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EIGHT SPECIES OF ANURAN AMPHIBIANS (AMPHIBIA, ANURA) FOUND IN UKRAINE: COMPARATIVE MORPHOLOGY AND CLASSIFICATION OF LARVAL DEVELOPMENT STAGES

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Eight Species of Anuran Amphibians (Amphibia, Anura) Found in Ukraine: Comparative Morphology and Classification of Larval Development Stages. Tkachenko, O. V. — Morphological characteristics typical for larval development stages of eight species of anuran amphibians found in Ukraine have been compared. Outer morphological features common for these species have been identified. Morphological features are suggested for determining the stages of larval development of various anuran amphibian's species, as well as the numbering of the stages after the larvae exit from the spawn membranes.
Key words: external gills, sucker, fin, operculum, the dental formula, hind limbs development, larval development stage.

Introduction

Over the years anuran amphibian larvae have been consistently used in experimental research in the fields of morphology, physiology, cytology, genetics and evolutionary studies. Various authors suggested charts demonstrating systemic data concerning larval development stages. However, these charts primarily address specific species like European grass frog, *Rana temporaria* (Hertwig, 1898; Kopsh, 1952; Dabagian, Sleptsova, 1975; Hertwig, Schneider, 1989) or Common European toad, *Bufo bufo* (Pysanets, 1989), rather than provide generalized data. As these charts facilitate studying certain species they appear to be unproductive for studying the others. Limiting the number of stages is insufficient for comparative analysis of several species for each of them and marked by specific ontogenetic characteristics. The manual on studying Amphibians (Lada, Sokolov, 1999) suggests using the chart by P. Terentiev (1950) for the study of anurans, although it addresses only 11 stages (from 20 to 30) of larval ontogenesis stages.

The most detailed chart (Gosner, 1960) provides generalized information on different American Amphibian species larval development. It is used for studying embryonic and larval development of American (del Pino et al., 2004) and other species (Sidorovska et al., 2002) of anuran amphibians. In recent publications summarized description of European species is absent.

Our primary objective is to consider external morphological peculiarities of several Amphibian species larvae found in Ukraine, define their specific morphological features and suggest a corresponding index of larval development stages in a sequence following larvae exit from the spawn membranes.

Materials and methods

Eight species of anuran amphibians were studied comparatively focusing on the material collected in Ukraine (table 1). The spawn was collected in natural ponds as well as obtained via artificial stimulated interbreeding.

The larvae were kept in plastic troughs of 10 L volume and 10 cm height of water column at room temperature. Water was substituted daily by desilting (for at least 24 hours) tap water. Since the beginning of active feeding till the metamorphosis stage the larvae were fed with boiled dandelion leaves. During the metamorphosis stage the troughs were biased to provide young animals' easier exit.

Fixed larvae were measured and their morphological features were described using the index of development stages suggested in (Gosner, 1960). Physiological processes like external gills development, fins development, operculum formation, hind limbs development, the beginning of fin resorption, front limbs visibility under the skin, adult-like mouth formation were disregarded. Their morphological structures were photographed with a digital camera equipped with a MBC-1 binocular at ×8 enhancement ratio. Electronic slide gauge was used to measure their linear parameters.

Table 1. Species and quantity of the investigated anuran amphibians larvae

Species	Date of obtaining	Place of collecting	Method of obtaining the spawn	Number of larvae analyzed
Bombina bombina Linnaeus, 1761	21.04.2004	Chernihiv, Kordivka	artificial inter-breeding	160
B. variegata Linnaeus, 1758	05.05.2005	Zakarpattia, Uzhhorod District, Kamjanytsia village, Plyshka Mountain	artificial inter-breeding	172
Pelobates fuscus Laurenti, 1768	07.04.2009 23.04.2010	Chernihiv, Bobrovytsia Volyn Region, «Shatski Lakes» resort, Liutsymer Lake	Collecting from a natural pond	982
P. vespertinus Pallas, 1771	21.04.2011	Kharkiv Region, Zmijiv District, Haydary village, Iskov pond	Collecting from a natural pond	1883
Hyla orientalis Bedriaga, 1890 "1889"	14.04.09, 28.04.11	Chernihiv, Bobrovytsia	artificial inter-breeding	806
Bufo bufo Linnaeus, 1758	09.04.2008	Chernihiv, Kordivka	Collecting from a natural pond	1374
B. viridis Laurenti, 1768	20.04.12, 27.04.12	Chernihiv, Bobrovytsia	Collecting from a natural pond	391
R. arvalis Nilsson, 1842	12.04.2004 12.04.2005	Chernihiv, Kordivka Chernihiv Region, Chernihiv District, Riabtsy village	Collecting from a natural pond	630

Results and discussion

Larval morphology at various development stages. Larvae of all 8 species leave the spawn membranes at stages 17–20. Up until stage 20 that occurs approximately within 2–3 days[1] they live in a stationary manner as their adhesion apparatus (a sucker or external gills) is well developed.

At stages 21–22 the fin develops and external gills reach their maximum size, larval mouth apparatus begins to develop involving the emergence of lips and corneous jaws (although various species differ in its final configuration — table 2).

Operculum develops intensively and opercular aperture is formed at stages 23–25. It is located on the left side along the bodily centerline of 6 considered species; except for the fire-bellied toad opercular hole appears on the abdomen side.

Table 2. Morphological features of 8 species of anuran amphibians in temporal dynamics

Species	*Bombina bombina*	*B. variegata*	*Pelobates fuscus*	*P. vespertinus*	*Hyla orientalis*	*Bufo bufo*	*B. viridis*	*Rana arvalis*
Stages of external gills' maximum development	22		21		21–22	21		22
Stages of larval mouth formation / dental formulae	25 1:1+1/3 27 dental rows are doubled	24 2/3 29 dental rows are doubled	26–34 The formula transforms from 1:1+1/2+2:1 to 1:3+3/4+4:1 as the number of dental rows increases	25–41	26 1:1+1/3	25 1:1+1/3		26 1:1+1/1+1:2 or 1:1+1/1+1:3

[1] The case when hatching occurs at stages 17–18; though some species demonstrate stage 20 developments while being still within the spawn.

Beginning with stage 26 the larvae form the rudiments of hinder limbs, start swimming freely, their body and tail start growing intensively. This growth encompasses pre-metamorphic stages 40–41 marked by the fin's cloacal part's reduction and front limb's visibility under the skin.

During stages 42–44 larvae form "adult-like" mouth, the tail undergoes intensive resorption and the animal gradually leave the water. At stage 45 they exercise the adult nutrition model; at stage 46 the remnants of the tail disappear thus completing the metamorphosis.

The suggested classification of larval development stages results from the comparative study of 8 species common external morphological indices: the degree of external gills, fins, operculum, hind limbs development, reduction of the tail cloacal part, visibility of the front limbs under the skin, the formation of adult-like mouth.

We also considered the following complementary indices: structure and the degree of development / reduction of the sucker and larval mouth, front limbs emergence, the degree of tail resorption, body linear size. As these indices are specific for each species we disregard them while identifying the above mentioned development stages.

Considering the obtained data and the charts of normal development (Gosner, 1960), we speak of 29 stages of larval development starting from their leaving the spawn membranes up till the completion of metamorphosis.

The first three stages are marked by larvae external gills development. At these stages most species leave the spawn membranes, while their suckers are well developed (fig.1).

At stages 4 and 5 the fin begins developing (fig. 2), while operculum does so at stages 6–8; at the same stages the larval mouth apparatus undergoes development (fig. 3).

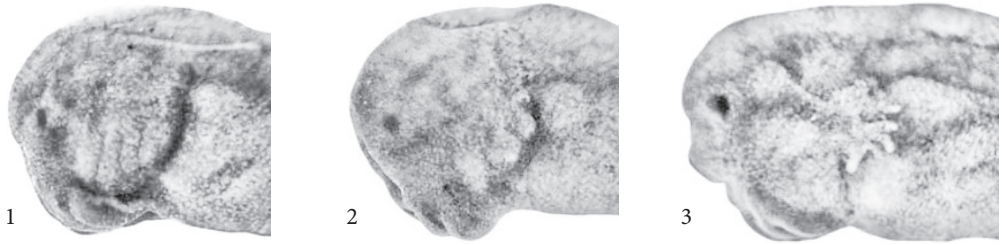

Fig. 1. Stages of external gills' development: 1 — external gills ridges get separated; 2 — gills branches embryos; 3 — emergence of gills filaments on external gills branches (filaments development may vary; operculum has not started developing yet).

Fig. 2. Stages of fin development: 4 — the tail fin is developed but not yet transparent; 5 — the body of tail fin is transparent (at this stage operculum may cover the base of gills filaments).

Fig. 3. Stages of operculum's development: 6 — operculum touches the belly skin or accretes it, gills can be seen from both sides; 7 — operculum completely covers gills from one (right) side; 8 — external gills are completely covered by operculum.

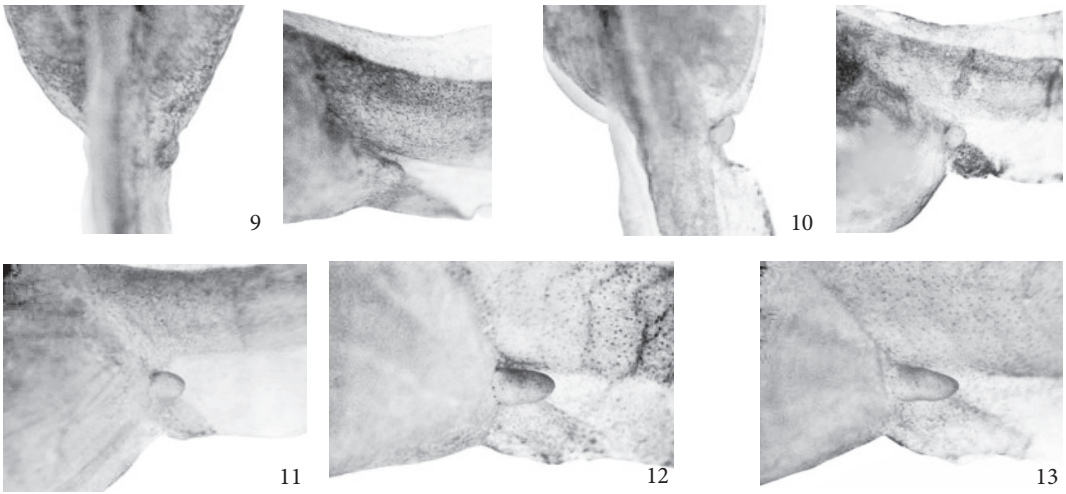

Fig. 4. Stages defined according to limb bud's length and diameter correlation: 9–l < 1/2d; 10–l ≥ 1/2d; 11–l ≥ 1d; 12– l ≥ 11/2d; 13–l = 2d.

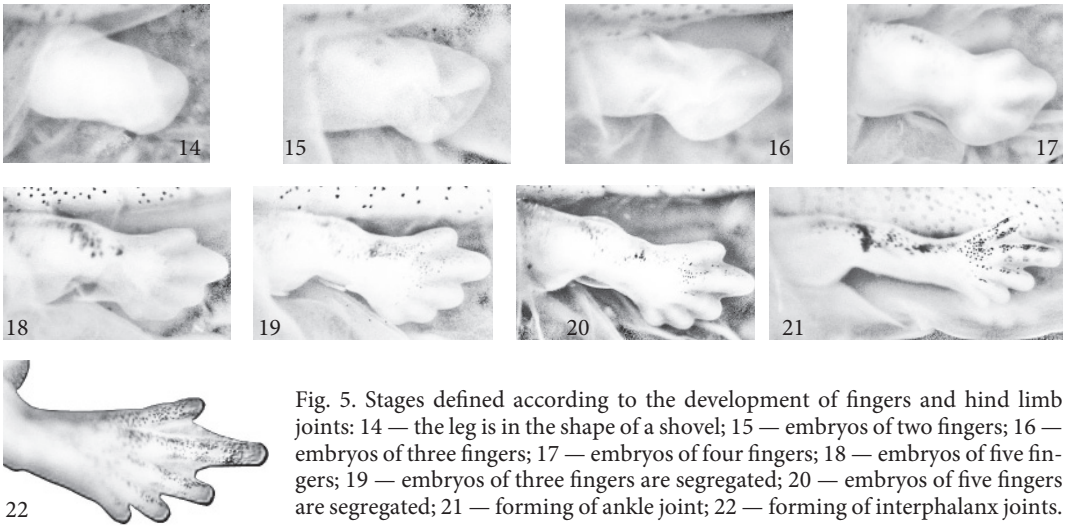

Fig. 5. Stages defined according to the development of fingers and hind limb joints: 14 — the leg is in the shape of a shovel; 15 — embryos of two fingers; 16 — embryos of three fingers; 17 — embryos of four fingers; 18 — embryos of five fingers; 19 — embryos of three fingers are segregated; 20 — embryos of five fingers are segregated; 21 — forming of ankle joint; 22 — forming of interphalanx joints.

Then the stages (9-13) of hind limbs buds development unfold. Their dynamics is determined by the limbs length-to-diameter correlation (fig. 4) and development of fingers and joints (stages 14–22) (fig. 5).

Processes related to metamorphosis are explicit at stages 23 and 24 marked by the reduction of the cloacal tail piece and visibility of front limbs under the skin (fig. 6).

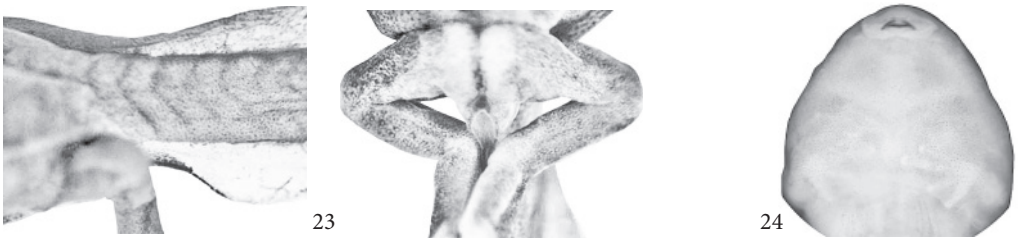

Fig. 6. Stages characterizing the beginning of metamorphosis: 23 — resorption of the fin's cloacal piece; 24 — front limbs are seen through the skin.

Larval development ends in reduction of larval mouth and formation of adult-like mouth (stages 25–28). At these stages most species demonstrate fast tail resorption; at stage 25 front limbs become free. Metamorphosis ends at stage 29 with the tail's complete resorption (fig. 7).

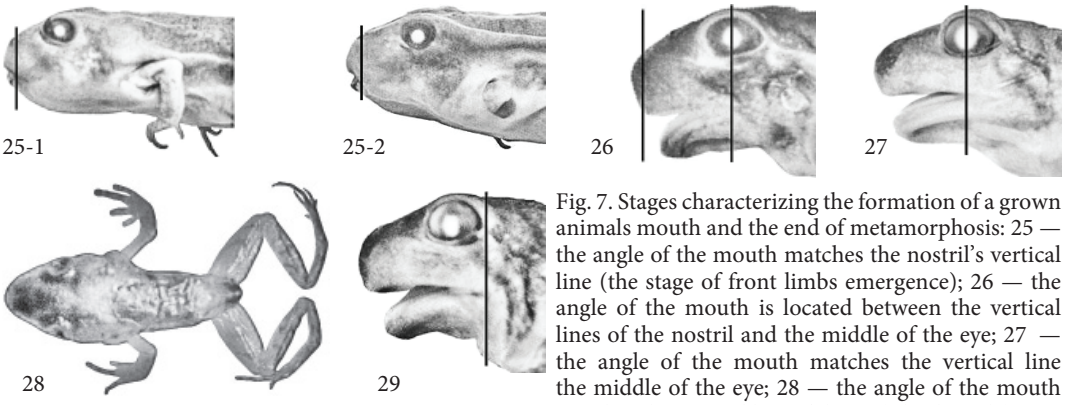

Fig. 7. Stages characterizing the formation of a grown animals mouth and the end of metamorphosis: 25 — the angle of the mouth matches the nostril's vertical line (the stage of front limbs emergence); 26 — the angle of the mouth is located between the vertical lines of the nostril and the middle of the eye; 27 — the angle of the mouth matches the vertical line the middle of the eye; 28 — the angle of the mouth matches the vertical line of the eye's back angle's vertical line; 29 — metamorphosis is complete.

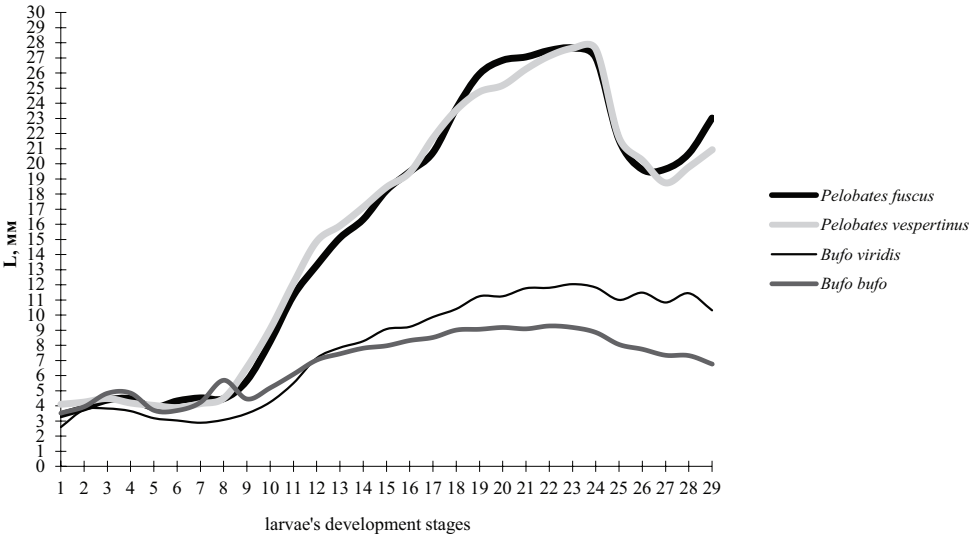

Fig. 8. Dynamics of four species' anuran amphibians body linear parameters.

The effectiveness of the discussed stages distribution is supported by comparing the dynamics of linear parameters of *Pelobates fuscus*, *P. vespertinus*, *Bufo viridis* and *B. bufo* larvae's bodies (Tkachenko, 2009, 2012) (fig. 8).

At initial eight stages the mean values of the compared species body length are virtually the same. Starting with stage 9, *Pelobates* grow faster reaching their maximum size at stages 19–25. At these stages they are more than 3 times larger than *Bufo* larvae. At the beginning of metamorphose (stage 24) *Pelobates* bodies shrink. Their growth renews at stage 27 as the younglings of the current year shift to the life on dry land.

Bufo larvae grow more consistently though there are certain differences concerning different species. At stages 1–12 the bodies of *B. bufo* larvae are longer than the larvae of *B. viridis* while at stages 12–29 the correlation is reversed.

At three initial stages larvae exist in a static manner as their nutrition is provided by yolk bags, thus their sucker is well-developed. Sucker's structural peculiarities facilitate species' identification (fig. 9).

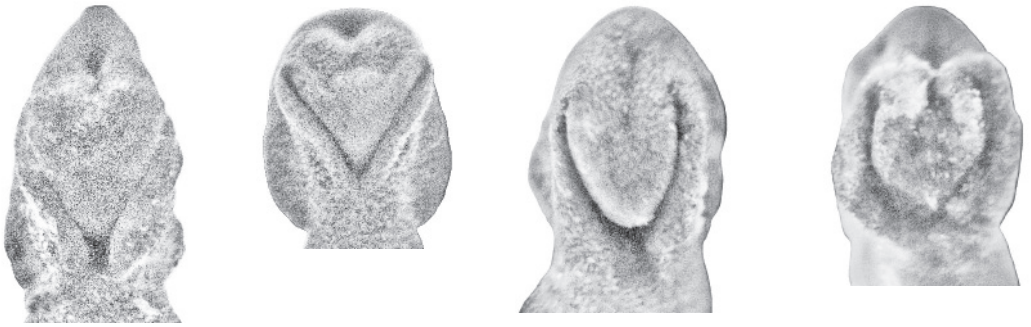

Fig. 9. The structure of four species' anuran amphibian's sucker at the first stage of development (view from below).

Conclusion

Larvae of the 8 studied species of anuran amphibians have similar morphological structures successively substituting each other over the period of larval development. Various species demonstrate specific features of the said structures.

We divide the whole water phase of ontogenesis (starting with larvae leaving the spawn membranes up until the end of metamorphosis) into 29 stages according to their basic morphological transformations. Using the larvae outer morphological markers facilitates defining stages of development of both live objects and fixed material.

The suggested classification of anuran amphibian's larval development stages allows comparing morphological features of larvae belonging to various species. The results of respective comparative research can be applied to designing diagnostic keys to be employed while identifying species at any stage of the water phase of their ontogenesis.

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References

Dabagian, N. V., Sleptsova, L. A. 1975. Common frog *Rana temporaria* L. *In: Objects of biology of development.* Science, Moscow, 442–462.

del Pino, E. M., Avila, M. E., Perez, O. D., Benitez, M.-S., Alarcon, I., Noboa, V., Moya, I. M. 2004. Development of the dendrobatid frog *Colostethus machalilla*. *Int. J. Dev. Biol.*, **48**, 663–670.

Gosner, K. L. 1960. A simplified table for staging anuran embryos and larvae. *Herpetologica*, **16**, 183–190.

Hertwig, I., Schneider, H. 1989. A revised staging table for better identification of embryos and larvae of the grass frog *Rana t. temporaria* L. (Amphibia, Anura). *Zool. Jb. Anat.*, **118**, 309–324.

Kopsch, F. 1952. Die *Entwicklung des braunen Grasfrosches Rana fusca Roesel, dargestellt in der Art der Normentafeln zur Entwicklungsgeschichte der Wirbeltiere.* G. Thieme, Stuttgart, 1–70.

Lada, G. A., Sokolov, A. S. 1999. *Methods of studying amphibians. Scientific and methodical manual.* Tambov G. R. Derzhavin state University, Tambov, 1–75.

Pysanets, Ye. M. 1989. Basic approaches towards studying g. *Bufo*. *In: A manual for studying amphibians and reptiles.* Kyiv, 46–72.

Sidorovska, V., Ljubisavljevic, K., Dzukic, G., Kalezic, M. L. 2002. Tadpole morphology of two spadefoot toads (*Pelobates fuscus* and *P. syriacus*) (Amphibia, Anura, Pelobatidae). Spixiana, München, 25/2, 183–191.

Terentiev, P. V. 1950. *A Frog*. Soviet science, Moscow, 1–348.

Tkachenko, O. V. 2009. Peculiarities of common toad's larval development (*Bufo bufo* Linnaeus, 1758) (Amphibia, Anura) on the territory of Ukraine. *Papers of the Ukrainian herpetologic society*. Kyiv, 2, 8189.

Tkachenko, O. V. 2012. Transformations of larvae of Common spadefoot toads (Amphibia: Anura: Pelobatidae) found in Ukraine (preliminary data). *Issues of herpetology. Papers of the 5th Congress of A. N. Nikolsky herpetologic society (Minsk, Belarus, April 25–28, 2012).* Law and economics, Minsk, 318–321.

Zabroda, S. N., Iljenko, E. P. 1981. Common spadefoot toads — *Pelobates fuscus* (Laur.) found in Ukrainian Polissia: peculiarities of reproduction and larval development. *Vestnik Zoologii*. 4, 66–71 [In Russian].

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