

Further investigations on *Discoglossus* bioacoustics: Relationships between *D. galganoi galganoi*, *D. g. jeanneae* and *D. pictus scovazzi*

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Abstract. Advertisement calls of *Discoglossus galganoi jeanneae* from two localities in southern Spain and *D. pictus scovazzi* from Ceuta were analyzed. Call duration as well as ratio of pulse group intensities and durations clearly group *D. pictus scovazzi* with the other subspecies of *D. pictus* (*pictus* and *auritus*). The calls of the taxon *jeanneae* show some differences to calls of populations of *D. g. galganoi* from northern Spain, but are more similar to calls of *D. g. galganoi* than to those of *D. pictus scovazzi*. These data support the status of *jeanneae* as subspecies of *galganoi*.

Introduction

The taxonomy of the genus *Discoglossus* and the relationships and differentiation among the species and subspecies of this genus have been subject of several studies during the last decades (Lanza et al., 1984; Capula et al., 1985; Busack, 1986; Clarke and Lanza, 1990; Glaw and Vences, 1991; Capula and Corti, 1993; Fritz et al., 1994). Presently eight valid taxa are recognized (Lanza et al., 1986).

Species-specific differences between the advertisement calls of *Discoglossus pictus* and *D. sardus* were first revealed by Weber and Schneider (1971) and Weber (1974). In a previous publication (Glaw and Vences, 1991) we confirmed these differences and described the advertisement calls of *D. montalentii* and *D. galganoi*, which also showed several species-specific characteristics. We found no significant differences between the advertisement calls of *D. pictus auritus* from France and *D. pictus pictus* from Sicily.

Except for the possibly extinct *D. nigriventer* from Israel, the calls of only two *Discoglossus* taxa remained unknown: *Discoglossus pictus scovazzi* from Morocco and *Discoglossus galganoi jeanneae*. The relationships of these taxa are of special interest since Busack (1986) described *D. jeanneae* as a full species, and regarded *D. jeanneae* as

more closely related to the *Discoglossus* from Morocco (*D. pictus scovazzi*) than to those from the Iberian peninsula north of the Guadalquivir river (*D. galganoi*). This assumption was the base for Busack's (1986) biogeographic scenario regarding the dessication of the Guadalquivir river basin and the formation of the Strait of Gibraltar.

However, Lanza et al. (1986) did not find high genetic distances between *jeanneae* and *Discoglossus galganoi* from northern Spain, and therefore regarded *jeanneae* as subspecies of *D. galganoi*.

In this study we describe the advertisement calls of *D. galganoi jeanneae* and *D. pictus scovazzi*, and discuss the relationships of these taxa with *Discoglossus galganoi* and *D. pictus* using the bioacoustic data.

Material and methods

For this study advertisement calls of the following *Discoglossus* males were analyzed:

- 1 *Discoglossus galganoi galganoi* from Foncuberta (Orense, Spain)
- 1 *Discoglossus galganoi jeanneae* from near Facinas (Cadiz, Spain), the type locality of *jeanneae*
- 1 *Discoglossus galganoi jeanneae* from a locality south-west of Ronda, between Malaga and Cadiz (Spain)
- 3 *Discoglossus pictus scovazzi* from Ceuta (Spanish town in northern Africa, bordering Morocco).

Calls were recorded in captivity. Most specimens called spontaneously, only the *jeanneae* specimen from Facinas was stimulated by injection of gonadotropic hormones. Recording temperatures are given in table 1.

Calls were digitized and analyzed by the first author on an IBM-compatible Computer with the programs DSCOPE and SOUND ANALYZER (developed by Prof. W. Walkowiak, Köln). Sonagrams and oscillograms were made with a MEDAV Spektro 3.2 sound analyzing system.

Discoglossus calls generally consist of an expiratory pulse group followed by an inspiratory pulse group (Weber, 1974). In the following text call duration is used for the period from the beginning of the first pulse group to the end of the second pulse group. The ratio of intensities of expiratory and inspiratory pulse groups (intensity ratio exp. : insp.) is here defined as quotient of the heights (measured in mm) of the maximum peaks of the respective pulse groups on the oscillogram. For additional discussion of recording conditions and sonagram interpretation see Glaw and Vences (1991).

Results

The call durations found in the investigated specimens are given in table 1.

Discoglossus pictus scovazzi: The calls of all three specimens were very similar to the human ear. The impression was that of a typical *pictus* call as described in Glaw

Table 1. Duration of advertisement calls in *Discoglossus*. Call duration is given as milliseconds (ms) and refers to the period between beginning of expiratory pulse group and end of inspiratory pulse group. It is given as mean value \pm standard deviation (maximum and minimum values in brackets). n is the number of analyzed calls. Differences are significant (t -test; $P < 0.001$) between all populations except for the comparison *D. pictus pictus*/*D. pictus auritus*.

Population	n	Call duration	Recording temperature
<i>D. g. galganoi</i> , La Coruña [1]	127	587 \pm 107 (288-1004)	19-20°C
<i>D. g. galganoi</i> , Orense	54	506 \pm 106 (324-788)	?
<i>D. g. jeanneae</i> , Facinas	117	394 \pm 77 (222-560)	22°C and 18°C
<i>D. g. jeanneae</i> , Ronda	99	450 \pm 97 (196-736)	21°C
<i>D. pictus scovazzi</i> , Ceuta	101	207 \pm 53 (94-352)	21-22°C
<i>D. pictus auritus</i> , S-France [1]	78	257 \pm 43 (145-393)	18°C and 20°C
<i>D. pictus pictus</i> , Sicily [1]	105	245 \pm 83 (98-441)	22°C

[1] data from Glaw and Vences (1991), shown there in fig. 4

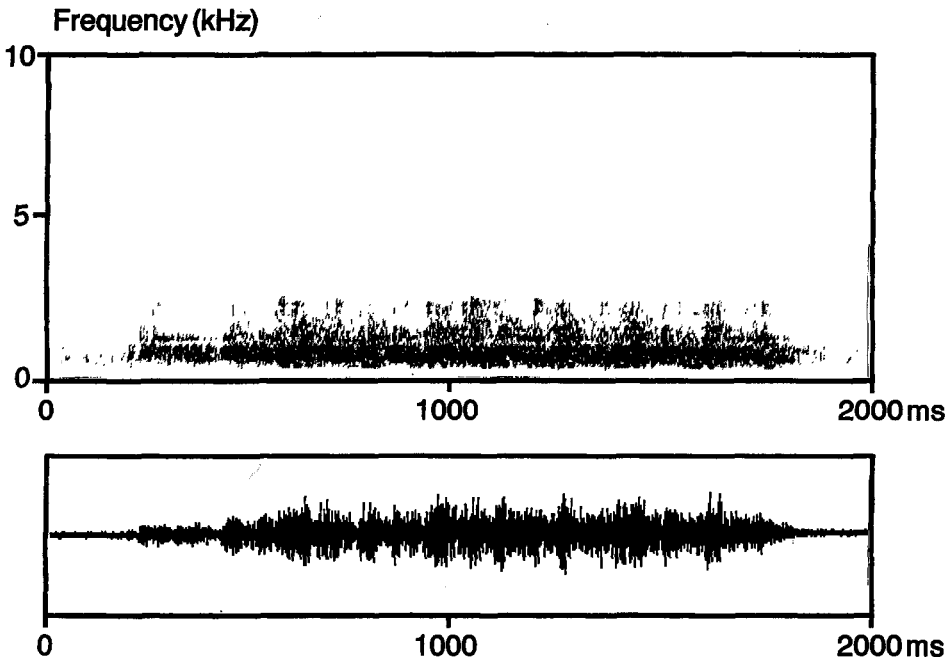


Figure 1. Sonogram and oscillogram of a call series of *Discoglossus pictus scovazzi* from Ceuta. The series consists of 10 calls; no intervals are spaced between calls, so that the exact limits between calls and pulse groups are difficult to recognize.

and Vences (1991), and this was confirmed by the analysis: Calls were unharmonious, call duration was low (even somewhat lower than in *D. pictus pictus* and *D. pictus auritus*; see table 1). Mostly several calls were arranged in a series, no silent intervals could be found between the calls of one call series. Figure 1 shows a sonogram and

an oscillogram of a typical call series with 10 calls. Inspiratory and expiratory pulse groups had less often the same duration than in other *pictus* subspecies; however there was no recognizable trend which would have resulted in one pulse group being regularly shorter than the other (ratio of pulse group durations exp. : insp. was between 0.7 and 1.3 in 46% of the cases, smaller than 0.7 in 26%, larger than 1.3 in 28%; $n = 90$). Both pulse groups had mostly the same intensity, as is typical for *D. pictus* (intensity ratio exp. : insp. was smaller than 0.9 in 16% of the cases, between 0.9 and 1.1 in 66%, larger than 1.1 in 18% of the calls; $n = 70$).

Discoglossus galganoi galganoi from Orense: The calls of this male were very similar to the previously studied population of La Coruña (the distance between both populations is about 125 km), but mean call duration was somewhat lower (see table 1). Only in a few cases ($n = 3$) was it possible to distinguish between expiratory and inspiratory pulse group on the oscillograms, but in these cases the second (inspiratory) pulse group was always much shorter (quotient about 3.5/1). The calls were unharmonious. Distinct silent intervals of about 100 ms occurred between the calls of a series.

Discoglossus galganoi jeanneae from near Ronda: The distribution area of *jeanneae* was defined as Spain, south of the Guadalquivir river by Busack (1986). We therefore consider the specimen from Ronda as *jeanneae*. The collecting site is located about

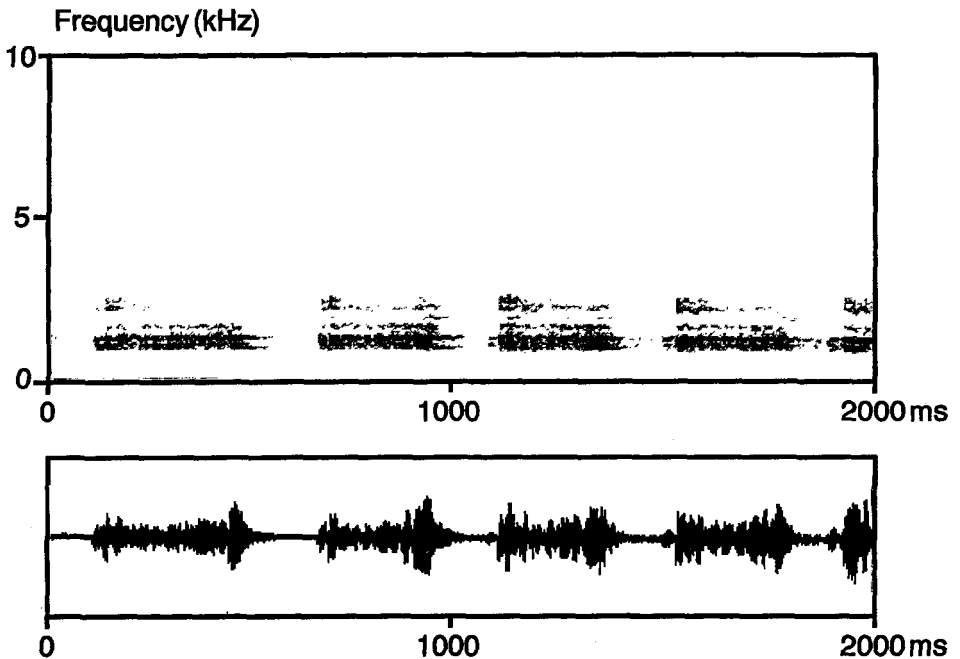


Figure 2. Sonagram and oscillogram of parts of a call series of *Discoglossus galganoi jeanneae* from near Ronda (Malaga). Distinct intervals are spaced between calls, which consist of only one (expiratory) pulse group.

70 km from the type locality of *jeanneae*. The calls of the recorded male were similar to those described from northern Spain. They were unharmonious, and when arranged in series a distinct silent interval (30-100 ms) was present between the calls (fig. 2). When two pulse groups were recognizable on the oscillogram ($n = 9$), the first (expiratory) pulse group was always much longer than the second (inspiratory) pulse group (ratio 2:1 to 6:1) as it is typical for *D. galganoi*. However the calls were shorter than those from northern Spain (see table 1).

Discoglossus galganoi jeanneae from near Facinas (type locality of *jeanneae*): The calls from one male of this population were also similar for the human ear to those from northern Spain. However, a detailed analysis (sonagram and oscillogram shown in fig. 3) revealed several differences. Calls were shorter (even shorter than those from the Ronda specimen; table 1), they were often arranged in series without distinct silent intervals between them (but these intervals occurred in some series), and the second (inspiratory) pulse group was often of similar duration or even longer than the first (expiratory) pulse group (ratio of pulse group durations exp.:insp was smaller than 0.7 in 32%, between 0.7 and 1.3 in 31%, and larger than 1.3 in 37% of the calls; $n = 40$). However, the intensity of the inspiratory pulse group was mostly lower than that of the expiratory pulse group,

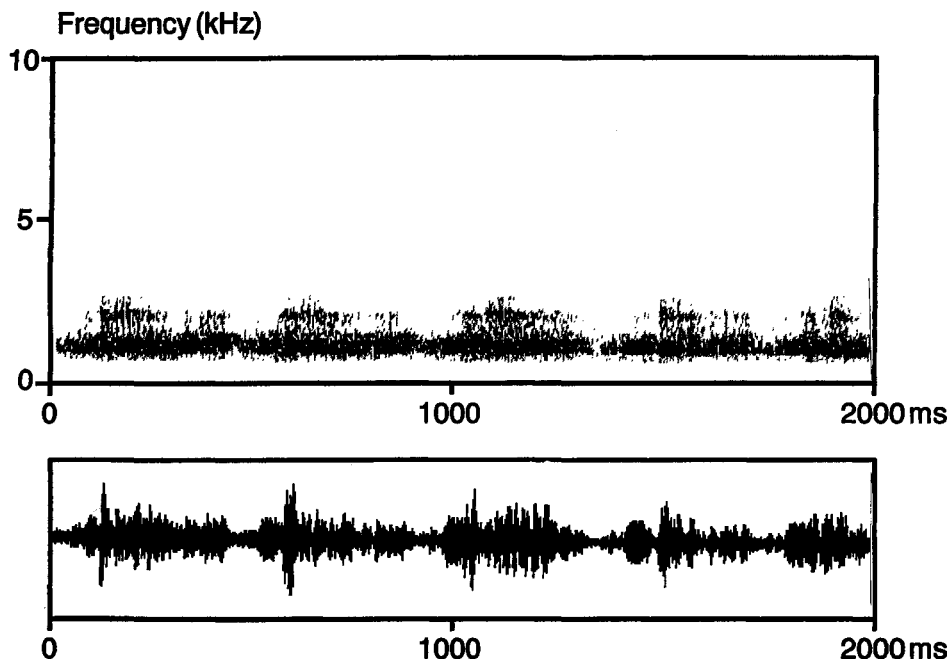


Figure 3. Sonagram and oscillogram of parts of a call series of *Discoglossus galganoi jeanneae* from near Facinas. Only short intervals are spaced between calls. Calls consist of two pulse groups; in the two former calls it can be recognized that the first (expiratory) pulse group has a longer duration and higher intensity than the second (inspiratory) pulse group.

as is typical for *galganoi*: ratio of pulse group intensities exp. : insp. was smaller than 0.9 in only 10% of the cases, between 0.9 and 1.1 in 33%, larger than 1.1 in 57% ($n = 24$). Calls were unharmonious.

Discussion

Busack (1986) related his new species *jeanneae* with *D. pictus* from Morocco, whereas Lanza et al. (1986) considered it as subspecies of *D. galganoi*. Data of comparative cellular DNA content also grouped *jeanneae* clearly with *D. galganoi* (Fritz et al., 1994); however the latter study had no material of *D. pictus scovazzi* available for comparison.

The call analysis shows clear differences between *jeanneae* and *pictus scovazzi*. Mean call duration in *jeanneae* from the type locality near Facinas is nearly twice as long as in *pictus scovazzi*. Unfortunately calls recorded at different temperatures had to be included in the calculation of the mean value of *jeanneae* from the type locality due to a restricted number of recorded calls. But even considering the maximum possible error (all *jeanneae* calls recorded at 18°C, all *pictus scovazzi* calls recorded at 22°C) the temperature effect on the call duration would result in a 25.6% to 26.6% decrease (calculated after the regressions of Weber, 1974, for *D. pictus* calls), assuming the null hypothesis that both call durations would be identical under similar temperature conditions. The mean call duration of *D. g. jeanneae* was 394 ms, the mean call duration of *D. p. scovazzi* was 207 ms, thus 47.5% lower (versus about 26% expected by the null hypothesis, see above). We therefore conclude that in fact the calls of *jeanneae* are more similar to *galganoi* calls than to *pictus scovazzi* calls regarding call duration. This is further supported:

- a) by the calls of the *jeanneae* specimen from near Ronda which are of similar duration to those of the Facinas specimen, and which were all recorded at 21°C;
- b) by the fact that about 40 calls of the *jeanneae* specimen from Facinas which were recorded at 22°C are distinctly longer than *pictus scovazzi* calls to the human ear, although duration of several of these calls could not be exactly measured due to the noisy recording.

There are also some similarities between *pictus scovazzi* and *jeanneae* that could not be found in any *galganoi* population: the lack of distinct intervals between the calls of a call series, the lack of a clear duration difference between first and second pulse group, and possibly a somewhat lower mean call duration. However it must be stressed that the respective data for *jeanneae* refer to only two specimens, one of which was stimulated by hormones. Call recordings of more specimens from the type locality Facinas would allow to clarify if the call characteristics of the single recorded male are common in this population, or if they are due to the artificial recording conditions.

Also the call of the specimen from Ronda had a somewhat lower mean call duration than *galganoi* specimens from northern Spain, although most call characteristics of this specimen were very similar to those of northern *galganoi*.

It is apparent that the *Discoglossus* from southern Spain (*jeanneae*) show some bioacoustic differences compared with those from northern Spain (especially in call duration). It should be investigated whether these differences are due to a continuous cline through Iberia, or due to a differentiated northern and southern group of populations. Nevertheless the bioacoustic data group the taxon *jeanneae* with *galganoi* rather than with *pictus* from northern Africa, what is supported by the data of cellular DNA-content (Fritz et al., 1994) and genetic distances (Lanza et al., 1986). In our opinion this situation is best reflected by a subspecific status of the populations south of the Guadalquivir river as *Discoglossus galganoi jeanneae*.

Busack (1986) proposed a biogeographic hypothesis in which an ancestral *D. pictus-jeanneae* stock populated a landmass containing the present southern Iberia and northern Morocco during the lower Miocene, whereas the ancestral *D. galganoi* stock populated northern Iberia. During this period Atlantic waters were flowing to the Mediterranean through what is now the basin of Guadalquivir river, dividing the southern Iberia/Morocco landmass from northern Iberia. In the Pliocene the Guadalquivir basin was filled by sedimentation, and the Strait of Gibraltar was formed, dividing southern Iberia and Morocco (see Busack, 1986 for references). The formation of the Strait of Gibraltar, following Busack (1986), divided ancestral *D. pictus-jeanneae* populations into two populations which evolved to become *D. jeanneae* and *D. pictus*.

Busack's scenario, with a secondary contact between the differentiated species *D. jeanneae* and *D. galganoi*, is contradicted by electrophoretic data (Lanza et al., 1986), data on DNA content (Fritz et al., 1994), and the bioacoustic data presented in this study. All these data group *jeanneae* nearer to *D. galganoi* than to *D. pictus*.

On the other hand, some electrophoretic differences between *jeanneae* and *D. galganoi* do exist. Nei's genetic distance between both taxa is 0.39 following Busack (1986) (based on comparison of 8 *jeanneae* specimens with only 1 *D. galganoi*), and 0.07 following Lanza et al. (1986). Morphological differentiation of *jeanneae* is rather high compared to *D. galganoi* but lower compared to *D. pictus* (Busack, 1986; Capula and Corti, 1993), and some call differences to *D. galganoi* from northern Spain exist (this study).

If discussing things in terms of Busack's scenario, also an alternative explanation for these characteristics of *Discoglossus* from southern Spain is possible. Assuming that *D. pictus* and *D. galganoi* already diverged 9-10 million years ago, as suggested by albumin data (Maxson and Szymura, 1984), two differentiated *Discoglossus* species would have populated the landmasses today representing Iberia and northern Africa during lower Miocene: The ancestral stock of *D. pictus* inhabited the northern Africa/southern Iberia landmass, and the ancestral *D. galganoi* stock was distributed in northern Iberia.

On this basis it is possible that after the formation of the Strait of Gibraltar a part of the ancestral *D. pictus* population remained isolated in southern Iberia. The dessication of the Guadalquivir Basin would have allowed the intrusion of ancestral *D. galganoi* into southern Iberia, which subsequently displaced the ancestral *D. pictus*. During this contact between the small remnant *D. pictus* populations and the invading large populations of

D. galganoi, hybridizations between both forms possibly occurred. Such a hypothetical past introgression of ancestral *pictus* alleles into the ancestral *galganoi* gene pool could be an explanation of the differentiation of the southern *galganoi* populations, today to be named *D. galganoi jeanneae*.

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