Acta Herpetologica 14(1): 65-68, 2019
DOI: 10.13128/Acta_Herpetol-24806

Age structure of a population of *Discoglossus scovazzi* Camerano, 1878 (Anura - Discoglossidae) in extreme environmental conditions (High Atlas, Morocco)

Mohamed Amine Samlali, Abderrahim S'khifa, Tahar Slimani*

*Cadi Ayyad University, Faculty of Sciences Semlalia, Biodiversity and Ecosystems Dynamic Laboratory, PO Box 2390, Marrakech 40 000, Morocco. *Corresponding author.* Email: slimani@uca.ac.ma

Submitted on: 2019, 25th February; Revised on: 2019, 12thApril; Accepted on: 2019, 29th April
Editor: Fabio M. Guarino

Abstract. The age structure and growth of the Moroccan Painted Frog (*Discoglossus scovazzi* Camerano, 1878) in a population living in the High Atlas at the upper altitudinal limit of the species' range was estimated for the first time by skeletochronology. Individual age was determined by counting the lines of arrested growth (LAGs) from cross-sections of the phalanges. Both males and females reached sexual maturity at 3-4 years of age at which point the speed of osteogenesis and body growth slow down**.** Males and females have maximum lifespans of six and five years, and average sizes of 47. 50 mm (n = 21, SD = 1.40) and 39.70 mm (n = 53, SD = 0.90) respectively. We detected a positive relationship between age and size, suggesting that the oldest individuals are always bigger and heavier. Sizes corresponding to the same age class are very heterogeneous reflecting divergent conditions and growth strategies.

Keywords. Endemic species, skeletochronology, life-history traits, extreme environment, Morocco.

Degradation and fragmentation of natural habitats due to human activities have harmful effects on amphibian populations (Collins and Crump, 2009; Hamer and Mcdonnell, 2008, 2010; Collins and Fahrig, 2017). The central plateau of the Oukaimeden massif in the High Atlas region of Morocco has been heavily impacted by overgrazing and the banks of its two main rivers are seriously degraded by the bulk removal of sand for construction (Ait Babahmad, 2012; Ait Zidan, 2018). Both human activities have degraded water habitats and altered the richness and abundance of their amphibian communities. Morphological abnormalities have been recorded in adults and tadpoles (Ait Babahmad, 2012; Lansari, 2018), especially in the frog *Pelophylax saharicus*, which is widespread in the massif. This habitat degradation could cause a demographic collapse leading to local disappearances (Mckinney, 2002; Löfvenhaft et al., 2004; Bionda et al., 2013; Babini et al., 2015; Green and Bailey, 2015; Zhelev et al., 2017), especially in species that are relatively sensitive to human activities due to habitat specialization or specific life history (Rubbo and Kiesecker, 2005; Hamer and Mcdonnell, 2008, 2010).

The Moroccan Painted Frog (*Discoglossus scovazzi* Camerano 1878, family Discoglossidae) is listed as Least Concern in the IUCN Red List of Threatened Species (Salvador et al., 2009; Başkale et al., 2018). Endemic to Morocco, the species is generally common, especially in sub-humid and humid areas. They are quite cryptic outside the breeding season, with adults preferring the proximity of small bodies of water such as temporary ponds, low-flow streams and sources. Although the species may tolerate a slight modification of its habitat, its sensitivity to the particular conditions and the quality of its aquatic and terrestrial environment remains largely unknown (Reques et al., 2013). Because the demographic changes are in response to environmental conditions (Sinsch et al., 2004;

ISSN 1827-9635 (print)
ISSN 1827-9643 (online)

Sinsch et al., 2007; Spear et al., 2009), the purpose of this study is to estimate the age structure, age at first reproduction and longevity of a high mountain population of *Discoglossus scovazzi*, by using skeletochronology.

Discoglossus scovazzi is an anuran of small body size known from a large number of aquatic biotopes throughout Morocco from sea level up to the Atlas Mountains, excluding the Saharan areas. It reaches its upper altitudinal limit in the High Atlas at Oukaimeden, over 2600 m a.s.l. (Bons and Geniez, 1996).

This study is based on mark-recapture tracking of 74 adult individuals (21 males and 53 females) between 2016 and 2018. The study took place at the population's breeding site, which consists of small pools of water fed by a permanent watercourse. Solitary and discreet during most of the year, *Discoglossus scovazzi* begins reproduction in early spring (Ait Babahmad, 2012; Beukema et al., 2013; Samlali, 2016). Egg-laying is spread over 8 to 10 days from early April to mid-June. During this period, the water temperature is about 13 °C and the outside temperature is about 10 °C.

The population is located at 2660 m of altitude in the Oukaimeden massif (31°11'N, 07°50'W) about 75 km South-East of Marrakech. The climate is typically Mediterranean with cold winters and an average annual temperature of 9.5 °C. The average maximum temperature of the hottest month (July) is 22.2 °C and the average minimum temperature of the coldest month (January) is -3.3 °C. The average annual precipitation is circa 518 mm and snow fall mainly between November and March. The dry period lasts about four months (June-September). The vegetation consists of spiny xerophytes and a herbaceous layer, essentially graminaceous, relatively rich during rainy periods but heavily overgrazed. The population breeds in pits (diameter 6-7 m and at least one meter deep) that resulted from sand extraction and used as waterers in the bed of a permanent river (Assif-N-Irène). Intensive use for livestock watering negatively impacts water quality and accelerates drying (Ait Babahmad, 2012; Samlali, 2016).

Sampling by hand occurred from February to June. We measured the frogs from snout to cloaca with calipers and weighed them using a field balance (accuracy: 0.1 g). We determined sex by the presence of nuptial callosities and calling in males. After measurements, we collected the third toe of the left hind leg and we immediately released the frog at the place of its capture. The samples are kept in absolute alcohol.

We sectioned phalanges from the toe to count lines of arrested growth (LAGs) following the method by Castanet and Smirina (1990), which is based on the detection of LAGs generated by the cold season (each LAG is interpreted as one year of age). In order to avoid the errors of the estimation of the age induced by the medullary resorption (Francillon-Vieillot, 1987; Fretey and Le Garff, 1992; Tsiora and Kyriakopoulou-Sklavounou, 2002; Guarino et al., 2003; 2008; Liao and Lu, 2010; Liao, 2011; Huang et al., 2013; Bionda et al., 2015; Jin et al., 2017), we selected, for each individual, diaphysis sections in which the size of the medullar cavity was at its minimum and that of the periosteal bone at its maximum (Oromi et al., 2016). On the other hand, bone remodeling and the close rapprochement of the outer most lines, especially in the oldest individuals (Wagner et al., 2011) could also compromise the age estimation. In these cases, at the insertion site of the phalangeal ligament allows to discern the peripheral LAGs and to reliably count them (Bionda et al., 2018). The number of lines of arrested growth (LAGs) in each section is counted by three authors (M.A.S., A.S. and T.S.). Since all the individuals studied were collected during the breeding season, the age at sexual maturity was revealed by the presence of LAGs suddenly becoming very closely adjacent, reflecting the slowing of growth after sexual maturity.

All variables were first tested for normality (Kolmogorov-Smirnov test) and the difference between sexes was tested using ANOVA. In addition, the relationship between size and age was analyzed by Spearman correlation test. We used the significance level of alpha = 0.05 in all tests.

A total of 74 reproductive adults (21 males and 53 female) were studied. Individuals were collected at breeding sites during the reproduction periods in 2016, 2017 and 2018. The cross-sections show the presence of strongly stained growth lines in periosteal bone in most individuals (Fig. 1). Since the frogs were collected in March and April, we considered the perimeter of the bone as corresponding to an indicator LAG of last winter. We clearly detected a decrease in the distance that

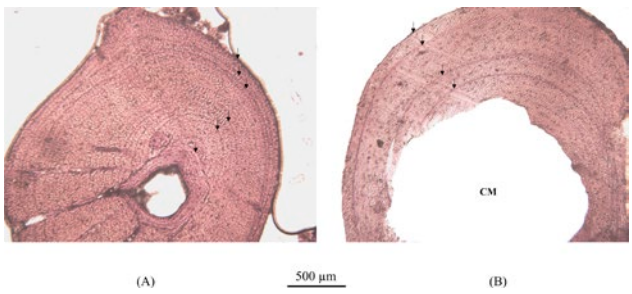

Fig. 1. Transverse sections at diaphysal region of phalanx bones of *Discoglossus scovazzi*. (**A**) Male with 6 visible LAGs. (**B**) Female with 4 visible LAGs (CM: medullary cavity.

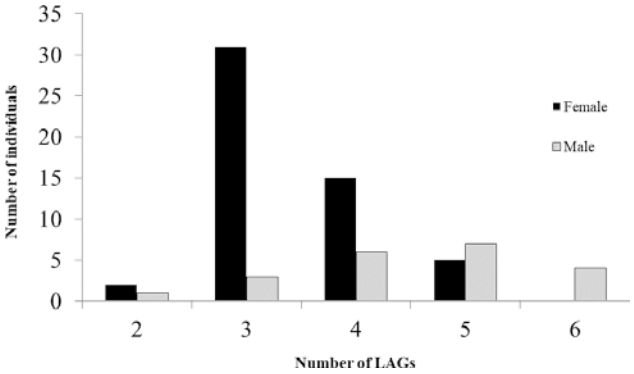

Fig. 2. Age distribution of the reproductive population of *Discoglossus scovazzi* at Oukaimeden in 2016-2018.

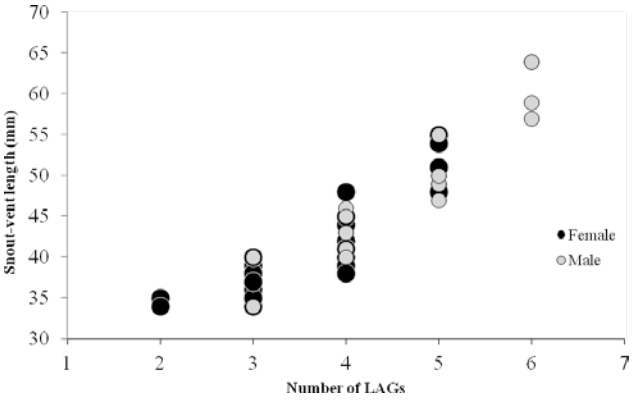

Fig. 3. Relationship between estimated age (X-axis, in year) and body size (Y-axis, snout-vent length in mm) in the studied population.

separating the LAGs suggesting that sexual maturity is reached at 3-4 years in both males and females (Fig.1 A, B). At the breeding site, we sampled some females younger than three years, although three-year individuals were the most frequently observed (Fig. 2). Highest estimated age is six years in males and five years in females.

Body size varied from 34.0 to 64.0 mm in males (mean ± SD = 47.5 ± 1.4 mm, n = 21) and from 33.0 to 56.1 mm in females (39.7 ± 0.9 mm, n = 53), with a mass of 14.5 ± 5.17 g in males and 8.05 ± 3.45 g in females. The observed pattern of sexual dimorphism in body size was significant ($F_{1, 72} = 21.4$; $P < 0.0001$). Because we did detect a significant relationship between age and body size in males (r = 0.786, P = 0.018, n = 21) and in females (r = 0.847, P = 0.003, n = 53) (Fig. 3), the oldest individuals were always the largest ones. Indeed, anurans often exhibit continuous growth, particularly females because of the strong relationship between fecundity and body size (Lengagne et al., 2007).

In most anuran species, males are smaller than females (Shine, 1979). For *D. scovazzi,* the sexual size dimorphism is inversed (García-Paris et al., 2004; Oromi et al., 2016). The variation in size dimorphism results from differences in the age structure between sexes (Monnet and Cherry, 2002; Liao et al., 2013a, b). In fact, in species where females exhibit smaller sizes (*Rhinella achalensis*, *Rana cascadae* and *Hylarana nigrovittata*), males mature at later ages (Monnet and Cherry, 2002), suggesting that delayed maturity in males produces size dimorphism as stated in the Rensch's rule (Liao et al., 2015). However, as in *D. pictus* (Oromi et al., 2016), we did not find differences in the age structure between the sexes in *D. scovazzi* which suggests that these two species failed to support Rensch's rule. In some anuran species, increased male body size is an important determinant of male mating success (Wells, 2007; Liao and Lu, 2011). Furthermore, large males are more likely to breed than smaller ones if competition for females is intense (Oromi et al., 2016). However, little information is available on the mating tactics of *D. scovazzi* and other factors might explain the sexual size dimorphism in this species.

Our study provides original data on the structure of a population at the upper altitudinal limit of this endemic species of Morocco. The earliest age at which sexual maturity was attained in most individuals of both sexes is about 3 years. However, the three-year-old frogs, theoretically fewer numerous than those aged two as a result of annual mortality, are more frequent at breeding sites than younger females. As observed in Brongersma's Toad in arid environments (Fattah et al., 2014), this asymmetrical age structure in females of *Discoglossus scovazzi* in the High Atlas suggests that this difference in young females is due to the fact that not all the females reach sexual maturity before three years, or that some females reach sexual maturity at the age of two years.

Compared to its Mediterranean congener, *D. pictus*, studied by Oromi et al. (2016) in North Africa, the late age at maturity in our population suggests a low growth rate in juveniles, strongly constrained by the harsh climatic conditions of this alpine environment (low temperature, snowfall, strong wind), or the modification of aquatic environments (due to excessive sand removal) and the deterioration of their physico-chemical quality (due to livestock).

The low values in terms of longevity (5-6 years) and reproductive potential (about two years) recorded in this population are related to climatic conditions and ongoing changes in habitat quality. Several other hypotheses could, however, be formulated, including moving larger adults /older to other sites, the low survival of larger individuals /older, or the case of an unstable population maintained by smaller / younger adults. This highlights a need monitoring.

ACKNOWLEDGEMENTS

This research was financed by funds from Hassan II Academy of Science and Technics (ICGVSA project, PI Slimani T.). It was authorized by the High Commissariat for Water and Forest (Decision Number 05/2013 HCE-FLCD/DLCDPN/DPRN/CFF) - Morocco. We would like to thank the two anonymous reviewers who provided important comments to improve the manuscript.

REFERENCES

Ait Babahmad, A. (2012): Diversité en amphibiens dans le massif de l'Oukaimeden (Haut Atlas Central). "Implications en termes de conservation dans le cadre du changement climatique global". Master II. Faculté des Sciences Semlalia.

Ait Zidan, A. (2018): Ecologie et Traits d'histoire de vie d'*Atlantolacerta andreanszkyi* (Werner, 1929) dans un environnement contraignant dans le Haut Atlas Central à l'Oukaimeden. Thermorégulation et structure de population. Master II. Faculté des Sciences Semlalia.

Babini, M.S., Bionda, C.L., Salas, N.E., Martino, A.L. (2015): Health status of tadpoles and metamorphs of *Rhinella arenarum* (Anura, Bufonidae) that inhabit agroecosystems and its implications for land use. Ecotoxicol. Environ. Saf. **118**: 118-125.

Başkale, E., Ulubeli, S. A., Kaska, Y. (2018): Age structures and growth parameters of the Levantine frog, *Pelophylax bedriagae*, at diferent localities in Denizli, Turkey. Acta Herpetol. **13**: 147-154.

Beukema, W., Depous, P., Donaire-Barroso, D., Bogaerts, S., Garcia-Porta, J., Escoriza, D., Arribas, O.J., El Mouden, E.H., Salvador, C. (2013): Review of the systematics, distribution, biogeography and natural history of Moroccan amphibians. Zootaxa **60**: 1-60.

Bionda, C.L., Babini, S., Martino, A.L., Salas, N.E., Lajmanovich, R. C. (2018): Impact assessment of agriculture and livestock over age, longevity and growth of populations of common toad *Rhinella arenarum* (anura: Bufonidae), central area of Argentina. Global Ecol. Conserv. **14**: 1-13.

Bionda, C.L., Kost, S., Salas, N.E., Lajmanovich, R., Sinsch, U., Martino, A.L. (2015): Age structure, growth and longevity in the common toad, *Rhinella arenarum*, from Argentina. Acta Herpetol. **10**: 55-62.

Bionda, C.L., Lajmanovich, R., Salas, N.E., Martino, A.L., Di Tada, I. (2013): Demografía poblacional de *Rhinella arenarum* (Anura: Bufonidae) y *Physalaemus biligonigerus* (Anura: Leiuperidae) en agroecosistemas de la provincia de Cordoba, Argentina. Rev. Biol. Trop. **61**: 1389-1400.

Bons, J., Geniez, P. (1996): Amphibiens et Reptiles du Maroc (Sahara Occidental compris), Atlas biogéographique. Asociación Herpetológica Española, Barcelona, Spain.

Castanet, J., Smirina, E.M. (1990): Introduction to the skeletochronological method in amphibians and reptiles. Ann. Sci. Nat., Zool. Biol. Anim. **11**: 191-196.

Collins, J.P., Crump, M. (2009): Extinction in Our Time. Oxford: University Press, New York, USA.

Collins, S.J., Fahrig, L. (2017): Responses of anurans to composition and configuration of agricultural landscapes. Agric. Ecosyst. Environ. **239**: 399-409.

Fattah, A., Slimani, T., El Mouden, E.H., Grolet, O., Joly, P. (2014): Age structure of a population of *Barbarophryne brongersmai* (Hoogmoed, 1972) (Anura, Bufonidae) inhabiting an arid environment in the Central Jbilets (West-Morocco). Acta Herpetol. **9**: 237-242.

Francillon-Vieillot, H. (1987): La croissance des os longs chez les Amphibiens. Son utilisation comme critère d'âge. Unpublished doctoral dissertation, Universite Paris VII, Paris.

Fretey, T., Le Garff, B. (1992): Apports de la squelettochronologie dans la démographie du crapaud commun, *Bufo bufo* (L.) (*Anura*, *Bufonidae*) dans l'Ouest de la France. In: Tissus durs et âge individuel des vertébrés, pp. 409-419. Baglinière J.L., Castanet J., Conand F., Meunier F.J., Eds, ORSTOM, INRA, Paris.

García-Paris, M., Montori, A., Herrero, P. (2004): Amphibia. Lissamphibia. In: Fauna Ibérica 24, pp. 346-352. Ramos, M.A., Alba, J., Bellés, X., Gosálbez, J., Guerra, A., Macpherson, E., Serrano, J.,Templado, J., Eds., Consejo Superior de Investigaciones Científicas, Madrid.

Green, A.W., Bailey, L.L. (2015): Reproductive strategy and carry-over effects for species with complex life histories. Popul. Ecol. **57**: 175-184.

Guarino, F.M., Di Gia, I., Sindaco, R. (2008): Age structure by skeletochronology in a declining population of *Rana temporaria* from northern Italy. Acta Zool. Acad. Sci. Hung. **54**: 99-112.

Guarino, F.M., Lunardi, S., Carlomagno, M., Mazzotti, S. (2003): A skeletochronological study of growth, longevity and age at sexual maturity in a population of *Rana latastei* (Amphibia, Anura). J. Biosci. **28**: 775-782.

Hamer, A.J., Mcdonnell, M.J. (2008): Amphibian ecology and conservation in the urbanizing world: a review. Biol. Conserv. **141**: 2432-2449.

Hamer, A.J., Mcdonnell, M.J. (2010): The response of herpetofauna to urbanization: Inferring patterns of

persistence from wildlife databases. Austral. Ecol. **35**: 568-580.

Huang, Y., Zhu, H.Q., Liao, Y.M., Jin, L., Liao, W.B. (2013): Age and body size of the toad *Bombina maxima* in a subtropical high-altitude population. Herpetol. J. **23**: 229-232.

Jin, L., Chen, C., Liao, W.B. (2017): Altitudinal variation in body size and age in male spot-legged treefrog (*Polypedates megacephalus*). Russ. J. Ecol. **48**: 476-48.

Lansari, A. (2018): Variations Environnementales et Plasticité Phénotypique chez la Grenouille Verte d'Afrique du Nord, *Pelophylax Saharicus* (Boulenger, 1913). Identification Taxonomique et Considérations Biogéographiques. Unpublished doctoral dissertation. Faculté des Sciences Semlalia.

Lengagne, T., Arthaud, F., Cormier, M., Joly, P. (2007): Cost of sexually embracing a large female offset by the number of eggs fertilised for small male *Bufo bufo* L. Biol. J. Linn. Soc. **92**: 755-762.

Liao, W. B., Liu, W.C., Merilä, J. (2015): Andrew meets Rensch: Sexual size dimorphism and the inverse of Rensch's rule in Andrew's toad (*Bufo andrewsi*). Oecologia. **177**: 389-399.

Liao, W.B., Lu, X. (2010): Age structure and body size of the Chuanxi Tree Frog *Hyla annectans chuanxiensis* from two different elevations in Sichuan (China). Zool. Anz. **248**: 255-263.

Liao, W.B., Lu, X. (2011): Proximate mechanisms leading to large male-mating advantage in the Andrew's toad *Bufo andrewsi*. Behaviour. **148**: 1087-1102.

Liao, W.B. (2011): A skeletochronological estimate of age in a population of the Siberian Wood Frog, *Rana amurensis*, from northeastern China. Acta Herpetol. **6**: 237-245.

Liao, W.B., Zeng, Y., Yang, J.D. (2013b): Sexual size dimorphism in anurans: roles of mating system and habitat types. Front. Zool. **10**: 65.

Liao, W.B., Zeng, Y., Zhou, C.Q., Jehle, R. (2013a): Sexual size dimorphism in anurans fails to obey Rensch's rule. Front. Zool. **10**: 10.

Löfvenhaft, K., Runborga, S., Sjögren-Gulveb, P. (2004): Biotope patterns and amphibian distribution as assessment tools in urban landscape planning. Landscape Urban. Plan. **68**: 403-427.

Ma, X., Lu, X. (2009): Sexual size dimorphism in relation to age and growth based on skeletochronological analysis in a Tibetan frog. Amphibia-Reptilia. **30**: 351-359.

Mckinney, M.L. (2002): Urbanization, biodiversity, and conservation. Bioscience. **52**: 883-890.

Monnet, J., Cherry, M.I. (2002): Sexual size dimorphism in anurans. Proc. R. Soc. Lond. Ser. B-Biological Sci. **269**: 2301-2307.

Oromi, N., Pujol-Buxóc, E., San Sebastián, O., Llorente, G.A., Aït Hammou, M., Sanuya, D. (2016): Geographical variations in adult body size and reproductive life history traits in an invasive anuran, *Discoglossus pictus*. Zoology **119**: 216-223.

Reques, R., Pleguezuelos, J.M., Busack, S.D., de Pous, P. (2013): Amphibians of Morocco, including Western Sahara: a status report. Basic Appl. Herpetol. **27**: 23-50.

Rubbo, M.J., Kiesecker, J.M. (2005): Amphibian breeding distribution in an urbanized landscape. Conserv. Biol. **19:** 504-511.

Salvador, A., Donaire-Barroso, D., Slimani, T., El Mouden, E.H., Geniez, P., Perez-Mellado, V., Martínez-Solano, I. (2009): *Discoglossus scovazzi*. The IUCN Red List of Threatened Species 2009: e.T55272A11266253. [*accessed on: 3 December 2018*]

Samlali, M.A. (2016): Dynamique spatio-temporelle d'une population de Discoglosse peint (*Discoglossus scovazzi* Camrano 1978) dans le massif de l'Oukaimden (Haut Atlas Central - Maroc). Master II. Faculté des Sciences Semlalia.

Shine, R. (1979): Sexual selection and sexual dimorphism in the Amphibia. Copeia **1979**: 297-306.

Sinsch, U., Leskovar, C., Drobig, A., König, A., Grosse, W.R. (2004): Life-history traits in green toad (*Bufo viridis*) populations: indicators of habitat quality. Can. J. Zool. **85**: 665-673.

Sinsch, U., Oromi N., Sanuy, D. (2007): Growth marks in natterjack toad (*Bufo calamita*) bones: histological correlates of hibernation and aestivation periods. Herpetol. J. **17**: 129-137.

Spear, P.A., Boily, M., Giroux, I., Deblois, C., Leclair, M.H., Levasseur, M., Leclair, R. (2009): Study design, water quality, morphometrics and age of the bullfrog, *Rana catesbeiana*, in sub-watersheds of the Yamaska River drainage basin, Quebec, Canada. Aquat. Toxicol. **91**: 110-117.

Tsiora, A., Kyriakopoulou-Sklavounou, P.A. (2002): Skeletochronological study of age and growth in relation to adult size in the water frog *Rana epeirotica*. Zoology. **105**: 55-60.

Wagner, A., Schabetsberger, R., Sztatecsny, M., Kaiser, R. (2011): Skeletochronology of phalanges underestimates the true age of long-lived Alpine newts (*Ichthyosaura alpestris*). Herpetol. J. **21**: 145-148.

Wells, K.D. (2007): The Ecology and Behaviour of Amphibians. Chicago University Press, Chicago.

Zhelev, Z.M., Tsonev, S.V., Arnaudova, D.N. (2017): Health status of *Pelophylax ridibundus* (Pallas, 1771) (Amphibia: Ranidae) in a rice paddy ecosystem in southern Bulgaria: body condition factor and fluctuating asymmetry. Acta Zool. Bulg. **8**: 169-176.