.7	54.2 ± 9.4	23.0–77.0	93.2 ± 13.5	52.0–125.0	11	GFHC
A. jamaicensis	57.8 ± 25.5	23.0–92.2	2.5 ± 0.7	1.3–3.6	67.6 ± 13.8	51.8–86.8	54.8 ± 11.3	40.0–77.6	89.1 ± 12.4	74.0–106.2	20	GFHC
A. lituratus	68.5 ± 35.5	29.3–140.5	2.3 ± 0.6	1.5–3.6	63.0 ± 8.8	34.4–75.6	50.6 ± 6.4	30.0–60.0	80.3 ± 10.3	43.0–94.0	20	GFHC
Uroderma bilobatum	45.5 ± 39.6	13.2–141.9	1.6 ± 0.4	0.9–2.1	74.7 ± 10.6	58.0–96.9	62.1 ± 7.2	56.4–76.2	89.1 ± 9.7	74.0–111.2	9	GFHC

TABLE 1. Continued

Taxon	IPI (ms)		DUR (ms)		FMAXE (kHz)		FMIN (kHz)		FMAX (kHz)		*n*	Guild
	x̄ ± SD	min–max	x̄ ± SD	min–max	x̄ ± SD	min–max	x̄ ± SD	min–max	x̄ ± SD	min–max		
Platyrrhinus helleri	30.7 ± 12.7	16.8–61.1	1.3 ± 0.1	1.0–1.4	99.0 ± 6.4	88.9–107.9	85.4 ± 6.1	76.0–92.0	118.1 ± 8.6	101.1–131.0	5	GFHC
Chiroderma trinitatum	50.3 ± 26.5	19.8–68.0	1.5 ± 0.3	1.1–1.8	96.9 ± 4.6	91.8–100.6	76.0 ± 10.4	64.0–83.0	110.3 ± 5.1	106.0–116.0	3	GFHC
C. villosum	57.7 ± 24.2	41.0–103.9	1.4 ± 0.3	0.9–1.9	91.8 ± 5.8	84.3–99.7	81.3 ± 2.9	78.0–87.0	112.9 ± 8.6	105.0–130.0	4	GFHC
Stumirinae												
Sturnira tildae	34.4 ± 8.5	28.8–49.0	1.9 ± 0.7	1.1–2.7	70.8 ± 9.0	55.8–79.0	56.2 ± 8.4	42.0–64.0	92.8 ± 12.7	76.0–111.0	5	GFHC
VESPERTILIONIDAE												
Myotis nigricans	24.0 ± 6.2	19.5–33.1	2.2 ± 0.1	2.0–2.3	66.2 ± 7.9	61.3–78.0	51.3 ± 1.3	50.0–53.0	125.0 ± 7.5	115.0–133.0	4	AIIC
Eptesicus brasiliensis	74.7 ± 23.3	50.5–56.8	3 ± 1.6	1.8–4.1	41.1 ± 1.3	40.1–45.0	30.5 ± 6.4	26.0–35.0	71.5 ± 14.8	61.0–82.0	2	AIIC
Rhogeessa io	38.4 ± 28.6	17.1–81.7	2.8 ± 0.6	2.1–3.8	52.4 ± 3.7	48.2–57.7	39.6 ± 3.9	33.0–42.0	99.6 ± 6.5	95.0–111.0	4	AIIC

* — The calls of *M. molossus* described here are not typical 'search phase calls' and contain much steeper FM components than is usually reported for this species. This difference is likely due to the fact that this species is normally recorded in large open spaces. In this case recordings were taken on forest trails

bat (*M. megalotis*), the white-bellied big-eared bat, (*M. minuta*), and Niceforo's big-eared bat (*T. nicefori*), are all small insectivorous species, but like the rest of the Phyllostomidae recorded in this study, emit multiharmonic FM sweeps characterised by short durations (1.3 ± 0.1, 1.4 ± 0.7, 1.5 ± 0.4, 1.6 ± 0.2, 1.6 ± 0.1 ms, respectively), broad bandwidths and relatively high frequencies (74.6 ± 7.8, 80.8 ± 14.2, 98.1 ± 15.6, 61.2 ± 26, 75.3 ± 1.8 kHz, respectively).

Discriminant Function Analysis

Quadratic discriminant analysis was applied to 11 individual species and 25 species grouped together according to guild classification.

Individual species

When using quadratic discriminant function analysis for individual species classification, an overall classification of 68.8% was reached (Table 2). The model included all five parameters: TBC, DUR, FMAXE, FMIN and FMAX. Classification rates ranged from 100% for *R. naso*, *S. bilineata*, *M. molossus* and *P. parnellii* to 29.2% for *C. perspicillata*.

MANOVA indicated significant discrimination of the model (Wilk's = 0.010, $F_{50,\ 856} = 29.71$, $P < 0.001$). The first discriminant function explained 80.1% of the variation, whilst the first three discriminant functions combined accounted for 98.6%. Though predictor variables were correlated, removing any one of the parameters could not increase accuracy. When parameters were removed in turn, overall accuracy decreased as follows: 64.9% (TBC removed), 68.3% (DUR removed), 67.3% (FMAX removed), 59.4% (FMAXE removed), and 55.4% (FMIN removed).

Guilds

Quadratic discriminant analysis classified an overall 65.8% of calls into the correct guild groupings (Table 3). Individual guild accuracy ranged from 100% in open air and clutter adapted aerial insectivores, to 43% in frugivores. This model included all five parameters. A MANOVA indicated significant discrimination of the model (Wilk's = 0.045, $F_{25,\ 1030} = 53.88$, $P<0.001$), that the first discriminant function accounted for 91.1% of the variation, whilst the first three discriminant functions combined accounted for 99.6%. The overall model accuracy was not improved by removing any of the variables, though equal classification accuracy was obtained by removing FMAXE (65.9%). When

removing all other variables, classification accuracy decreased as follows: 60.6% (FMAX removed), 59.9% (DUR removed), 60.3% (TBC removed) and 57.1% (FMIN removed).

Discussion

Quadratic discriminant function analysis for a community of 11 individual species of neotropical bats belonging to four families resulted in a classification rate of 68.1%. This is lower than most other published work. Russo and Jones (2002) obtained a classification rate of 81.8% in a model which comprised nineteen species of five families. Neotropical bat communities are very diverse, comprising in excess of 50 species in just one habitat type (Handley *et al.*, 1991), and the species considered here are only a section of the community present. In total, 49 species of bats have been recorded from the VMFR and thus calls of the 29 species dealt with here represent less than two-thirds of the community (Clarke *et al.*, 2005*a*, 2005*b*; authors' unpublished data). The classification rate for a larger community is likely to be lower, as increased number of species result in lower classification rates for DFA (Biscardi *et al*., 2004). Aerial insectivorous species such as *S. bilineata*, *S. leptura*, *M. molossus* and *P. parnellii* resulted in higher classification rates (93.3–100%) than did gleaning frugivores and nectarivores (27.9–75%). This result supports previous research that found that bats emitting FM/QCF and FM/CF/FM calls are typically more accurately classified than species emitting FM calls (Russo and Jones 2002), possibly because narrower bandwidths result in reduced frequency overlap.

These findings suggests that rapid biodiversity assessments (RBAs) with time-expansion detectors alone would not provide an accurate and complete picture of the resident bat community, especially where members of the family Phyllostomidae are concerned. Inherent biases involved in using the detector as a tool for RBAs include varying detection levels due to varying intensities of echolocation signals emitted by different species (Waters and Jones, 1995). Even within the same guild, some bats were consistently easier to record than others. Attempts to obtain recordings from *A. jamaicensis* and *A. lituratus* always yielded data, whilst recordings from some of the smaller frugivorous or nectarivorous species such as *P. helleri*, *U. bilobatum*, *G. soricina* and *A. cinereus* were often not successful. Moreover, in a recent study comparing sampling methods in a Mediterranean bat community, using

Table 2. Quadratic discriminant function analysis classification for 11 common neotropical bat species, based on five parameters of their echolocation calls (interpulse interval, duration, frequency at maximum energy, minimum frequency and maximum frequency). Overall classification rate was 68.1% (n = 232). Species and families examioned: *M. molossus* (MOLossidae), *R. naso*, *S. leptura*, *S. bilineata* (EMBallonuridae), *P. parnellii* (MORmorpidae), *A. cinereus*, *C. perspicillata*, *G. soricina*, *U. bilobatum*, *A. jamaicensis*, *A. lituratus* (PHYllostomidae)

DFA classified as	True groups										
	M. molossus MOL	*R. naso* EMB	*S. leptura* EMB	*S. bilineata* EMB	*P. parnellii* MOR	*A. cinereus* PHY	*C. perspicillata* PHY	*G. soricina* PHY	*U. bilobatum* PHY	*A. jamaicensis* PHY	*A. lituratus* PHY
Molossus molossus	12	0	0	0	0	0	0	0	0	0	0
Rhynchonycteris naso	0	7	1	0	0	0	0	0	0	0	0
Saccopteryx leptura	0	0	14	0	0	0	0	0	0	0	0
S. bilineata	0	0	0	8	0	0	0	0	0	0	0
Pteronotus parnellii	0	0	0	0	9	0	0	0	0	0	0
Artibeus cinereus	0	0	0	0	0	16	5	3	1	2	0
Carollia perspicillata	0	0	0	0	0	1	12	0	2	2	0
Glossophaga soricina	0	0	0	0	0	4	3	21	0	0	0
Uroderma bilobatum	0	0	0	0	0	1	9	0	10	1	3
Artibeus jamaicensis	0	0	0	0	0	2	4	3	1	13	2
A. lituratus	0	0	0	0	0	2	8	0	1	2	15
Total N	12	7	15	8	9	26	41	27	15	20	20
N correct	12	7	14	8	9	16	12	21	10	13	15
% correct	100	100	93.3	100	100	61.5	29.2	77.8	66.7	65	75

TABLE 3. Quadratic discriminant function analysis classification for six guilds of Neotropical bat species, based on habitat type, feeding mode and diet (Schnitzler and Kalko, 1998). The guilds represented are aerial insectivores feeding in open spaces (AIOS; one species), aerial insectivores feeding in intermediate (or back-ground) clutter (AIIC; six species), aerial insectivores feeding in highly cluttered habitats (AIHC; one species), gleaning insectivores feeding in highly cluttered habitats (GIHC; six species), frugivores (GFHC; nine species) and nectarivores (GNHC; two species) which both feed in highly cluttered habitats. Overall classification rate was 65.8% (n = 287)

DFA classified as	True groups					
	Aerial insectivores (open space)	Aerial insectivores (intermediate clutter)	Aerial insectivores (high clutter)	Gleaning insectivores (high clutter)	Gleaning frugivores (high clutter)	Gleaning nectarivores (high clutter)
AIOS	12	1	0	0	0	0
AIIC	0	44	0	0	7	1
AIHC	0	0	9	0	0	0
GIHC	0	0	0	32	66	3
GFHC	0	1	0	1	65	2
GNHC	0	0	0	3	13	27
Total N	12	46	9	36	151	33
N correct	12	44	9	32	65	27
% correct	100	95.7	100	88.9	43	81.8

any one of three techniques (roost inspections, mist-netting and acoustic sampling) resulted in either under- or overrepresentation of certain groups of species (Flaquer *et al.*, 2007). We therefore recommend the use of both acoustic and capture (mist-nets and harp traps) techniques to survey the largest possible number of species in bat community studies. This supports findings from previous research in the Neotropics (Kalko and Handley, 2001; MacSwiney *et al*., 2008).

Quadratic DFA classified 65.9% of calls from 25 species into the appropriate guild. Higher classification rates were observed for aerial insectivores feeding in high, intermediate and low levels of clutter, whilst gleaning frugivores displayed the lowest at 46.1%. Again, this rate is low when compared to other similar studies, where classification to the genus level reached 95.7% (Vaughan *et al.*, 1997; Russo and Jones, 2002). It is possible that this result may be due in part to the fact that the gleaning frugivores guild comprised a higher number of species (9) and thus incorporated more variability than any other guild.

There is ample evidence that Neotropical bats partition resources by having specialised eco-morphological adaptations. Fine-grained resource partitioning based on differences in size and use of foraging areas, as well as differences in activity pattern and foraging strategies, are thought to play key roles in structuring these species-rich communities, and facilitate long-term species co-existence (Weinbeer and Kalko, 2004). There may be two reasons for low classification rates for the Phyllostomidae: (1) within this family overlap of guilds in habitat preferences, flight characteristics and diet are more extensive than previously appreciated or (2) echolocation in the Phyllostomidae may not reflect fine-grained resource partitioning.

Several authors have reported considerable plasticity in the foraging behaviour and habitat use within the same species of bats (Fenton, 1989; Faure and Barclay, 1994; Siemers *et al.*, 2001). Siemers *et al.* (2001) found that *Myotis nigricans* exploited both open spaces and edge and gap habitats, adjusting its signal to different constraints in different environments. A number of studies have found that some species exhibit flexibility in their foraging behaviour, by using both gleaning and aerial insect capture (*Megaderma lyra* — Marimuthu and Neuweiler, 1987; *Rhinolophus ferrumequinum* and *R. hipposideros* — Jones and Rayner, 1989; *Hipposideros ruber* — Bell and Fenton, 1984). The advantages of displaying such behavioural plasticity may include

adaptability to prey availability or successful competition over species restricted to particular habitats or feeding modes. In any case, these findings suggest considerable niche overlap and are a reminder that assigning species to strict guilds may be misleading (Fenton, 1990; Faure and Barclay, 1994).

It is also possible that, although shaped by ecological requirements, echolocation may not reflect some of the variables involved in guild division (i.e., foraging strategy, diet and clutter levels) in such a way as to result in clear cut species classification amongst guilds. Studies on bats foraging in closed habitats for instance show that diet may have little influence on echolocation as other senses (such as olfaction, vision and hearing) may play important roles in finding food (Tuttle and Ryan, 1981; Bell 1985). The low rate of classification for the Phyllostomidae may thus be due to a combination of ecological plasticity and lack of fine-grained resource partitioning reflected in the echolocation design of this family.

The echolocation calls of 12 species of phyllostomid bats (*A. cinereus*, *A. lituratus*, *C. trinitatum*, *C. villosum*, *C. minor*, *L. brachyotis*, *M. hirsuta*, *M. megalotis*, *M. minuta*, *T. nicefori*, *U. bilobatum and P. helleri*) are described here for the first time. Quadratic discriminant function analysis of Neotropical forest bat echolocation calls resulted in a relatively low classification rate (68.1% of calls from 11 species and 65.9% of calls from 25 species into the appropriate guild), suggesting that considerable niche overlap and a relatively loose relationship between echolocation design and ecological specialisation may exist for the Phyllostomidae in particular(which displayed the lowest classification rates in the community). Bat detectors alone are therefore not a reliable means of inventory for Neotropical bat communities. We recommend that mist nets and harp traps continue to be used in combination with bat detectors to obtain more complete survey results.

Acknowledgements

This research was funded by a research grant from The Leverhulme Trust, ref. no. F/00 152/B. Dorothea Pio thanks the University of Aberdeen for funding. We thank Ruari Allan, Daveka Boodram, Katherine Fisher, Anita Hogan, Luke Rostant, and Sheeba Sreenivasan for assistance in the field. Finally, we are indebted to Nadra Gyan and Krishnna John, Forestry Division, and to Petrotrin Ltd. for providing accommodation in Trinidad. We are indebted to Bruce W. Miller and another anonymous referee for their helpful comments on an earlier version of this manuscript.

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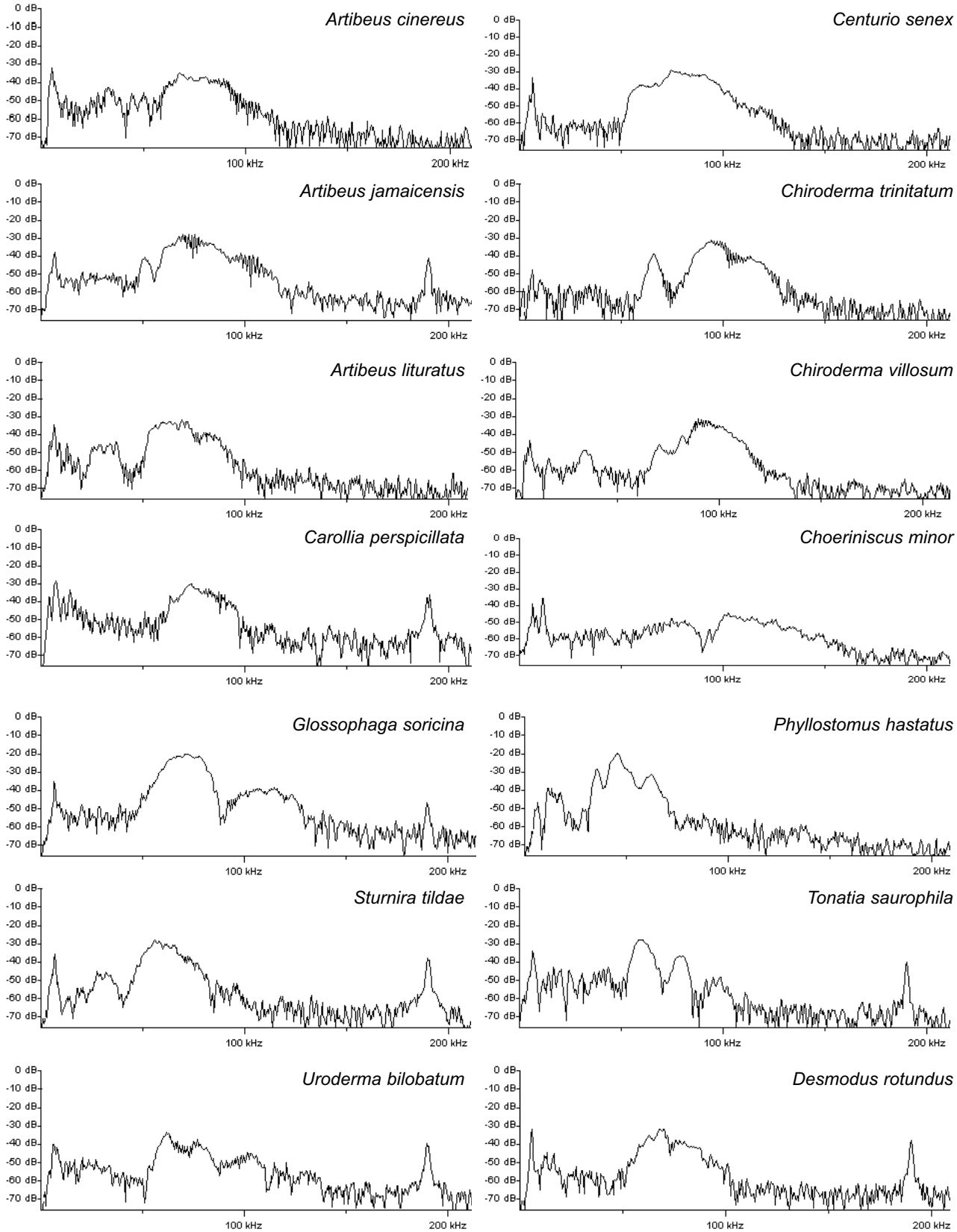
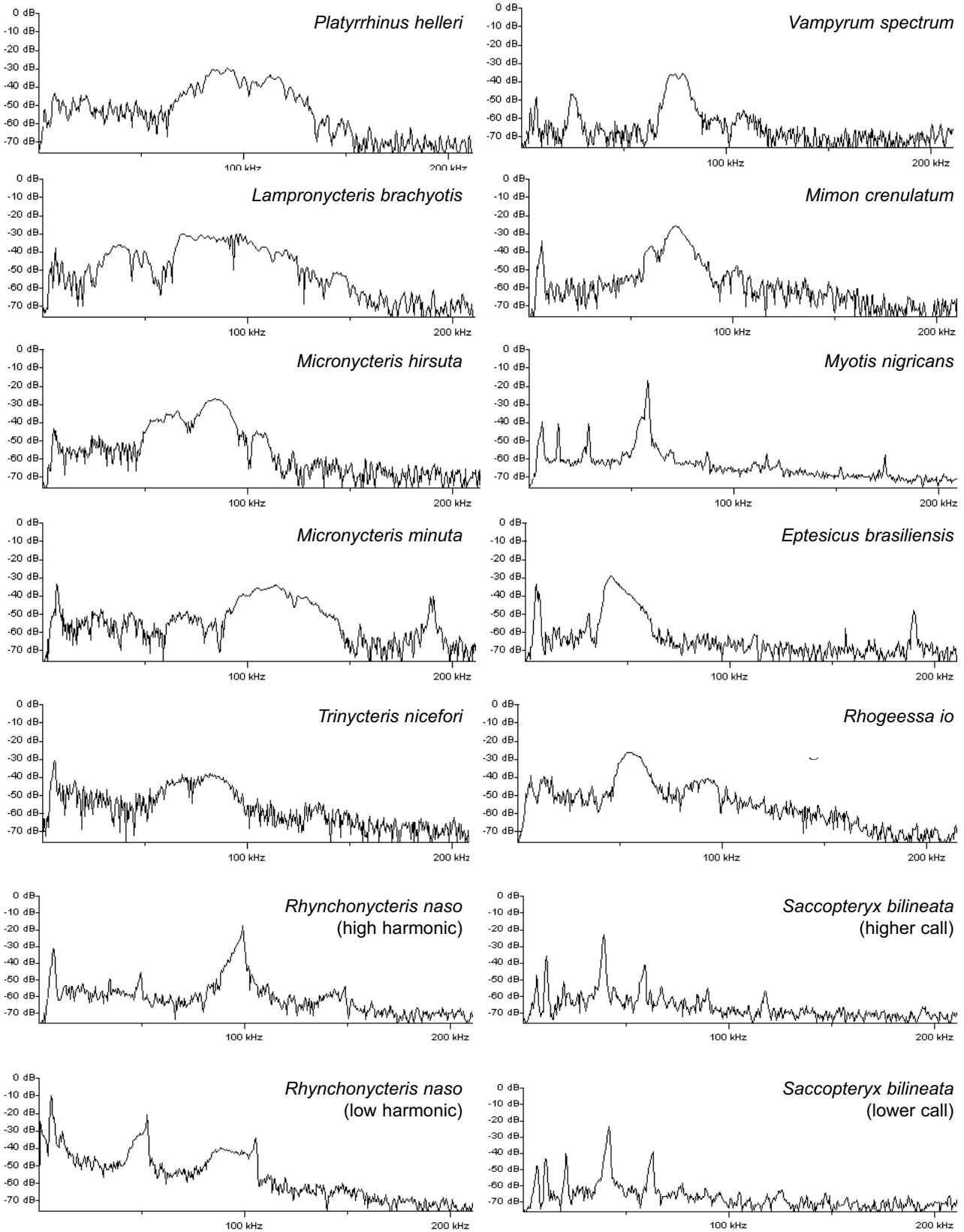
Received 17 July 2009, accepted 20 March 2010

Appendix

Power spectra of each echolocation call in Figure 1



APPENDIX. Continued



APPENDIX. Continued