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# Cases of abnormal amplexus in anurans (Amphibia: Anura) from Bulgaria and Greece

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**Abstract.** The current study reports 29 cases of abnormal amplexus in some anuran species (*Bombina variegata, Bufo bufo, Epidalea viridis, Pelophylax ridibundus, Rana temporaria* and *Rana dalmatina*) from Bulgaria and one from Greece (*Rana dalmatina* and *Rana graeca*). Totally six different types of aberrations from the normal amplexus in anurans were detected: multiple amplexus (numerous males and one female); amplexus from the abdominal side; amplexus between two males; amplexus between alive male and dead female; amplexus between specimens from different species and amplexus between a male and inanimate object. The possible reasons for the occurrence of the various cases of abnormal amplexus are discussed.

**Key words:** mating behavior, amplexus, aberrations, Anura, Amphibia, Bulgaria, Greece.

## Introduction

Anurans show a wide variety of mating behavior. Almost all temperate anuran species are external fertilizers and most of them breed in water. During mating males typically grasp females in a behavior known as amplexus. Amplexus is thought to help to align both male and female reproductive tracts and help to achieve successful fertilization (Hutchence, Sheffield, UK, pers. comm. 2004).

Despite the fact that quite a lot of studies on the breeding behavior of the anuran amphibians are conducted in Bulgaria (Belcheva 1959, Hristova 1962, Beškov 1970, 1972, Beschkov 1978, Beshkov 1974, 1977, 1988, Beshkov & Angelova 1981, Beshkov et al. 1986, Nöllert et al. 1986, Stojanov 1997, Beshkov & Stoyanov 2000, Undjian 2000, Mollov 2005, Petrov et al. 2006, Schlüter 2006) there are still some aspects that are poorly studied. There is a gap of information concerning the aberrations from the normal amplexus.

The aim of the current study is to determine the types of aberrations from the normal amplexus occurring in Bulgaria and Greece and report the recorded cases, as well as discuss the possible causes and biological and ecological purpose of these aberrations.

### Material and Methods

For the purposes of the current study various freshwater basins (rivers, streams, creeks, ponds, swamps, lakes, reservoirs, irrigation canals etc.) in Bulgaria and Greece were repeatedly visited during the reproduction period of anuran amphibians (February-June) in the period 1985-2008. The mating behavior of the amphibians was observed and documented. Any aberrations from the normal amplexus (one male gasping one female from the same species) were recorded and photographed (when possible).

## Results

Based on the summarized data from our observations (29 cases from Bulgaria and 1 from Greece) we can distinguish the following types of aberrations from the normal amplexus in anurans: multiple amplexus (numerous males and one female); amplexus from the abdominal side; amplexus between two males; amplexus between alive male and dead female; amplexus between specimens from different species and amplexus between a male and inanimate object (Table 1).

## Discussion

Aberrations from the normal amplexus in anurans are not a precedent. Multiple amplexus has been observed by various authors in different species: *Rana iberica* in Spain (Ayres 2008); *Rana dalmatina* in France (Lode & Lesbarreres 2004); *Lithobates sylvatica* in USA (Conrad

**Table 1.** Cases of abnormal amplexus in anurans from Bulgaria and Greece.

| Type of aberration | Species | Date / Locality / Observer(s) | Notes |
|---|---|---|---|
| Multiple amplexus | *B. variegata* | 08.VII.1995 / Kresna George near the road fork for Stara Kresna Village (South-Western Bulgaria) / B. Naumov | four males and one female |
| | | 02.V.2003 / Mostovska Sushitsa River, Mostovo Village, Western Rhodopes Mts. (Bulgaria) / I. Mollov | 7 males and 1 female (Fig. 1); 4 males on 1 female (Fig. 2) |
| | | 18.V.2006 / Chakalarovo Village, Eastern Rhodopes Mts. (Bulgaria) / G. Popgeorgiev | two males and one female |
| | | 11.V.2004 / Kolets Village, Eastern Rhodopes Mts. (Bulgaria) / G. Popgeorgiev | two males and one female |
| | *B. bufo* | 04.III.2007 / Kolets Village, Eastern Rhodopes Mts. (Bulgaria) / G. Popgeorgiev | seven males and one female |
| | | 17.III.2007 / Kolets Village, Eastern Rhodopes Mts. (Bulgaria) / G. Popgeorgiev, N. Tzankov | 9 males and one female (Fig. 3). |
| | | 05.IV.2007 / The Dendrariuma Area, Vitosha Mt. (Bulgaria) / N. Tzankov | many males and one female |
| | | IV.2001 / Makotsevska Reka River, near Makotsevo Village (Bulgaria) / A. Stoyanov | three males and one female |
| | *R. temporaria* | 22.03.2007 / Dendrariuma Area, Vitosha Mt.; Urvich Area, Lozenska Mt. and Muhalnitsa Swamp, near Botevgrad Town (Bulgaria) / B. Naumov | two males on one female (Fig. 4). |
| | *R. dalmatina* | Multiple observations in the period 1993-1995 / Germanski Swamps, Lozenska Mt. (Bulgaria) / B. Naumov. | two males and one female |
| | | 27.III.2006 / Gorno Bryastovo Village, Eastern Rhodopes Mts. (Bulgaria) / G. Popgeorgiev | two males and one female |
| | *R. temporaria* - ♂ (many) and one *P. ridibundus* - ♀. | 16.III.2007 / Protected area "Muhalnitsa Swamp", near Botevgrad Town / N. Tzankov . | - |
| Amplexus from the abdominal side | *B. variegata* | 29.VI.2007 / Slavyanka Mt. (South-Western Bulgaria) / G. Popgeorgiev, B. Naumov, N. Tzankov, A. Stoyanov | see Fig. 5 |
| | *B. bufo* | 24.IV.2005 / Gornoto Ezero Lake in the Dendrariuma Area, Vitosha Mt. (Bulgaria) / B. Naumov | the male has embraced the female in the axilar area from the abdominal side. |
| Amplexus between alive male and dead female | *R. temporaria* | 22.03.2007 / Gornoto Ezero Lake, Dendrariuma Area, Vitosha Mt. (Bulgaria) / B. Naumov | the male has embraced a dead female (killed with a nail) in the inguinal area (Fig. 6). |
| | *R. temporaria* ♂ x *P. ridibundus* ♀ | 28.II.2008 / Muhalnitsa Swamp, near Botevgrad Town (Bulgaria) / B.Naumov | a male *R. temporaria* has embraced a dead female *P. ridibundus* (Fig. 7). |
| Amplexus between two males | *E. viridis* | 02.III.2008 / Small swamp in nature landmark "Mladezhki halm" in Plovdiv City (South Bulgaria) / I. Mollov | - |
| | *R.temporaria* | III.2003 / Temporary floods in the riverbed of Iskar River, near Vedena Village, Sofia District (Bulgaria) / A. Stoyanov | - |
| | *B. bufo* | III.1999 / Small flood of Iskar River, near its entrance in Pasarel Village, Sofia District (Bulgaria) / A. Stoyanov | - |
| | *B. bufo* ♂ x *P. ridibundus* ♂ | 14.IV.1985 / Gornoto Ezero Lake, Dendrariuma Area, Vitosha Mt. (Bulgaria) / B. Naumov | a ♂ *B. bufo* has embraced a ♂ *P. ridibundus* in the axilar area. |
| | *B. bufo* ♂ x *P. ridibundus* ♂ | 08.IV.1987 / Dalbochitsa River, near Gabrovnitsa Village (Bulgaria) / V. Beshkov, D. Dobrev, Z. Arnaudov, B. Petrov | a ♂ *B. bufo* has embraced a ♂ *P. ridibundus* in the axilar area. |
| | *R. temporaria* ♂ x *B. bufo* ♂ | IV. 2007 / Dendrariuma Area, Vitosha Mt. (Bulgaria) / A. Stoyanov | a male *R. temporaria* has embraced a male *B. bufo* upside down in the inguinal area (Fig. 8). |
| Amplexus between specimens from different species | *B. bufo* ♂ x *E. viridis* ♀ | 22.III.2006 / Ostar Kamak Village, Eastern Rhodopes Mts. (Bulgaria) / G. Popgeorgiev, O. Todorov | - |
| | *B. bufo* ♂ x *P. ridibundus* ♀ | 02.IV.2007 / Dendrariuma Area, Vitosha Mt. (Bulgaria) / N. Tzankov | - |
| | *R. temporaria* ♂ x *P. ridibundus* ♀ | 01.IV.2004 / Boyansko Ezero Lake, Vitosha Mt. (Bulgaria) / N. Tzankov | A young male, probably late, because all the other frogs have left the lake. |
| | *R. dalmatina* ♂ x *R. temporaria* ♀ | 25.III.2007 / Urvich Area, near Iskar River (Bulgaria) / N. Tzankov | - |
| | *R.a temporaria* ♂ x *R. dalmatina* ♀ | 25.III.2007 / Boyansko Ezero Lake, Vitosha Mt. (Bulgaria) / N. Tzankov | - |
| | *R. temporaria* ♂ x *B. bufo* ♀ | 22.III.2007 / Dendrariuma Area, Vitosha Mt. (Bulgaria) / N. Tzankov | - |
| | *R. dalmatina* ♂ x *R. graeca* ♀ | 03.IV.2007 / A small creek north-west from Lithopos Village, near Kerkini Dam Lake (Northern Greece) / N. Tzankov | - |
| Amplexus between a male and inanimate object | *B. bufo* | III. 1997 / Makotsevska Reka River, near Makotsevo Village (Bulgaria) / A. Stoyanov | A male toad has embraced a plastic cup with the size of a female toad from the same species. |

**Figure 1-8.** **(1)** *B. variegata* – multiple amplexus of seven males and one female, 02.V.2003, Mostovska Sushitsa River, Mostovo Village (Western Rhodopes Mts), Photo: I. Mollov. **(2)** *B. variegata* – multiple amplexus of four males and one female, 02.V.2003, Mostovska Sushitsa River, Mostovo Village (Western Rhodopes Mts.), Photo: I. Mollov. **(3)** *B. bufo* – multiple amplexus, nine males on one female, 17.III.2007, small dam lake near Kolets Village (Eastern Rhodopes Mts.). Photo: G. Popgeorgiev. **(4)** *R. temporaria* – multiple amplexus, two males on one female, 22.03.2007, Dendrariuma Area, Vitosha Mt. Photo: B. Naumov. **(5)** *B. variegata* – amplexus from the abdominal side, 29.VI.2007, Slavyanka Mt. (South-Western Bulgaria). Photo: G. Popgeorgiev. **(6)** *R. temporaria* - amplexus between alive male and dead female, 22.03.2007, Gornoto Ezero Lake, Dendrariuma Area, Vitosha Mt. Photo: B. Naumov. **(7)** *R. temporaria* ♂ x *P.ridibundus* ♀ - amplexus between alive male and dead female from different species, 28.II.2008, Muhalnitsa Swamp, near Botevgrad Town. Photo: B.Naumov. **(8)** A male *R. temporaria* has embrased a male *B. bufo* upside down in the inguinal area. IV. 2007, Dendrariuma Area, Vitosha Mt. Photo: A. Stoyanov.

2008); *Bombina variegata* in Romania (Gherghel et al. 2008a) and others. Multiple amplexus is especially common in explosive breeding amphibians where a large number of breeding adults can be present in the breeding site for a short time period (days to weeks). In these species, females are important resources for males because the males are present in larger numbers than females, even from the start of the breeding season and the males breed with more females, whereas the females leave the pond in short time after the eggs were laid (Hartel et al. 2007). Thus the operational sex ratio (OSR) may be strongly male biased in these cases resulting in an intense competition between males to obtain a mate. This competition may result in multiple amplexus, takeovers, and frequently, the death of females, as it was observed for example in *B. bufo*.

The strongly male-biased OSR is the probable reason for the occurring of male-male amplexus, which occurs quite rarely and usually continues for a short period of time. According to Marco et al (1998) the male *B. boreas,* for example, does not discriminate between sexes before attempting amplexus.

Amplexus between alive male and dead female, as well as amplexus between males and between different species and even the peculiar case of amplexus with inanimate object could be explained with the study conducted by Susumu Ishii in Japan. He found that when adult male toads (*Bufo japonicus*) find any pliable object as large as an adult female toad during the breeding season, they mount it and try to clasp it. If the object they have mounted doesn't respond, they keep holding on for hours. If the object is a female ready to mate, then amplexus proceeds normally. However, if the object mounted is a female not ready to produce eggs, she will vibrate her body. When the clasping male feels the vibration, he suddenly stops the amplexus (Conrad 2008).

Another mechanism for interruption of the amplexus between two males is a release call (Marco et al. 1998). According to Marco & Lizana (2002) during mate search male *B. bufo* do not discriminate species, sex or size. After a male clasps another male toad, a release call is released and the amplexus lasts only for a few seconds. The release call in amplected male *B. bufo* seems to be the only element of sex recognition for male *B. bufo*, and consequently, sex recognition only occurs after an attempted amplexus. Apparently, male *B. bufo* of its own species does not interpret the release call of frogs as a release call and therefore toad-frog amplexus can be prolonged, regardless of the very different morphology of the frogs. Thus, the reproductive strategy for male *B. bufo* may be to clasp quickly every moving animal of similar size and then determine whether it is a female. Once a female is encountered, the male and female mate and there is little competition from other males for the female. This strategy is efficient if males have a mechanism to determine when they have clasped another male and if the probability of clasping an individual of another species is low.

Marco & Lizana (2002) have also observed in the field males in amplexus for long periods (many hours) with dead male or female toads which in their opinion confirm the importance of the release calls in the identification of sex or even the species of the mates. Male toads that clasp frogs could waste time and energy and miss opportunities to mate with gravid toad females. Also, toad-frog amplexus would have high costs for frogs. Due to the fact that toads usually breed earlier than most frog species it is a rare case of abnormal multi-species amplexus.

Another explanation for the multi-species amplexus could be found in the work of Rugh (1935). According to this author amplexus between different species could be induced chemically. Perhaps somehow in nature anurans, using this strategy get confused and embrace in amplexus a female from different species. Also according to the same author there is reluctance in some species to embrace a specimen from different species despite the chemical stimulation. For example *Bufo fowleri* is "disposed" for amplexus with another male, but some *Rana* species are not. According to our observations however, the frogs from genus *Rana* are in fact very inclined to embrace a male or female from another species, while the green frogs (*Pelophylax sp.*) are not. Perhaps the reason for this lays in the fact that all *Pelophylax* species in Bulgaria breed a lot later than the other species of frogs and toads. Other reported cases of multi-species amplexus are found in the works of Storm (1952), Brown (1977), Reading (1984), Gerghel et al. (2008b) and others.

Another type of aberration from the normal amplexus, which wasn't observed by us, is a female-female amplexus, which was reported by Malashichev (1999) for the yellow-bellied toad (*Bombina variegata*).

In our opinion further studies are needed to fully understand the discrete mechanisms and purpose of the various aberrations from the normal amplexus in the Anura.

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