Identification of twenty-two bat species (Mammalia: Chiroptera) from Italy by analysis of time-expanded recordings of echolocation calls

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Abstract
Spectral and temporal features of echolocation calls emitted by 22 bat species from Italy (three rhinolophids, 18 vespertilionids and the molossid Tadarida teniotis) are described. Time-expanded recordings of calls from 950 bats of known identity were examined. Rhinolophus ferrumequinum, R. hipposideros, R. euryale and T. teniotis could be identified by measuring the call frequency of highest energy (FMAXE). Quadratic discriminant function analysis with cross-validation was applied to calls from the remaining 18 species. A function based on start frequency (SF), end frequency (EF), FMAXE and duration (D) provided a correct overall classification of approximately 82%. A classification model at genus level that also comprised middle frequency (MF) and inter-pulse interval (IPI) reached 94% correct classification. Two separate discriminant functions were devised for species emitting FM (frequency modulated) and FM/QCF calls (i.e. calls consisting of a frequency-modulated component followed by a terminal part whose frequency is almost constant) respectively. The former function included SF, EF, FMAXE and D and provided an overall classification rate of 71%; the latter comprised EF, MF, D and IPI, and reached 96%. The functions may be applied to bat habitat surveys in southern Italy since they cover most of the species occurring in the area.

Key words: bat, Chiroptera, echolocation, identification, Italy

INTRODUCTION
All 31 European bat species occur in Italy, on the basis of both historical and recent records (Lanza, 1959; Lanza & Finotello, 1985; Lanza & Agnelli, 1999; Russo & Jones, 2000).
To identify bat species in flight, Italian researchers have mostly used heterodyne (see Parsons, Boonman & Obrist, 2000) bat detectors, and more recently time expansion (Pettersson, 1999; Parsons et al., 2000) devices. Their main intent was to map species distribution (e.g. Violani & Zava, 1991; Fornasari et al., 1999). In these studies, however, identification was generally either based on subjective criteria (i.e. it relied on the listener’s ability and experience) or carried out by comparing field observations with call descriptions and recordings from other geographical areas (Ahlén, 1981, 1990; Barataud, 1996). In no study was an estimate of the confidence in identification provided.
It is widely documented that echolocation calls may be similar between species, and that calls show a large within-species plasticity resulting from geographical location (Thomas, Bell & Fenton, 1987), habitat structure, flight height (Miller & Degn, 1981; Zbinden, 1989; Schumn, Krull & Neuweiler, 1991; Obrist, 1995; Jensen & Miller, 1999), and various other physiological and environmental influences (Neuweiler et al., 1987; Heller & Helversen, 1989; Jones, Gordon & Nightingale, 1992; Huffman & Henson, 1993; Jones & Ransome, 1993; Jones, Morton et al., 1993; Jones & Kokurewicz, 1994; Jones, Sripathi et al., 1994; Guilën, Juste & Ibàñez, 2000; Russo, Jones & Mucedda, 2001).
These factors may have a significant effect on identification. Furthermore, in regions such as Italy where a large number of bat species occurs, the use of qualitative criteria in identification brings a higher, uncontrollable risk of misclassification.
Some preliminary attempts were made to devise quantitative identification methods for frequency-divided echolocation calls from Italian bats (Pretoni & Martinoli, 1999). Russo & Jones (1999) showed the diagnostic importance of time-expanded social calls from Italian Pipistrellus kuhlii, and Russo & Jones (2000) described time-expanded echolocation and social calls from Italian common pipistrelles, providing evidence that Pipistrellus pipistrellus and Pipistrellus pygmaeus occur in sympatry in the country.

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Discriminant function analysis (DFA) has been applied successfully to identify bats by their echolocation calls in several areas in Europe (Zingg, 1990; Vaughan, Jones & Harris, 1997; Parsons & Jones, 2000) and North America (Krusic & Neefus, 1996; Murray et al., 1999). More recently, methods such as synergetic pattern recognition algorithms performing identification in real-time (Obrist et al., in press) and artificial neural networks (Parsons, 2000; Parsons & Jones, 2000) have also been employed.

Models dealing with a large number of species (especially with very similar calls) may show reduced identification performance. Models so far devised for European bats have not included > 12 species (Zingg, 1990; Vaughan et al., 1997; Parsons & Jones, 2000).

The aims of our study were: (1) to provide the first comprehensive description of time-expanded echolocation calls from Italian bat populations; (2) to test the performance of a DFA on a larger number of bat species; (3) to devise an objective method of species identification for Italian bats, i.e. a method that is independent from the researcher's subjectivity and ability and that quantifies the degree of certainty of identification. Since most species occurring in peninsular southern Italy were analysed, a classification method which could be applied to bat habitat surveys within this area is also offered.

**MATERIALS AND METHODS**

**Species recorded and study areas**

Bats from 22 species were recorded. The database of bat calls featured most species occurring in peninsular central and southern Italy. Of the species whose current presence is documented for Italy, our function did not cover: those whose occurrence is limited to the Alps area (Eptesicus nilssonii, Vespertilio murinus; Mitchell-Jones et al., 1999; Rhinolophus mehelyi, whose populations are mostly confined to Sardinia (Lanza, 1959; Muccteda et al., 1994–95); and Myotis bechsteinii, Nyctalus lasiopterus and Pipistrellus nathusii. Of the last 3 species, M. bechsteinii and N. lasiopterus are rare throughout the country, and few confirmed recent records exist (Vergari, Dondini & Agnelli, 1997; Vergari, Dondini & Ruggieri, 1998), while P. nathusii is uncommon in southern Italy (Lanza, 1959). In the north P. nathusii was recently documented as breeding (Martini, Preatoni & Tosi, 2000).

The study area lay between latitudes 40°09'N and 44°15'N and most field work was carried out in southern Italy, i.e. in Campania, Puglia, Abruzzo, Molise and Lazio. Several bat species, however, were exclusively or mainly recorded further north: Tuscany, most of the Nyctalus leisleri, Plecotus auritus, Plecotus austriacus; Emilia-Romagna, all the Nyctalus noctula and several specimens of Myotis daubentonii and Myotis emarginatus. When possible, bats were recorded at different sites so intraspecific geographical and population variability are represented in the data set.

The nomenclature of bats previously known as Pipistrellus pipistrellus follows Jones & Barratt (1999). Hence pipistrelles of the 45 kHz phonic type (Jones & Parigi, 1993) are referred to as P. pipistrellus, and those of the 55 kHz phonic type are termed P. pygmaeus.

**Recording conditions and equipment**

Recordings were made under 3 conditions:

1. **During emergence from roosts where bats of known identity occurred.** Each site was visited only once to avoid pseudo-replication (Hurlbert, 1984). Calls were recorded 20–30 m away from the roost exit, so the usually broadband calls emitted immediately after emergence were not included in our dataset.

2. **When bats were released from the hand after capture.** The bats were mist-netted at foraging sites or while leaving the roost or, on a few occasions, captured inside the roost. Some bats were released in clutter, others in the open. As a result, both calls affected by cluttered environments and those typically emitted in the open were represented in the sample. The first calls in a sequence seem to have been influenced by release because they were generally steeper in spectrograms and shorter than those emitted away from the release point, and thus were not analysed. On all but 2 occasions, data collected on a single visit to each roost were used. The only Pl. austriaeus roost was visited twice in 2 consecutive years: in 1999, 28 bats were caught inside the roost and recorded in the open; in 2000, 25 bats were caught and recorded in clutter. The bats were not marked, so it is not certain that some bats were not recorded twice. However, this risk seems to be small even without considering mortality or migration, because the colony contained > 100 individuals. Likewise, the only large roost of Myotis myotis–Myotis blythii found was visited twice (June 1998 and September 2000). In this roost, calls of 30 M. blythii and 13 M. myotis captured in 2000, and calls of 15 M. blythii and 26 M. myotis captured in 1998 were used. Again, the risk of pseudo-replication is low as the number of bats captured each time was small, the colony contained several hundred individuals, and the visits were 2 years apart and at different times of the year. Moreover, only 5 M. myotis and 4 M. blythii captured in 1998 showed the same sex, age class and forearm length of bats captured in 2000.

3. **At foraging sites.** Tadarida teniotis is clearly audible to the unaided ear and easily identified (Zbinden & Zingg, 1986). In order to provide a description of echolocation from Italian T. teniotis populations, all bats were recorded in free-flight (Table 1). Each individual was recorded at a different site to eliminate the risk of pseudo-replication. Several P. pipistrellus and P. kuhlii, and most of the P. pygmaeus were also recorded in flight (Table 1) and identified by examining the species-specific structure of their social calls (Barlow & Jones, 1997a,b; Russo & Jones, 1999, 2000). In these
species, recordings were made at sites well apart and only 1 call sampled at each site was analysed. In this way only 1 call from each bat was represented in the sample. Finally, 9 passes of *Barbastella barbastellus* recorded in free-flight were added to the dataset to make this rare species feature sufficiently in the discriminant function analysis. Free-flying barbastelles were recorded at a drinking site where captures conducted over several months had shown that this species occurs frequently (to our best knowledge, it is the only site where barbastelles have been captured recently in the study area) and the recordings were identified by recognizing the characteristic alternation of the 2 call types this species sometimes emits (Barataud, 1996; Parsons & Jones, 2000; see also Results). The barbastelle roosts were probably located in the woodland adjacent to the recording site since bats were mostly mist-netted around emergence time. The call sequences for analysis among those obtained from several hours of recordings conducted on different nights and at different times of the night were randomly selected to minimize the risk of pseudo-replication.

Since our main aim was to devise a method for identifying foraging bats, whenever possible bats were recorded leaving the roost or in free-flight (identified by examining social calls), as these conditions are closer (or, for free-flying bats, identical) to those of a foraging bat. However, it was necessary to make recordings of several bats on hand-release, i.e. when only small colonies of a particular species were found, or when bats roosted either alone or in small numbers, making it necessary to capture them to ensure successful recordings (this was true for most *N. leisleri*, which occupied bat boxes); when the subjects to be recorded roosted together with bats from other species emitting similar calls (e.g. *M. myotis* and *M. blythii*); and when the species emitted faint calls (*Plecotus* spp.), so that the requisite for multivariate normality (MacArdle, 1994) ± a necessary prerequisite for multivariate normality ± a necessary pre-requisite for multivariate normality (MacArdle, 1994) ± it followed that the dataset did not conform to multivariate normal distribution. However, multivariate tests are robust to departures from normality (Dillon & Goldstein, 1994; Vaughan *et al.*, 1997; Parsons & Jones, 2000). Wilk’s λ values were obtained with a MANOVA to test for statistical significance of DFA models, and to assess discrimination power of each variable (Parsons & Jones, 2000). Correlation analysis (Spearman’s rank coefficient) was used to explore the strength of relationship between model variables. All tests were performed with MINITAB release 9.2 except Box’s M test which was performed with SPSS for Windows version 10. In all tests, values of *P* < 0.05 were considered significant.

For this species, 2 calls were analysed, i.e. 1 for each call structure, for 5 bats (4 hand-released, 1 in free-flight). Although a larger number of barbastelles alternated calls in flight, only the bats that were best recorded were chosen to limit replication of calls from the same individual in the sample.

The following 6 parameters were measured from each call: start frequency (SF, the frequency value measured at the beginning of the call), end frequency (EF, the frequency value measured at the end of the call), middle frequency (MF, the frequency of highest energy taken at half call duration), frequency of maximum energy (FMAXE), duration (D) and inter-pulse interval (IPI, the time interval between 2 consecutive calls).

D and IPI (ms) were measured from oscillograms, FMAXE (kHz) from power spectra, and all other spectral parameters (kHz) from spectrograms. In *Plecotus* spp., the highest energy may be in either the fundamental or in the second harmonic (e.g. Parsons & Jones, 2000), therefore FMAXE was taken from the harmonic with highest energy, while all other measurements were taken from the fundamental. For all other species, measurements were taken from the harmonic containing most energy, i.e. always from the fundamental in all other vespertilionid and *T. teniotis* calls and from the second harmonic in rhinolophid calls.

Statistical procedures

For each species, descriptive statistics (mean ± sd and range) are shown. Univariate inferential procedures (ANOVA for normally distributed variables, Mann–Whitney and Kruskal–Wallis tests for those that did not conform to normality) were used to test for differences between species whose calls had been recorded under identical and well controlled conditions.

Multivariate discriminant function analysis (DFA) with cross-validation was applied to call parameters from 18 species. Because several variables departed from univariate normal distribution – a necessary pre-requisite for multivariate normality (MacArdle, 1994) – it followed that the dataset did not conform to multivariate normal distribution. However, multivariate tests are robust to departures from normality (Dillon & Goldstein, 1984). Box’s M test showed that covariance matrices were not homogeneous (*P* < 0.001), and quadratic analyses were therefore used (Dillon & Goldstein, 1994; Vaughan *et al.*, 1997; Parsons & Jones, 2000). Wilk’s λ values were obtained with a MANOVA to test for statistical significance of DFA models, and to assess discrimination power of each variable (Parsons & Jones, 2000). Correlation analysis (Spearman’s rank coefficient) was used to explore the strength of relationship between model variables. All tests were performed with MINITAB release 9.2 except Box’s M test which was performed with SPSS for Windows version 10. In all tests, values of *P* < 0.05 were considered significant.

Sound analysis

The recordings were analysed with the software BatSound release 1 (Pettersson Elektronik AB, Uppsala) using a sampling frequency of 44.1 kHz, with 16 bits/sample, and a 512 pt. FFT with a Hamming window for analysis. A 112Hz frequency resolution was obtained for spectrograms and power spectra.

One echolocation call selected at random from each bat was analysed for all species except *B. barbastellus.*
RESULTS

Description of echolocation calls

Echolocation calls were recorded from 950 individuals: 46.3% were recorded during roost emergence, 45.8% on hand-release, and 7.9% in free-flight (Table 1).

Rhinolophids

The three species we examined, *Rhinolophus ferrumequinum*, *R. hipposideros*, and *R. euryale*, all emitted typical FM/CF/FM echolocation calls, i.e. calls with a long, strictly constant-frequency component (CF) preceded and followed by a brief, frequency-modulated sweep (FM; Fig. 1). Echolocation calls from *R. ferrumequinum* showed lower values for all frequency parameters, which did not overlap those of *R. hipposideros* and *R. euryale* (Table 2). As there was no overlap of FMAXE between species, this variable may be used for species identification; therefore, rhinolophids were not included in the discriminant function analysis.

A Kruskal–Wallis test and Dunn’s non-parametric post-hoc test showed that of the three species, only *R. ferrumequinum* differed in time parameters, as it produced calls with significantly longer D ($H = 13.43$, d.f. = 2, $P < 0.005$) and IPI ($H = 20.13$, d.f. = 2, $P < 0.001$). Statistical comparisons were possible as most rhinolophid calls were recorded on roost emergence (Table 1) in similarly cluttered situations.

Genera *Myotis*, *Plecotus*, *Barbastella*

The species in these genera all produced FM calls and could be grouped accordingly (Figs 2 & 3).

*Myotis nattereri* and *M. emarginatus* calls were characterized by the highest mean SF (>105 kHz) of all *Myotis* species; most calls of the former differed from those of the latter in having a clearly lower EF, and consequently a larger bandwidth (Table 2, Fig. 2). The two cryptic species *M. myotis* and *M. blythii* emitted similar calls. Comparable sample sizes of both species were obtained (Table 1), and since all subjects were recorded under identical and well-controlled conditions (i.e. hand-released in open habitat, mostly from the same roost), it was possible to make a comparison of call parameters. *Myotis myotis* showed significantly lower values of FMAXE (ANOVA, $F_{1,89} = 5.15$, $P < 0.001$), EF (Mann–Whitney $W = 1364.5$, $P < 0.0001$), and a longer IPI ($W = 2247.0$, $P < 0.05$); no differences between species were found in SF (ANOVA, $F_{1,89} = 3.10$, NS), MF ($W = 2061.5$, NS), and D (ANOVA $F_{1,89} = 1.60$, NS).

*Myotis capaccinii* and *M. daubentonii* also produced similar calls. A comparison between 16 *M. capaccinii* and 41 *M. daubentonii* — all hand-released — showed significant differences in EF (medians were: *M. capaccinii* = 38 kHz, *M. daubentonii* = 32 kHz. Mann–Whitney $W = 763.0$, $P < 0.0001$; see also Fig. 2) and D (means were: *M. capaccinii* = $4.0 \pm 0.81$ ms, *M. daubentonii* = $2.9 \pm 0.97$ ms, ANOVA $F_{1,55} = 15.48$, $P < 0.001$). All

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Table 1. Species recorded, numbers of recording sites and number of bats recorded. The numbers of bats recorded in each situation are also shown

<table>
<thead>
<tr>
<th>Species</th>
<th>n sites</th>
<th>n bats</th>
<th>Hand-released</th>
<th>Leaving roost</th>
<th>Free-flight</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Rhinolophus ferrumequinum</em></td>
<td>6</td>
<td>63</td>
<td>13</td>
<td>50</td>
<td>--</td>
</tr>
<tr>
<td><em>Rhinolophus hipposideros</em></td>
<td>3</td>
<td>34</td>
<td>5</td>
<td>29</td>
<td>--</td>
</tr>
<tr>
<td><em>Rhinolophus euryale</em></td>
<td>3</td>
<td>45</td>
<td>8</td>
<td>37</td>
<td>--</td>
</tr>
<tr>
<td><em>Myotis daubentonii</em></td>
<td>6</td>
<td>55</td>
<td>41</td>
<td>14</td>
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</tr>
<tr>
<td><em>Myotis capaccinii</em></td>
<td>3</td>
<td>49</td>
<td>16</td>
<td>33</td>
<td>--</td>
</tr>
<tr>
<td><em>Myotis mystacinus</em></td>
<td>3</td>
<td>13</td>
<td>13</td>
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<tr>
<td><em>Myotis emarginatus</em></td>
<td>6</td>
<td>52</td>
<td>33</td>
<td>19</td>
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</tr>
<tr>
<td><em>Myotis nattereri</em></td>
<td>3</td>
<td>12</td>
<td>12</td>
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<tr>
<td><em>Myotis myotis</em></td>
<td>3</td>
<td>42</td>
<td>42</td>
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</tr>
<tr>
<td><em>Myotis blythii</em></td>
<td>2</td>
<td>49</td>
<td>49</td>
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</tr>
<tr>
<td><em>Nyctalus noctula</em></td>
<td>1</td>
<td>42</td>
<td>3</td>
<td>39</td>
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</tr>
<tr>
<td><em>Nyctalus leisleri</em></td>
<td>2</td>
<td>13</td>
<td>11</td>
<td>2</td>
<td>--</td>
</tr>
<tr>
<td><em>Eptesicus serotinus</em></td>
<td>3</td>
<td>15</td>
<td>13</td>
<td>12</td>
<td>--</td>
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<tr>
<td><em>Pipistrellus pipistrellus</em></td>
<td>9</td>
<td>61</td>
<td>9</td>
<td>40</td>
<td>12</td>
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<tr>
<td><em>Pipistrellus pygmaeus</em></td>
<td>8</td>
<td>27</td>
<td>5</td>
<td>--</td>
<td>22</td>
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<tr>
<td><em>Pipistrellus kuhlii</em></td>
<td>7</td>
<td>107</td>
<td>38</td>
<td>58</td>
<td>11</td>
</tr>
<tr>
<td><em>Hypsugo savii</em></td>
<td>7</td>
<td>37</td>
<td>12</td>
<td>25</td>
<td>--</td>
</tr>
<tr>
<td><em>Plecotus auritus</em></td>
<td>2</td>
<td>26</td>
<td>26</td>
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</tr>
<tr>
<td><em>Plecotus australis</em></td>
<td>2</td>
<td>55</td>
<td>55</td>
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<tr>
<td><em>Barbastella barbastellus</em></td>
<td>1</td>
<td>15</td>
<td>6</td>
<td>--</td>
<td>9</td>
</tr>
<tr>
<td><em>Miniopterus schreibersii</em></td>
<td>4</td>
<td>117</td>
<td>35</td>
<td>82</td>
<td>--</td>
</tr>
<tr>
<td><em>Tadarida teniotis</em></td>
<td>21</td>
<td>21</td>
<td>--</td>
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<td>21</td>
</tr>
</tbody>
</table>

Total 950 435 440 75
other parameters did not differ significantly between species (mean SF: *M. capaccini* = 80.1 ± 9.81 kHz, *M. daubentoni* = 77.6 ± 9.27 kHz, ANOVA *F* <sub>1,55</sub> = 0.77, NS; median FMAXE: *M. capaccini* = 47.7 kHz, *M. daubentoni* = 46.8 kHz, W = 550.0, NS; mean MF: *M. capaccini* = 53.5 ± 2.06 kHz, *M. daubentoni* = 53.3 ± 3.71 kHz, ANOVA *F* <sub>1,55</sub> = 0.02, NS; median IPI *M. capaccini* = 70.1 ms, *M. daubentoni* = 70.8 ms, W = 463.5, NS).

*Plecotus auritus* and *P. austriacus* both emitted multi-harmonic echolocation calls (Fig. 3). *Plecotus auritus* was recorded in a range of different conditions (from moderate clutter to fairly open habitat) and *P. austriacus* under two well-controlled conditions (high clutter and completely open space). Although the two species samples separated quite well in a multivariate space (see below), the variety of recording conditions adopted made it difficult to control for habitat effects and thus to explore the differences between species of each variable statistically.
Barbastella barbastellus emitted two differently structured echolocation calls: one (type 1; see Table 2 \((n = 10)\), Fig. 3) consisting of a narrow-band FM sweep, the other characterized by a peculiar convex frequency-time course (type 2; see Table 2 \((n = 10)\), Fig. 3). Bats either emitted type 1 calls only, or alternated the two call types.

Genera Pipistrellus, Hypsugo, Miniopterus, Eptesicus, Nyctalus, Tadarida

Calls from these genera (here defined FM/QCF) were characterized by two components: one shows a steep frequency modulation (FM part) and is followed by another characterized by a shallow frequency modulation (quasi-constant frequency part, QCF; Figs 4 & 5). The relative importance of each component varied both between and within species. For instance, the FM portion was more developed in Pipistrellus spp. and Miniopterus schreibersii calls than in those from the other species; in T. teniotis and in N. noctula type 2 calls (Table 2) this portion was often absent. Within species, habitat structure significantly influenced call structure: the FM component was more pronounced in clutter, and reduced or sometimes omitted in open habitats, where the importance of the QCF portion increased considerably. Our database included calls from clutter and open habitats for most species, so both FM and QCF components were represented in calls (Table 2, Figs 4 & 5). Of the frequency parameters considered, EF was the one that overlapped least between Mi. schreibersii, Hypsugo savii and the three pipistrelle species considered (Table 2). This variable may therefore help species identification as it is often diagnostic. Echolocation calls from H. savii were often character-
ized by a narrow bandwidth and a longer duration (Table 2, Fig. 4).

Calls from *Eptesicus serotinus* and *N. leisleri* seemed to be quite similar in spectral and temporal features (Table 2, Fig. 5). However, since most *N. leisleri* were hand-released and most *E. serotinus* were recorded on emergence (Table 1), differences between species were not analysed statistically. Call parameters of *N. noctula* only occasionally overlapped those of *E. serotinus* and *N. leisleri*. All *N. noctula* were recorded flying high above ground and regularly alternated two distinct types of calls (types 1 and 2; Table 2, Fig. 5). These were easily recognizable from spectrograms as type 1 showed a sensibly more pronounced frequency modulated portion than type 2. Statistical analysis supported this qualitative distinction: type 1 calls \((n = 17)\) showed significantly higher values of SF (Mann–Whitney test, \(W = 353.5, P < 0.0001\), EF \((F_{1,40} = 41.27, P < 0.0001)\), FMAXE \((F_{1,40} = 57.04, P < 0.0001)\), MF \((F_{1,40} = 42.74, P < 0.0001)\), shorter D \((W = 707.5, P < 0.0001)\) and IPI \((W = 672.0, P < 0.0001)\) than type 2 ones \((n = 25)\).

*Tadarida teniotis* constantly emitted clearly audible echolocation calls (it was the species which called at the lowest FMAXE). In hunting grounds, feeding buzzes produced on prey approach were also clearly audible. This species also showed the longest IPI (up to 1 s; Table 2).

### Discriminant function analysis

Eighteen species were considered for DFA. Quadratic discriminant analysis was applied to: (1) the whole species \((18)\) dataset; (2) calls lumped together according to genera \((eight \ groups)\); (3) the species groups respectively emitting FM \((10 \ species)\) and FM/QCF \((eight \ species)\) calls, as done by Vaughan *et al.* (1997).

#### All species

The best model included SF, EF, FMAXE, and D and produced an overall classification rate of 81.8%: 648 out of 792 calls were correctly classified (Table 3). Random data classification would be 5.6% correct. A MANOVA showed that the model was significant (Wilk’s \(\lambda = 0.00273, F_{68,3027} = 155.911, P < 0.001\)) and that 77.5% of the variation was explained by the first discriminant function. The first three discriminant functions explained 98.9% of the total variation. Classification rates ranged from 38% (for *Myotis mystacinus*) to 98% (for *P. pipistrellus*). Classification rates > 70% were reached for 12 out of 18 species.

Wilk’s \(\lambda\) values illustrated the following decreasing discrimination power for the six variables: EF > MF > FMAXE > D > SF > IPI (Table 4). The removal of MF increased the DFA performance probably because it

### Table 3. Discriminant function analysis model for all species. Model relied on four parameters (SF, EF, FMAXE, D): abbreviations as Table 2). Overall correct classification rate was 81.8% \((n = 792)\). M. myo., *Myotis myotis*; M. bly., *M. blythii*; M. cap., *M. capaccini*; M. ema., *M. emarginatus*; M. dau., *M. daubentonii*; M. nat., *M. nattereri*; M. myst., *M. mystacinus*; Pl. aus., *Plecotus austriacus*; Pl. aur., *P. auritus*; B. bar., *Barbastella barbastellus*; P. kuh., *Pipistrellus kuhlii*; P. pip., *P. pipistrellus*; P. pyg., *P. pygmaeus*; H. sav., *Hypsugo savii*; E. ser., *Eptesicus serotinus*; N. lei., *Nyctalus leisleri*; N. noc., *N. noctula*; Mi. sch., *Miniopterus schreibersii*

<table>
<thead>
<tr>
<th>True group</th>
<th>Classified as</th>
</tr>
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<tbody>
<tr>
<td>M. myo.</td>
<td>M. bly.</td>
</tr>
<tr>
<td>M. cap.</td>
<td>M. ema.</td>
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<tr>
<td>M. dau.</td>
<td>M. nat.</td>
</tr>
<tr>
<td>M. mys.</td>
<td>Pl. aus.</td>
</tr>
<tr>
<td>Pl. aur.</td>
<td>B. bar.</td>
</tr>
<tr>
<td>P. kuh.</td>
<td>P. pip.</td>
</tr>
<tr>
<td>P. pyg.</td>
<td>H. sav.</td>
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<tr>
<td>E. ser.</td>
<td>N. lei.</td>
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<td>N. noc.</td>
<td>Mi. sch.</td>
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<table>
<thead>
<tr>
<th>Total (n)</th>
<th>42</th>
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</thead>
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<td>(n) correct</td>
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<tr>
<td>% correct</td>
<td>64</td>
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minimized correlation between variables. MF was highly positively correlated with SF and FMAXE (Spearman’s rank coefficient \( r_s = +0.9 \); Table 5). The model also excluded IPI, which showed the lowest discrimination power.

**Genus discrimination**

The best model for genus identification relied on all six variables and reached an overall correct classification of 94.1%: 745 out of 792 calls were correctly classified (Table 6). Random data classification would be 12.5% correct. A MANOVA showed that the model was significant (Wilk’s \( \lambda = 0.02474, F_{42,3657} = 104.542, P < 0.001 \)) and that 56.7% of the variation was explained by the first discriminant function. The first three discriminant functions explained 99.8% of the total variation. The model could not be improved further by removing any of the six variables. Their discrimination power according to Wilk’s \( \lambda \) values (in decreasing order) was: EF > MF > FMAXE > SF > D > IPI (see Table 4). Classification rates ranged from 60% (for *Eptesicus*) to 99% (for *Myotis*).

**Species from genera Myotis, Plecotus, Barbastella**

The best model included SF, EF, FMAXE, and D and produced an overall classification rate of 71.3%: 266 out of 373 calls were correctly classified to species (Table 7). Random data classification would be 10% correct. A MANOVA showed that the model was significant (Wilk’s \( \lambda = 0.04527, F_{36,1350} = 48.181, P < 0.001 \)) and that 82.1% of the variation was explained by the first discriminant function. The first three discriminant functions explained 98.8% of the total variation. Classification rates ranged from 38% (for *M. mysta-
Acoustic identification of Italian bats

Table 7. Discriminant function analysis for species emitting FM calls (genera Myotis, Plecotus, Barbastella: names of species abbreviated as Table 3). Model relied on four parameters (SF, EF, FMAXE, D: abbreviations as Table 2) and provided an overall correct classification rate of 71.3% (n = 373)

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<td>43</td>
<td>12</td>
<td>3</td>
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<td>1</td>
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<td>46</td>
<td>0</td>
<td>2</td>
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<td>0</td>
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<td>85</td>
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Table 8. DFA analysis for species emitting FM/QCF calls (genera Pipistrellus, Hypsugo, Eptesicus, Nyctalus, Miniopterus: names of species abbreviated as Table 3). Model relied on four parameters (EF, MF, D, IPI: abbreviations as Table 2) and provided an overall correct classification rate of 95.7% (n = 419)

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<td>97</td>
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<td>77</td>
<td>93</td>
<td>98</td>
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cinus) to 88% (for M. capaccinii). Classification rates > 70% were obtained for six out of 10 species. About 20% of M. blythii calls were misclassified as M. myotis, and about 12% of M. myotis signals as M. blythii. About 22% of Pl. austriacus calls were attributed to the sibling species Pl. auritus. According to Wilk’s λ values, the discriminating power of the six variables measured in descending order is as follows: EF > SF > MF > FMAXE > D > IPI (Table 4). Again, the removal of MF may have increased the DFA performance because it reduced correlation between variables. MF showed a high positive correlation to SF (r_s = + 0.9; Table 5). The removal of IPI, a parameter making little contribution to discrimination, must have improved the model by simplifying it.

Species from genera Pipistrellus, Hypsugo, Miniopterus, Eptesicus, Nyctalus

The best model comprised EF, MF, D and IPI and produced an overall classification rate of 95.7%: 401 out of 419 calls were correctly classified (Table 8). Random data classification would be 12.5% correct. A MANOVA showed that the model was significant (Wilk’s λ = 0.00603, F_{28,142} = 164.399, P < 0.001) and that 95.1% of the variation was explained by the first discriminant function. The first three discriminant functions accounted for 99.9% of the total variation. Classification rates ranged from 77% (for N. leisleri) to 98% (for P. kuhlii, P. pipistrellus, Mi. schreibersii). Classification rates > 70% were obtained for all eight species. According to Wilk’s λ values, the discriminating power of the six variables measured in descending order is as follows: EF > FMAXE > MF > D > SF > IPI (see Table 4). Despite the high discriminating power of FMAXE, its presence in the model degraded the DFA performance. This probably happened because of its very strong correlation with EF and MF (r_s approximated 1; see Table 5). SF had little discrimination power, and was strongly correlated with EF and MF (r_s = + 0.7 and + 0.8 respectively; see Table 5).
DISCUSSION


Four species (the three rhinolophids and *T. teniotis*) from our study region could be identified with no ambiguity using FMAXE. For rhinolophids, this might not always be the case as populations from different geographic areas show large differences in call frequencies (Heller & Helversen, 1989). Heller & Helversen (1989) documented some frequency overlap between *R. hippocideros* and *R. euryale* from Greece. Similarly, Barataud (1996) reported that a 5% overlap may occur between call frequencies of these rhinolophids. Problems in identification may arise in areas where *R. mehelyi* also occurs, as its FMAXE may overlap that of *R. euryale* and *R. hippocideros* (Heller & Helversen, 1989; Russo et al., 2001). *Rhinolophus ferrumequinum* showed lower FMAXE, longer D and IPI than the other two rhinolophids: this was predictable since *R. ferrumequinum* is considerably larger than the other two species, and larger species tend to produce longer calls, spaced out over longer time intervals, at lower frequencies (e.g. Jones, 1999).

Among calls from *Myotis* spp., those of *M. nattereri* were often identifiable because of their broad bandwidth as also documented by other studies (e.g. Vaughan et al., 1997; Parsons & Jones, 2000; Siemers & Schnitzler, 2000). Such calls allow the species to detect prey very close to acoustic clutter-producing background (Siemers, 2000). Such calls allow the species to detect prey very close to acoustic clutter-producing background (Siemers, 2000). Echolocation signals from most other *Myotis* species, however, showed similar structures and large overlap in spectral and temporal parameters (e.g. Krusis & Neehus, 1996; Vaughan et al., 1997), probably, as Parsons & Jones (2000) pointed out, because of the close phylogenetic relatedness existing among such species. Small but significant differences were found between *M. myotis* and *M. blythii*. Such differences might be related to the ecological segregation and limited size differences occurring between these cryptic species (Arlettaz, 1995; Arlettaz et al., 1997). Since *M. myotis* is on average slightly larger than *M. blythii* (e.g. Schober & Grimmberger, 1997), one might expect it to call at lower frequencies (Heller & Helversen, 1989; Barclay & Brigham, 1991; Jones, 1999), as our study showed. A generally clear difference in EF was also observed between *M. daubentonii* and *M. capaccinii*, as reported – but neither quantified nor explored statistically – by Barataud (1996).

In general, species that emit FM/QCF calls were easier to tell apart than those producing FM calls, as also verified by other studies (Zingg, 1990; Vaughan et al., 1997; Parsons & Jones, 2000). Often this discrimination could even be accomplished by measuring only EF or FMAXE, because of the generally limited range overlap of these variables. This was not always the case, however: in particular, echolocation calls of *N. leisleri* and *E. serotinus* often showed similar values of the variables studied. All calls of *N. leisleri* had to be obtained from hand-released bats: hence, they were often more frequency modulated than calls emitted by the species in open space, and this might have partly increased the degree of overlap with *E. serotinus* calls. The similarity of calls of the two species was also stressed by Vaughan et al. (1997), whose discriminant function for FM/QCF calls often misclassified their signals. In open habitats, *N. leisleri* often alternates between two call structures in the same way as *N. noctula*, while *E. serotinus* does not (Waters, Rydell et al., 1995; Russ, 1999a,b; Waters, Jones & Furlong, 1999).

Because one of the aims of this work was to devise an identification tool for bats from southern Italy, where *P. nathusii* is either rare or absent, calls from this species were not included in the DFA. In fact, during extensive acoustic survey work (D. Russo & G. Jones, pers. obs.) mainly carried out in Campania (southern Italy) in 1998–2000, the distinctive social calls emitted by *P. nathusii* (Barlow & Jones, 1996) were never recorded. The addition of *P. nathusii* to the analysis would probably decrease the discrimination rate for FM/QCF species, particularly because call parameters from this species largely overlap those of *P. kuhlii* (Zingg, 1990).

In this study, DFA provided a high classification rate for most species, despite the large number of species included. Classification success was similar to or higher than that obtained by previous studies where discriminant analysis was applied to a smaller number of European bat species (Zingg, 1990; Vaughan et al., 1997; Parsons & Jones, 2000). Had it been possible to record more calls for some of the species considered, the overall correct identification rate would perhaps have been higher. Models dealing with groups of species performed better than the model covering all species. Their drawback is that they involve a degree of subjectivity in attributing an unknown call to either the FM or FM/QCF group by visual inspection of the spectrogram shape. In our experience, the difference between such groups is normally clear and such a preliminary classification straightforward. Because of this first subjective examination, however, this procedure cannot be used to devise a fully automatic identification system – an attractive goal for the future (Jones, Vaughan & Parsons, 2000).

Our models classifying to species level included fewer parameters than those considered in previous studies (Zingg, 1990; Vaughan et al., 1997; Parsons & Jones, 2000). Highest classification rates were achieved by models that best balanced the inclusion of variables with a high discriminating power with the removal of highly correlated ones. Strong correlations are common
between echolocation call spectral features (S. Parsons, pers. comm.). As verified, the inclusion of a variable that highly correlates with others in a DFA model may degrade classification performance as the parameter will add noise rather than increase discrimination power, already provided by its covariate. As in other studies (e.g. Vaughan et al., 1997; Parsons & Jones, 2000), the species that emitted FM calls were more frequently misclassified than those producing FM/QCF calls, and the lowest classification rates occurred for *N. leisleri* and *M. mystacinus*. Low classification rates for British *M. mystacinus* were obtained with both DFA and neural networks (Vaughan et al., 1997; Parsons & Jones, 2000).

There was a high degree of discrimination achieved in species identification given the large number of species involved. Moreover, the inclusion of calls recorded in cluttered situations makes our models conservative because call types from different species flying in clutter, especially FM/QCF bats, tend to converge in design making identification more difficult (Jones, Vaughan et al., 2000). Even higher levels of discrimination may be possible for bats foraging in open habitats. The application of DFA to separate call databases from cluttered and open situations may also be considered to reduce the influence of confounding variables and therefore enhance the resolution power for some species.

The per cent identification rate obtained for each species in DFA (and in neural network applications, e.g. Parsons & Jones, 2000) offers an objective method of determining the probability that a species has been identified correctly. Researchers may find it useful to fix the ‘quality threshold’ in practical applications, such as distributional or habitat use work. Especially when investigating bat distribution (i.e. when even a single record may be important), a conservative approach (i.e. rejecting species identification when classification rates score < 80–90%) may ensure a minimal risk of mistaken species recognition. The researcher may consider the option of lumping in to groups (e.g. *Myotis* sp.) species whose discrimination falls below the set threshold.

Devising an identification system based on either DFA or other methods (Parsons & Jones, 2000; Obrist et al., in press) for a certain geographical area requires considerable effort. Many researchers will find this both time- and resource-consuming, and will probably continue to rely on less sophisticated methods of acoustic identification. Such research should adopt extremely conservative criteria, given the well-known high variability of echolocation calls that our study has also confirmed. Identification should be limited to a restricted number of species, minimizing the risk of misclassification. Identification criteria should always rely on accurate measurements of diagnostic features, defined from a thorough knowledge of the geographical and habitat variability of echolocation calls. Moreover, such criteria should always be clearly stated, see e.g. McAney & Fairley (1988) for *R. hipposideros*, Russo & Jones (1999) for *P. kuhlii*, and Waters, Jones et al. (1999) for *N. leisleri*. The use of social calls should be used as an aid to identification whenever these are proved to be diagnostic, as in European *Pipistrellus* species (Barlow & Jones, 1996, 1997a,b; Russ, 1999a; Russo & Jones, 1999, 2000; Jones, Vaughan et al., 2000).

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