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Bioacoustic differentiation in Painted frogs (*Discoglossus*)

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Abstract. Advertisement calls of the *Discoglossus*-species *D. pictus, D. sardus, D. galganoi*, and *D. montalentii* were studied. In comparison with all other species, the advertisement call of *D. montalentii* is completely different. The frequency spectrum displays a harmonic structure and resembles a *Bombina*-call, while advertisement calls of the other *Discoglossus* species are mostly unharmonious. *D. pictus auritus* and *D. pictus pictus* show a great similarity of call patterns. *D. galganoi* is characterized by the longest call duration. The call of a red-backed *Discoglossus* with the vague locality "Sardinia", here named "*D. spec.*", differed substantially from the calls of *D. sardus* and any other studied specimen. The complex situation regarding the distribution of different Tyrrhenian *Discoglossus* phenotypes is discussed in this context.

Introduction

Protein electrophoresis and immunological techniques such as micro-complement fixation have been used increasingly by taxonomists. In the past seven years, three new species of the Mediterranean genus *Discoglossus* have been described, mainly by biochemical differences: *D. montalentii* from Corsica (Lanza et al., 1984), *D. galganoi* from Spain (Capula et al., 1985) and *D. jeanneae* from southern Spain (Busack, 1986). Lanza et al. (1986) recognized eight valid *Discoglossus* taxa:

D. pictus pictus: Sicily, Malta, and Gozo Islands.
D. pictus auritus: Southern France/NE-Spain (probably introduced), Algeria, Tunisia.
D. pictus scovazzi: Morocco.
D. sardus: Argentario (Tuscany), Islands of Corsica, Sardinia, Monte Cristo, Giglio, and Hyères.
D. galganoi galganoi: West and Central Spain, Portugal.
D. galganoi jeanneae: Spain south of the Guadalquivir river.
D. nigriventer: Northern Israel (probably extinct).
D. montalentii: Corsica.

Without locality, a reliable determination of these taxa only by morphological characters is nearly impossible. Advertisement calls, also an useful parameter for taxonomy, have only been described for *D. pictus auritus* from southern France and *D. sardus* from Hyères Islands (Weber and Schneider, 1971; Weber, 1974).

In addition to the completely divergent mechanism in pipids, three basic types of sound production occur in anurans, each one realized in one discoglossid genus. The call of *Bombina* is generated by inspiration (Zweifel, 1959; Lörcher, 1969) whereas *Alytes* produces its call by the expiratory airstream (Heinzmann, 1970). *Discoglossus* can use both inspiration and expiration for sound production (Weber, 1974). The mechanism of sound production in *Discoglossus*, therefore, is intermediate between *Bombina* and *Alytes*. Nothing is known about calls of the fourth discoglossid genus *Barbourula*.

Material and methods

Advertisement calls of the following males were investigated (body length and recording temperature are also indicated):

2 *D. pictus auritus* (Southern France), ca. 4.5 cm, 18°C and 20°C
2 *D. pictus pictus* (Palermo, Sicily), 5.1 and 5.5 cm, 22°C
2 *D. galganoi* (La Coruña, NW-Spain), 5.8 and 4.5 cm, 19 - 20°C
3 *D. sardus* (Mezzana, Corsica), ca. 5.5 cm, 17°C and 19°C
1 *D. sardus* (Sassari, Sardinia), 6.4 cm, 21°C
1 *D. spec.* (Sardinia?), 6.2 cm, 20°C
1 *D. montalentii* (Vizzavona, Corsica), 4.8 cm, 21°C

The male of *D. spec.* was supplied with the uncertain locality "Sardinia". Considering its divergent calls, we preferred not to use the name "*D. sardus*".

The calls were recorded in captivity. Most specimens called spontanously. *D. sardus* (Sardinia), *D. pictus pictus* and *D. montalentii* were stimulated by injection of gonadotropic hormones.

During the recordings we recognized that a number of factors have to be considered before giving a reliable interpretation of the calls. Weber (1974) has already mentioned the dependence of call duration on water temperature and size of the calling male. We also found a strong influence of the sexual state (estimated by the expression of nuptial pads) on the call parameters analyzed.

Our observations indicate a very complex mating behaviour in *Discoglossus*. The change of behaviour and calling was especially remarkable when other specimens were added to the calling male. In this situation calls are influenced by so many factors that a reliable comparison was not possible. For this reason we analyzed only highly motivated males which were kept alone. To give a statement on the biological importance of the call-types it would be necessary to correlate them with the displayed behavioral repertoire (e.g. water surface wave production).

Calls were digitized and analyzed on an IBM-compatible AT with the programs DSCOPE and SOUND ANALYZER (developed by Prof. W. Walkowiak). Definitions of call parameters were used according to Weber (1974). He distinguished mating calls of males, excitement calls, distress calls and warding-off calls of males and females and release calls of females. In this paper we considered only the mating (= advertisement) calls.

Lörcher (1969) recognized for calls of *Bombina bombina* and *B. variegata* that even-numbered harmonics are more intense than odd-numbered harmonics. The same was observed for *Bombina orientalis* (Van den Elzen, 1979) and *Alytes o. obstetricans* (Heinzmann, 1970), while the sonagram shown by Schneider (1966) for *A. obstetricans* shows different harmonic patterns.

The number of registered harmonics and their intensities strongly depend on recording distance and recording angle (Heinzmann, 1970). Walkowiak (1980) could not confirm the presence of harmonics in sonagrams of *B. bombina* mating calls (dynamic of sonagraph 15 dB). To allow better comparison, we show sonagrams of *A. obstetricans* and *B. variegata* calls, analyzed under the same conditions as the *Discoglossus*-calls (figs. 1g, 1h).

Results

The calls of Discoglossus

Except for *D. montalentii*, most *Discoglossus* advertisement calls can be divided into two pulse groups. The first pulse group is generated by expiration, the second by inspiration (Weber, 1974). Calls can be arranged in series. Single calls of a series may (*D. galganoi, D. spec.*) or may not (*D. pictus, D. sardus*) be separated by silent intervals. Short intervals can also occur between inspiratory and expiratory pulse groups (*D. spec., D. galganoi*).

Sonagrams of the different species are shown in fig. 1 and oscillograms in fig. 2. Each species showed typical envelopes on the oscillogram (fig. 3). On the other hand, there was a big variability of these envelopes within one species. Comparison of call duration is presented in fig. 4. This parameter and the differences of intensity and duration of both pulse groups (fig. 5) are useful for determination of the different species.

Species differentiation

- *D. pictus*: The investigated subspecies of *D. pictus* did not show differences in their mean duration of calls (*D. p. pictus*: 250 ms, *D. p. auritus*: 256 ms). Most calls were arranged in series without intervals between calls or pulse groups. Mean duration of expiratory pulse group (*D. p. pictus*: 113 ms, *D. p. auritus* 126 ms) and inspiratory pulse group (*D. p. pictus*: 106 ms, *D. p. auritus*: 125 ms) were also similar. A 1:1 relation of maximal amplitudes of inspiratory and expiratory pulse groups was found in most calls.

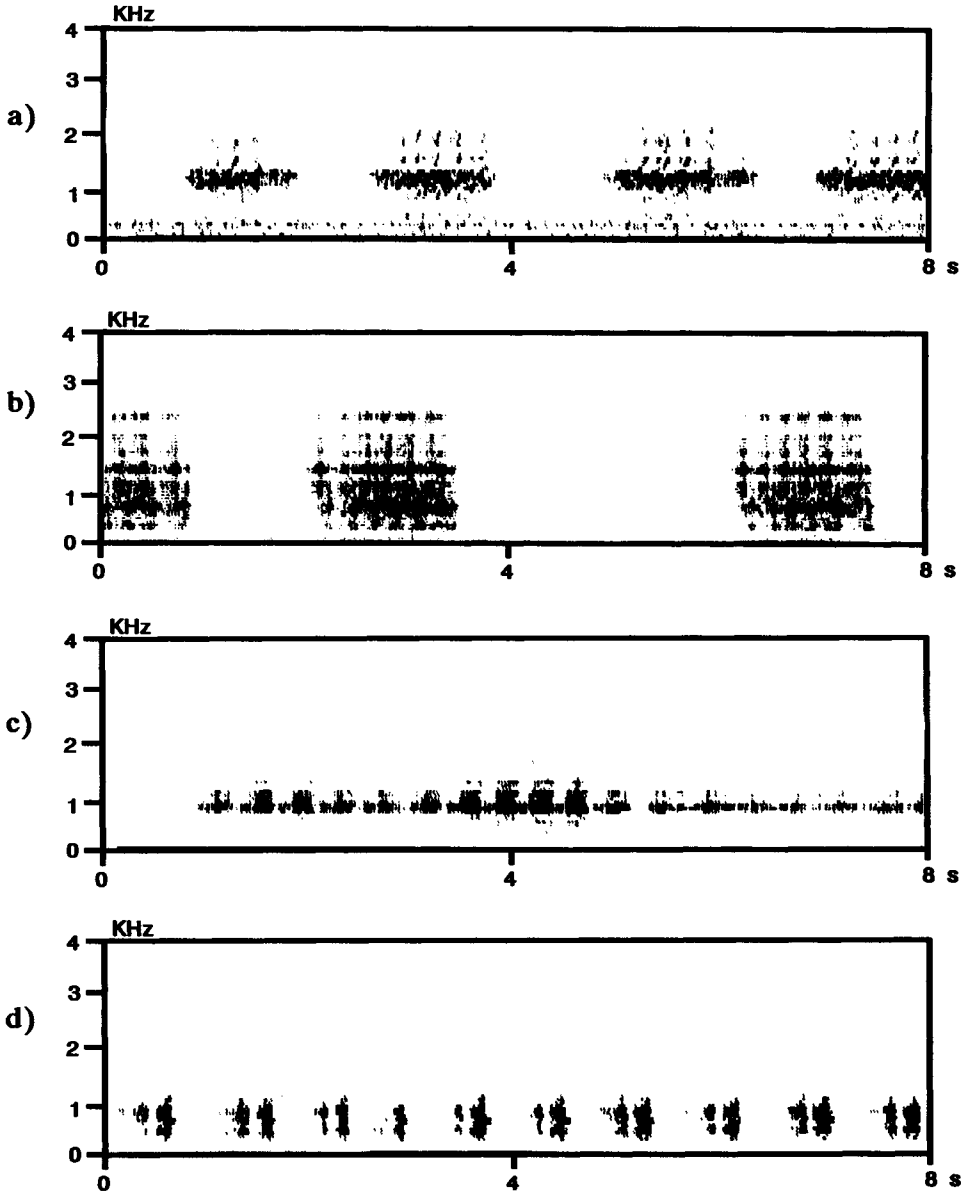
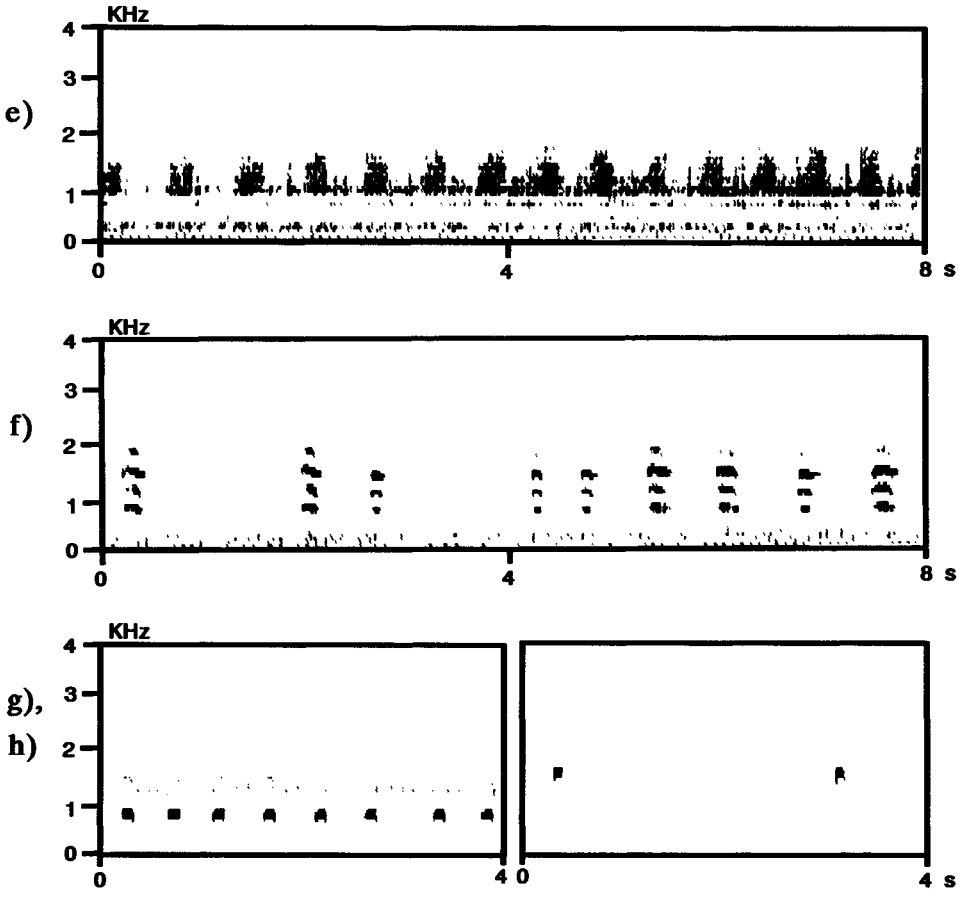


Figure 1. Sonagrams of different discoglossid advertisement calls. a) *Discoglossus pictus auritus*, b) *D. p. pictus*, c) *D. sardus* (Sardinia), d) *D. spec.*, e) *D. galganoi*, f) *D. montalentii*, g) *Bombina variegata* h) *Alytes obstetricans*. Sampling rate 8 KHz.



– *D. sardus*: For this species we found a longer call duration than for *D. pictus*. The expiratory pulse group was normally longer and more intense than the inspiratory pulse group. Specimens from Corsica and Sardinia showed similar call duration. However, analyzed calls differed in the relation of the pulse groups. Expiratory pulse groups of the Sardinian male were nearly always longer and more intense than the inspiratory pulse groups, while in the Corsican males also other accentuations were discovered.

In many calls of the Sardinian specimen the different pulse groups could not be distinguished in the oscillogram (fig. 2c), although a distinction is possible for the human ear. On the contrary, such hardly distinguishable calls of the Corsican specimens could mostly be separated on the oscillogram; just these calls showed less intense and shorter exspiratory pulse groups. Since the hardly distinguishable calls of the Sardinian male could not be analyzed, the comparison with the other taxa is limited.

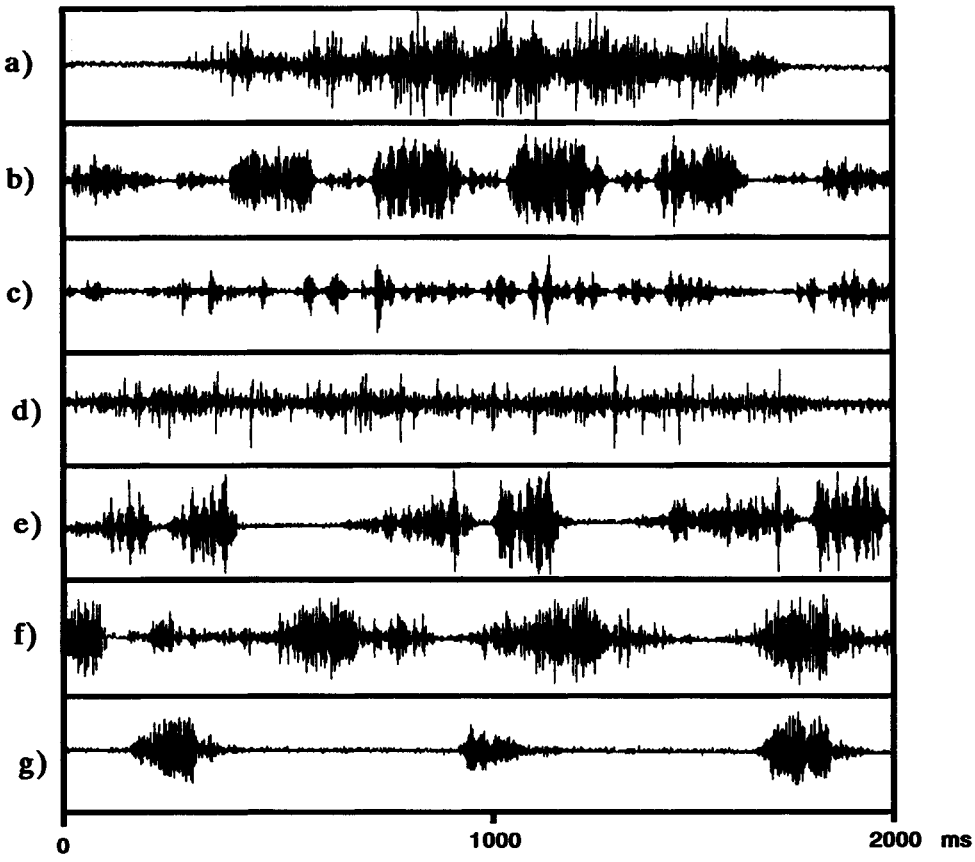


Figure 2. Oscillograms of *Discoglossus* advertisement calls. a) *D. p. pictus*, b) *D. sardus* (Sardinia), c) low emphasized call of *D. sardus* (Sardinia), d) low emphasized call of *D. sardus* (Corsica), e) *D. spec.*, f) *D. galganoi*, g) *D. montalentii*. Sampling rate 2 KHz.

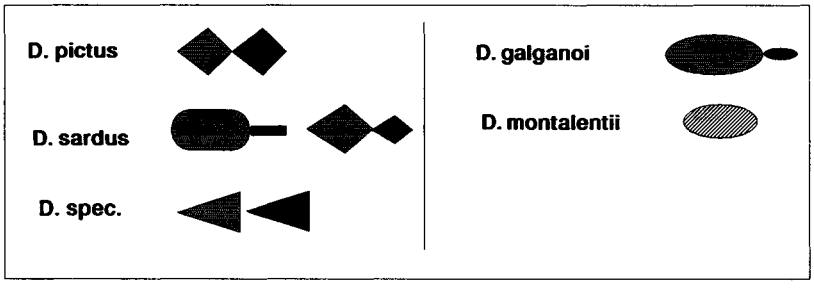


Figure 3. Typical envelopes of *Discoglossus* advertisement calls. Grey: expiratory pulse group. Black: inspiratory pulse group. Shaded: uncertain (in *D. montalentii*).

- *D. spec.*: Call duration of *D. spec.* was similar to *D. sardus*. On the other hand, the relation of pulse group intensities was in between those of *D. pictus* and *D. sardus*. Both pulse groups often had the same intensity. The main characteristics were the distinct intervals between pulse groups (average: 40 ms) and calls (50-1000 ms). These intervals were present in every call.
- *D. galganoi*: Call duration of *D. galganoi* was the longest of all forms investigated (up to 1000 ms). Although *D. galganoi* never showed low accentuated calls like *D. sardus*, it can sometimes be mistaken with this species. However, the human ear can distinguish both forms.
- *D. montalentii*: In comparison with other *Discoglossus* species, calls of *D. montalentii* were completely different. A distinction between expiratory and inspiratory pulse groups could not be made. We did not find out which of the two mechanisms is used for sound production. Call duration varied from 120 to 280 ms. In contrast to the other *Discoglossus* species—but in accordance with *Bombina* and *Alytes*—the calls were not noisy but display harmonic structure. The fundamental frequency was around 900 Hz, with 2-3 distinct harmonics (fig. 1f). The second harmonic was most emphasized.

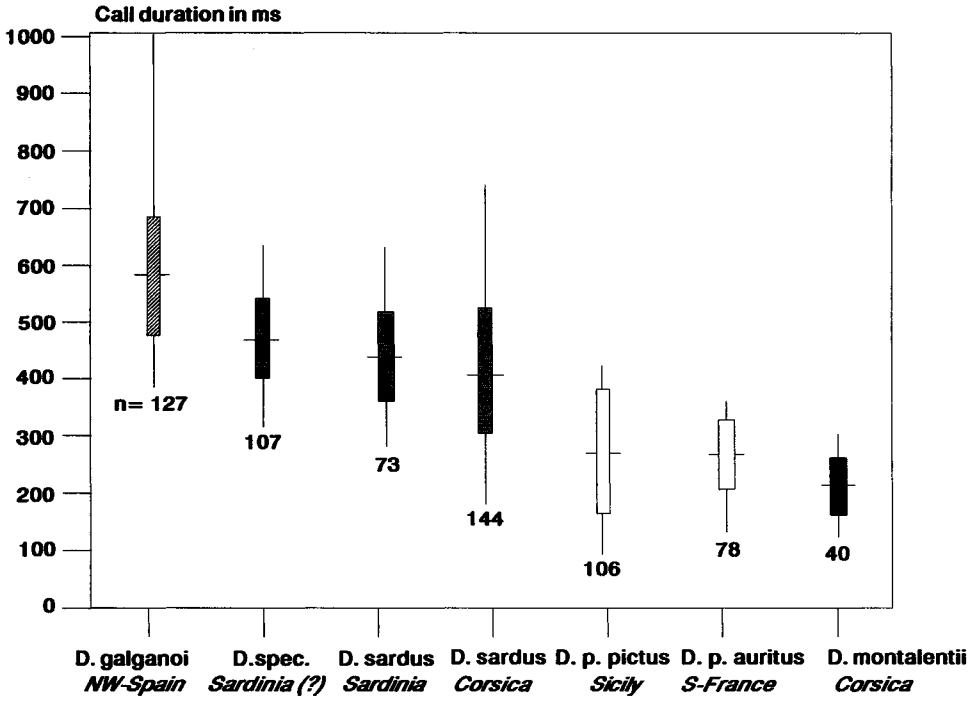


Figure 4. Duration of *Discoglossus* advertisement calls (Mean, Maximum, Minimum, Standard deviation).

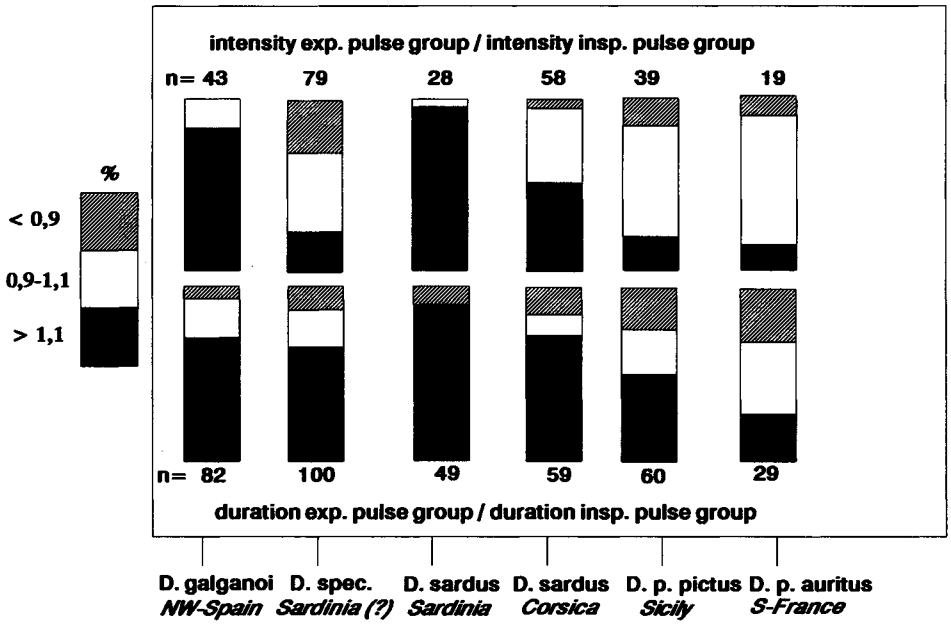


Figure 5. Relations of intensity and duration of expiratory and inspiratory pulse groups in *Discoglossus* advertisement calls. Above: Quotient of intensities of expiratory and inspiratory pulse group. Below: Quotient of durations of expiratory and inspiratory pulse groups. Shaded: Quotient <0.9. White: Quotient between 0.9 and 1.1. Black: Quotient >1.1.

Table 1. Significance values for the parameters shown in figs. 4 and 5. 1 = relation of pulse group intensities (χ^2 test). 2 = relation of pulse group durations (χ^2 test). 3 = call duration (*t*-test).

		D. pictus *Sicily*	D. pictus *S-France*	D. sardus *Sardinia*	D. sardus *Corsica*	D. spec. *Sardinia (?)*	D. galganoi *NW-Spain*
D. pictus	1						
Sicily	2	—					
	3						
D. pictus	1	<0.05					
S-France	2	>0.05	—				
	3	>0.05					
D. sardus	1	—	—				
Sardinia	2	—	—	—			
	3	<0.001	<0.001				
D. sardus	1	<0.05	<0.001	—			
Corsica	2	<0.001	<0.001	—	—		
	3	<0.001	<0.001	>0.05			
D. spec.	1	<0.001	<0.001	—	<0.001		
Sardinia (?)	2	<0.05	<0.01	—	<0.001	—	
	3	<0.001	<0.001	<0.001	<0.001		
D. galganoi	1	<0.001	<0.001	—	<0.01	<0.05	
NW-Spain	2	<0.001	<0.001	—	<0.001	<0.001	—
	3	<0.001	<0.001	<0.001	<0.001	<0.001	

Discussion

Systematic studies of various genera of frogs have shown that species sharing morphological and/or biochemical attributes also have structurally similar advertisement calls (Duellman and Trueb, 1986).

Our results are substantially in agreement with the taxonomy suggested by Lanza et al. (1986):

- *D. pictus, D. sardus, D. galganoi* and *D. montalentii* are recognized as distinct species. They can be distinguished by their calls. The same call differences which we found between *D. pictus* and *D. sardus* have also been noted by Weber and Schneider (1971) and Weber (1974) in *D. pictus* specimens from southern France and *D. sardus* specimens from Hyères Islands and considered as species-specific.
- *Discoglossus* from Sicily and southern France were considered as two subspecies (*D. p. pictus* and *D. p. auritus*). The analyzed call parameters of both forms coincide.
- Specimens from Sardinia and the lowland of Corsica were regarded as *D. sardus*. We found many similarities between their calls.
- *D. montalentii* is genetically the most divergent species. Morphological and osteological data confirm this view (Clarke and Lanza, in press). Its calls differ completely from all other *Discoglossus* species.

The only locality where two *Discoglossus* species occur sympatrically is Corsica. In sympatric species breeding at the same time and the same place, acoustic partitioning may exist (Duellman and Trueb, 1986). One possible function of acoustic partitioning in closely related species is the prevention of interbreeding; the sympatric occurence of *D. montalentii* and *D. sardus* is therefore another probable reason for their strong call-differences.

The status of *D. spec.* is unknown. The situation is complicated additionally by the vague locality. Our two specimens show a uniformly red-coloured back (not spotted). Colour patterns in *Discoglossus* are inherted in a Mendelian way (Lantz, 1947). Red backs are known for *D. pictus* and *D. sardus*. Capula (pers. com., 1990) did not find biochemical differences between "red" and "brown" *D. sardus*. Nevertheless, the problem could be more complex. In the Corsican lowlands, red phenotypes seem to be restricted to some specific localities; at other places, no red specimens have been found (Wolterstorff, 1899; Rogner, 1985). These lowland forms are not *D. montalentii*; until now, this species has only been known from higher elevations. Red *D. montalentii* have not been discovered to date (Capula, pers. com. 1990). On the other hand, Linnenbach (1984) found red *montalentii*-like specimens at high elevations, which differed morphologically from lowland frogs. Our *D. sardus* from Corsica and Sardinia came from populations in which no red phenotypes have been found. Therefore, calls of red *D. sardus* are unknown. Only further investigations can clarify the relation between the different phenotypes of the Tyrrhenian *Discoglossus*.

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