A Comparative Normal Table of Anuran Development: Towards a Unified Framework for Xenopus and Non-Model Species

Shouhong Wang

wangsh@cib.ac.cn

Chengdu Institute of Biology, Chinese Academy of Sciences

Yanmei Cai

Sichuan Normal University

Zerui Yang

Yibin University

Bin Wang

Chengdu Institute of Biology, Chinese Academy of Sciences

Jianping Jiang

Chengdu Institute of Biology, Chinese Academy of Sciences

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A Comparative Normal Table of Anuran Development: Towards a Unified Framework for *Xenopus* and Non-Model Species

Shouhong Wang[a,b], Yanmei Cai[c], Zerui Yang[d], Bin Wang[a,b], Jianping Jiang[a,b]*

[a]Mountain Ecological Restoration and Biodiversity Conservation Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, China

[b]University of Chinese Academy of Sciences, Beijing, China

[c]College of Life Science, Sichuan Normal University, Chengdu 610101, China

[d]Yibin University, Yibin 644000, China

*Corresponding author at: Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610213, China

E-mail address: wangsh@cib.ac.cn; jiangjp@cib.ac.cn.

Abstract

Background Developmental staging tables, primarily defined by specific external morphological characteristics, are fundamental tools in amphibian research. Although numerous staging tables have been reported for various frog species, significant differences are often observed, especially when compared to the canonical model organism *Xenopus*. To date, no comprehensive study has integrated *Xenopus* developmental stages with the other widely used anuran staging systems, such as the Gosner table.

Results To bridge this gap, we present the first comparative staging system that directly aligns the *Xenopus* developmental table with two widely used non-model anuran staging systems. This integrated framework was validated through detailed morphological observations across the entire embryonic development of three representative species—*Xenopus laevis*/*tropicalis*, *Microhyla fissipes*, and *Odorrana tormota*—each exemplifying a major staging paradigm. Our system comprehensively covers both early embryonic and postembryonic developmental stages.

Conclusions This integrated system provides a unified foundation for analyzing morphological development in anurans. It is expected to facilitate cross-species comparisons and promote interdisciplinary research in areas such as ecotoxicology, evolutionary developmental biology, and conservation physiology.

Keywords Developmental table, Morphology, Anura, *Xenopus*, *Microhyla fissipes*, *Odorrana tormota*

Background

Frog amphibians have long served as foundational models in experimental embryology and developmental biology, underpinning many landmark discoveries in the field [1-3]. Their large, robust eggs and embryos are easily accessible for micromanipulation, enabling researchers to gain mechanistic insights into early developmental processes [4-6]. Moreover, the complex biphasic life cycle of most frogs, transitioning from aquatic herbivorous larvae to terrestrial carnivorous adults via metamorphosis, offers a powerful natural system for investigating phenotypic plasticity, cell proliferation, differentiation, and apoptosis [7-11]. Additionally, their permeable skin and dual-phase lifestyle make them highly sensitive bioindicators for assessing environmental toxicity [12].

Developmental staging tables are indispensable for standardizing research across laboratories. They provide a temporal framework for understanding morphological transformations, such as cell cleavage patterns, organogenesis (e.g., eyes, limbs, tail), spiracle closure, and tail resorption- and are critical for defining developmental windows and ensuring experimental reproducibility [13, 14]. To date, over 100 anuran species have been documented with species-specific staging tables [15-23]. Although early embryonic and postembryonic development are largely conserved across anurans, significant interspecific variations necessitate the integration of disparate staging systems, especially when comparing non-model species with the well-established *Xenopus* framework.

As we know, 3 major staging systems are widely used for describing ontogenetic development in anurans. One is the extensively adopted system created by Gosner (1960), and has been applied in numerous studies [11, 17, 20, 24-28]. This system defines 46 developmental stages based on both external morphological and physiological characteristics, divided into two main phases: 1) early embryonic period (stages G1-25), spanning from fertilization to operculum completion stage; 2) the postembryonic period (G26-46), covering from operculum completion to full tail regression [29, 30]. A second system, similar to that of Gosner, comprises 45 developmental stages and likewise includes an early embryonic period (stages S1-28) and a postembryonic phase (S29-45) [8, 22, 31, 32]. In addition, the model organism *Xenopus* has its unique developmental staging system, constructed by Nieuwkoop and Faber (1965). This system encompasses 66 stages from fertilization through complete metamorphosis, also divided into an embryogenesis period (NF1-45) and larval developmental period (NF46-66) [18, 23, 33-35]. A straightforward comparison among these 3 staging systems is a crucial first step toward integrating the developmental timelines of *Xenopus* with non-model anurans, thereby providing a foundation for further comparative and mechanistic research.

In this study, we present a detailed comparative developmental table that integrates 3 major anuran staging systems, based on detailed morphological observations of *Xenopus laevis/tropicalis, Microhyla fissipes*, and *Odorrana tormota*. This unified framework enables precise cross-species staging and uncovers fundamental developmental differences between fully aquatic *Xenopus* and typical anurans, which

undergo aquatic-to-terrestrial transitions. By establishing standardized criteria, our work enhances the comparability of developmental research and offers deeper insights into embryological diversity, evolutionary adaptation, and underlying developmental mechanisms.

Materials and methods

Animal husbandry

Adult *M. fissipes* were collected from farmlands in Shifang City, Sichuan Province, China, while adult *O. tormota* were obtained from Huangshan Hot Springs. Wild-type adults of *X. tropicalis* and *X.laevis* were purchased from NASCO (Fort Atkinson, WI, USA; http://www.enasco) and subsequently reared in our laboratory. *M. fissipes* and *O. tormota* adults were housed individually in plastic containers (325 mm ×22 mm ×25 mm) with moistened sponges to maintain high humidity. Similarly, *Xenopus* adults were maintained in plastic containers with a water depth of 75 mm. All animal procedures were approved by the Animal Care and Use Committee of Chengdu Institute of Biology.

Induced breeding with LHRH-A3 and hCG in frogs

Induced breeding in both male and female *M. fissipes and O. tormota* was conducted following standard procedures [22]. Briefly, each frog received a single intraperitoneal injection of luteinizing hormone-releasing hormone A3 (LHRH-A3) at a dosage of 0.3 μg/g body weight. For sexually mature *X. tropicalis* and *X. laevis*, an initial priming injection of 20 U of human chorionic gonadotropin (hCG) was administered on day 1. The following day, males and females of both *Xenopus* received

subsequent hCG boosts: males administered 200 U, while females received 250 U for *X. tropicalis* and 650 U for *X. laevis*, respectively.

Tadpole feeding and observation during embryonic development

Fertilized embryos were collected and placed into larger plastic containers (325 mm ×405 mm × 115 mm; water depth: 80 mm) for rearing. Tadpoles were maintained at a temperature of 25 ± 0.5 °C under a 12:12 hour light/dark cycle. All tadpoles were fed spirulina powder (China National Salt Industry Corporation) twice daily, and the water was replaced every two days. Developmental stages of *O. tormota, M. fissipes, and Xenopus* were determined based on the staging tables reported by Gosner (1960), Wang et al. (2017) and Nieuwkoop and Faber (1965), respectively. Morphological characteristics of the tadpoles and the photographs of each animal were acquired using a stereo microscope (JSZ8T, Jiang Nan Yong Xin, Nanjing, China) equipped with the Mshot Image Analysis system (Mc50-N).

Results

Early embryonic development stage

The early embryonic development period of anurans is often described from the zygote formation to the operculum completion (G1/S1/NF1-G25/S28/NF45). This period encompasses several distinct stages, including fertilization, cleavage, blastula formation, gastrulation, neurulation, tail elongation, eye development, gill development, and the formation of the spiracle (Table 1).

Zygote and cleavage stage

Shortly after fertilization, the egg undergoes rotation, with the darkly pigmented

134 animal hemisphere moving to the upper side while the lighter vegetal hemisphere faces
135 downward. The cleavage phase is commonly characterized by the number of cells,
136 spanning from the 2-cell stage (G3/S3/NF2) to 32-cell stage (G7/S7/NF6). In contrast,
137 the blastula phase is typically distinguished based on cell size (Table 1, Fig.1).

138 Most anuran species, such as *Xenopu*s, *M. fissipes*, and *O. tormota*, exhibit a
139 "standard" cleavage pattern. This pattern is defined by the first two cleavages being
140 meridional, followed by a third latitudinal cleavage, and then an extensive series of
141 synchronous divisions in the animal hemisphere (Fig.1 A1-C1).

142 **Gastrula and neurula stage**

143 The onset of gastrula stage (G10/S11/NF9-G12/S14/NF11-13) is characterized by
144 blastopore formation, followed by dynamic changes in its size and shape (Table 1,
145 Fig.1A2-C2). During the late gastrula stage (G12/S14/NF11-13), the yolk plug
146 disappears, resulting in a horseshoe-shaped embryo (Table 1). Subsequent neural stages
147 are marked by the sequential development of the neural plate (G13/S15/NF14), the
148 elevation of neural folds (G14/S16/NF15-17), and the closure of the neural tube
149 (G16/S18/NF19-21) (Table 1, Fig.1A3-C3).

150 **Embryo and organogenesis stage**

151 During the embryo and organogenesis stages, key morphological structures
152 emerge sequentially. A tail bud becomes visible at the posterior end, followed by the
153 formation of a gray eye cup and progressive tail elongation around stages G17-18/S19-
154 21/NF22-32 (Table 1, Fig. 1A4-C4). Notably, a heartbeat is first detectable at
155 G19/S22/NF33-34. Subsequently, gill buds appear and expand, coinciding with embryo

hatching (G20/S23/NF35-36). During this period, developmental staging can also be assessed using intestinal morphology. For example, by G24/S26/NF44, the intestine develops a distinct S-shaped loop (Fig. 1A5-C5). Finally, the operculum subsequently forms, enveloping the gill buds and completing spiracle formation. This stage marks the onset of feeding and the transition to free-swimming tadpoles (Table 1, Fig.1 A5-C5).

Postembryonic development stage

Generally, the postembryonic development period, spanning from hindlimb bud emergence to tail resorption (G26/S29/NF48-G46/S45/NF66), is characterized by series of morphological transformations. These include limb bud development, hindlimb growth, toes differentiation and growth, head reshaping, and ultimately tail resorption. It should be noted that the endpoint of this period differs between species. In *Xenopus*, it concludes at the end of metamorphosis (NF66), whereas in most other anurans, it is defined by the complete disappearance of the tail (G46/S45) (Table 2).

Limb development and growth

Tadpoles at G26/S29/NF48 are clearly identified by the presence of gold iridophores around the gut and the emergence of hindlimb buds (Table 2, Fig.2A1-C1). Subsequent developmental stages from G27/S30/NF49 to G29/S32/NF51 are readily determined by the length/width ratio of the hindlimb bud. For instance, by G28/S31/NF50, the hindlimb bud length equals its width (Table 2). Additionally, the hindlimb bud exhibits an oar-shaped outline of the foot by G31/S33/NF52.

Next, from G32/S34/NF53 to G36/S36/NF54, developmental staging is determined by the degree of toe differentiation. The process begins at G32/S34/NF53 and completes at G36/S36/NF54, when all five toes are fully distinct (Table 2, Fig.A2-C2; Fig.A3-C3). This phase represents a key transition from pre- to pro-metamorphosis.

Further stages can be identified through toe morphology, including the appearance of metatarsal and subarticular tubercles. By G40/S40/NF58, tadpoles reach their maximum body and tail length. In *Xenopus*, forelimbs erupt from the ventral skin at this stage (NF58) (Table 2).

Tail resorption and head reshaping

These stages represent the transition from the climax of metamorphosis to the end of metamorphosis (G40/S40/NF58-G46/S45/NF66) (Table 2, Fig. 3). During this period, tail resorption and head narrowing occur progressively. Tail resorption initiates around G42/S42/NF60-61, accompanied by a slight reduction in tail length and narrowing of the head, coinciding with near-peak plasma T3 levels.

By G43/S42-43/NF62, around 2/3 of the tail is resorbed. Additionally, the head becomes narrower than the trunk, while the tail remains slightly longer than the body. In most anuran species except *Xenopus*, forelimbs erupt from the ventral skin at this stage (S42/G42) (Fig. 3B2-C2). Next, rapid tail resorption occurs between G44/S43/NF63 and G46/S45/NF66, eventually in the completion of metamorphosis and the formation of a tailless frog.

Discussion

In this study, we established the first integrated and comprehensive staging system that combines 3 major anuran developmental systems: the widely used Gosner (G) stages (G1-66), a second key anuran staging system (S1-45), and the classic Nieuwkoop and Faber (NF) Normal Table for the model species *Xenopus* (NF1-66). Our comprehensive framework covers both early embryonic (G1/S1/NF1-G25/S28/NF45) and postembryonic (G26/S29/NF48-G46/S45/NF66) development, where the aquatic tadpoles undergo metamorphosis into a juvenile frog [17, 18, 22]. This integrated framework offers a standardized and efficient tool for accurate developmental staging across diverse anuran species, offering a critical reference for ecological, conservation, and experimental studies where accurate and consistent developmental staging is crucial.

Larval period: rapid and efficient organization provides essential materials for researching organ development

Early tadpole development, characterized by external fertilization, cleavage, neurulation, tail elongation, eye development, is a rapid, complex, and tightly regulated process, exhibiting distinct stage-specific characteristics that render them highly valuable as developmental biology models [36-38]. Developmental time courses, however, varies among different species. For example, it takes around 20 minutes in *Xenopus* and 10 minutes in *M. fissipes* at 25°C to complete the fertilization [22, 39]. Embryonic development lasts 3-4 days in *X. tropicalis* at 25°C, compared to 4 days in *X. laevis* at 20°C [18, 34]. *M. fissipes* requires around 70 hours at 24-26°C, whereas *Kaloula borealis* completes embryogenesis in just 30 hours at room temperature [20,

22]. Yet, some species, such as those in the family Megophryidae, exhibit markedly prolonged early embryonic development; for example, *Vibrissaphora leishanensis* takes approximately 116 days (2,784 hours) at 6.3–11.5°C [40]. This diversity in developmental timing is fascinating and may represent a significant aspect of the evolutionary embryology.

At the single-cell embryo stage (G1/S1/NF1), techniques such as microinjection and fluorescence labeling enable highly efficient gene knockout or knockin [41-43], allowing precise functional genetic manipulation via targeted inhibition or overexpression. After fertilization, rapid cell divisions (cleavage) occur without an increase in overall egg volume, offering a powerful model for investigating the mechanisms and regulation of early mitotic cycles. In addition, these stages serve as models to assess the impact of external factors (e.g., temperature, chemical exposure) on cell division [32, 44]. Subsequent gastrulation is a fundamental process of metazoan embryogenesis, during which germ layers are formed through extensive cell movements and differentiation [45, 46]. Importantly, as the neural tube eventually develops into the central nervous system, including the brain and spinal cord, “Neural stages” (G13-16/S15-18/NF14-21) are crucial for studying early neurodevelopment [47, 48].

Once the neural tube has formed, it induces changes in its neighbors, and organogenesis continues [49]. The initiation of tail bud (G17/S19/NF22-25) provides a suitable window for investigating tail development and regeneration [6, 34, 50]. For example, Wang et al. (2021) showed that the tail could fully regenerative from the tail

bud stage to the onset of metamorphosis climax (NF60/61) except for a refractory period (NF45-47), which temporarily lost the regenerative ability in *X. laevis* [34, 51, 52]. This regenerative transition offers a useful model to deciphering molecular mechanisms governing regeneration competence.

In addition, by the tadpole stage, gill development supports efficient oxygen exchange, enabling locomotion and feeding [53]. This period is marked by active organogenesis, with upregulated expression of genes essential for the development and functional maturation of the heart, liver, kidneys, and other organs [32, 54]. Their transparency, permeable skin, and aquatic lifestyle of tadpoles also make them ideal for toxicological and environmental studies [55-57]. For example, Chen et al. (2025) examined triclosan-induced neurotoxicity in *Rana omeimontis* tadpoles (G22) [57], while Chai et al. (2023) explored heavy metals effects in *Bufo gargarizans* tadpoles (G26-38) [58]. Overall, frog larval development is an ideal and essential model for developmental, evolutionary, and conservation studies, as it is an independent process that is not influenced by maternal factors and develops rapidly.

Metamorphosis: an evolutionary transition from aquatic to terrestrial life

Frog metamorphosis is a postembryonic process marked by dramatic morphological changes, including de novo organ formation (such as limbs), remodeling of existing organs (including the intestine, brain, and pancreas) and regression of larval structures (e.g., gills and tail) [9, 59]. This fascinating event in frogs is primarily orchestrated by thyroid hormone (TH) via precise gene regulatory mechanisms [8, 60]. Thus, amphibian metamorphosis provides us with an excellent model for understanding

the fundamental mechanisms that govern postembryonic organ development and programmed tissue remodeling.

Numerous studies have been carried out in frogs during the post-embryonic development period, especially during the metamorphosis stage. There are a number of similarities between anuran metamorphosis and postembryonic development in mammals, including thyroid hormone secretion, organ maturation, tissue regression, a switch from fetal or larval to adult hemoglobin, and the transition from aquatic to a terrestrial environment (from uterus to delivery) [8, 53, 60-64]. Understanding these conserved developmental events in frogs can thus provide insights into human developmental disorders.

Frogs as model organisms for organ regeneration

Elucidating how to promote organ and appendage regeneration is a major goal of regenerative medicine [65]. Intriguingly, many frog organs, such as tail, heart, brain, and spinal cord, can achieve both scar-free healing and tissue regeneration during their larval stages, while this ability declines during metamorphosis as TH level rise [10, 33, 65, 66]. In addition, elevated TH levels have been reported to inhibit appendage regeneration, such as heart and tail [67, 68]. For example, Marshall et al. (2019) revealed that both TH excess and natural peaks during metamorphosis disrupt heart regeneration in *Xenopus*, altering fibrotic responses and extracellular matrix gene expression [33]. Concurrently, we and others have observed substantial cardiac remodeling during metamorphic climax (Additional file 1) [33, 69], including chamber separation, cardiomyocytes mature, and a decline in regenerative capacity. It remains

unclear whether these structural changes influence the cardiomyocyte dedifferentiation and impair regenerative ability [70]. Interestingly, neonatal mammals also exhibit transient cardiac regenerative potential that is lost postnatally as TH levels increase [71,72], suggesting an evolutionarily conserved role for TH in modulating tissue regeneration. Investigating the TH-mediated loss of regenerative capacity in frogs may therefore yield novel insights for regenerative medicine.

Conclusion

In summary, the integrated staging system proposed in this study establishes a robust and unified framework for comparative developmental research in anurans, facilitating more precise cross-species analyses between *Xenopus* and non-model frogs. By systematically aligning staging tables and morphological characteristics, our work uncovers both conserved developmental patterns and species-specific adaptations. Anuran embryonic development, including both early embryonic and postembryonic development, emerges as a powerful and independent model for investigating fundamental questions in developmental biology, evolution, regeneration, toxicology, and environmental adaptation. Importantly, we highlight the use of diverse frog species—not just the established model *Xenopus*—in molecular, cellular, toxicological, and evolutionary investigations. Future applications of advanced methods such as micro-CT, molecular profiling, and single-cell technologies will further elucidate the conserved and unique mechanisms underlying anuran development, supporting future discoveries in basic and translational science.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare no competing interests.

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Authors' contributions

Shouhong Wang: Writing–original draft, Writing–review & editing, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Yanmei Cai:** Data curation, Writing – review & editing. **Zerui Yang:** Data curation, Writing–review & editing. **Bin Wang:** Writing–review & editing, Supervision. **Jianping Jiang:** Writing – review & editing, Supervision.

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Figure Legends

Fig.1 The comparison analysis of representative embryonic developmental stages across 3 species: (A) *Xenopus,* (B) *Microhyla fissipes,* and (C) *Odorrana tormotus*; (A1-C1) Cleavage stages; (A2-C2) Gastrula stage; (A3-C3) Neurula stage; (A4-C4) Tail development; and (A5-C5) Operculum formation. Red arrows (a) show the development of optic vesicles, while red arrows (b) indicate tail bud development. Scale bars are 0.5 mm (A1-C4) and 2 mm (A5-C5).

Fig.2 The comparison analysis of representative limb bud developmental stages across 3 species: (A) *Xenopus*, (B) *Microhyla fissipes*, and (C) *Odorrana tormotus*; (A1-C1) Appearance of limb bud; (A2-C2) Development of limb bud; (A3-C3) Toe differentiation. Red arrows show the magnification of limb buds or differentiated toes. Scale bars are 2 mm.

Fig.3 Tail resorption stages in *Xenopus* (A)*, Microhyla fissipes* (B), and *Odorrana tormotus*(C). (A1-C1) Eruption of forelimbs (dorsal view); (A2-C2) Tail resorptions at metamorphosis climax (dorsal view); (A3-C3) Completion of metamorphosis (dorsal view). Scale bars are 2 mm.

Table Legends

Table 1. Comparison of early embryonic development among the major 3 staging tables, including Goner (1960) for most anurans, Wang (2017) for *Microhyla fissipes*, and Nieuwkoop and Faber (1956) for the model species *Xenopus*.

Table 2. Comparison of post-embryonic development among the major 3 staging tables, including Goner (1960) for most anurans, Wang (2017) for *Microhyla fissipes*, and Nieuwkoop and Faber (1956) for the model species *Xenopus*.

Figures

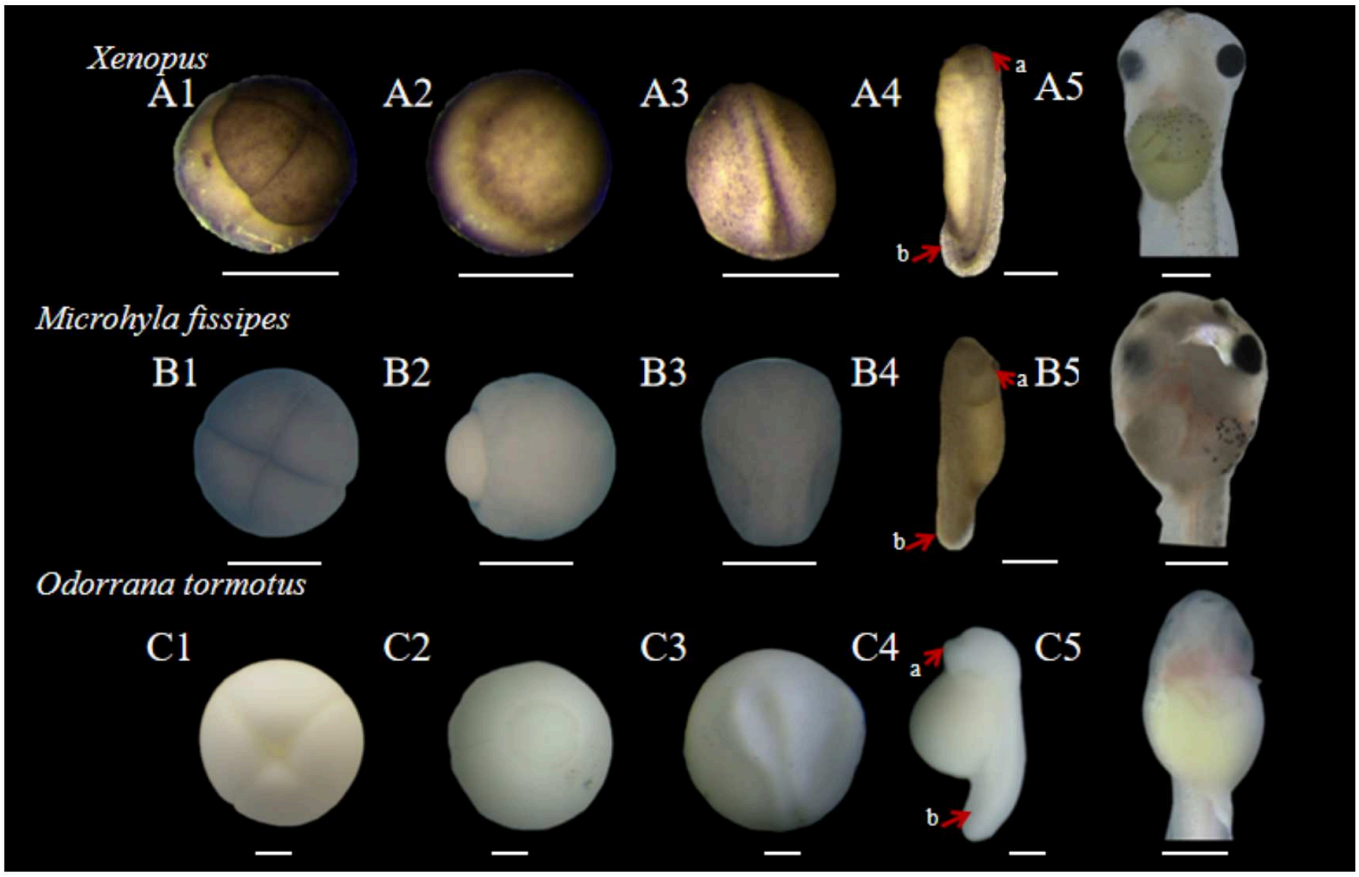

Figure 1

The comparison analysis of representative embryonic developmental stages across 3 species: (A) Xenopus, (B) Microhyla fissipes, and (C) Odorrana tormotus; (A1-C1) Cleavage stages; (A2-C2) Gastrula stage; (A3-C3) Neurula stage; (A4-C4) Tail development; and (A5-C5) Operculum formation. Red arrows (a) show the development of optic vesicles, while red arrows (b) indicate tail bud development. Scale bars are 0.5 mm (A1-C4) and 2 mm (A5-C5).

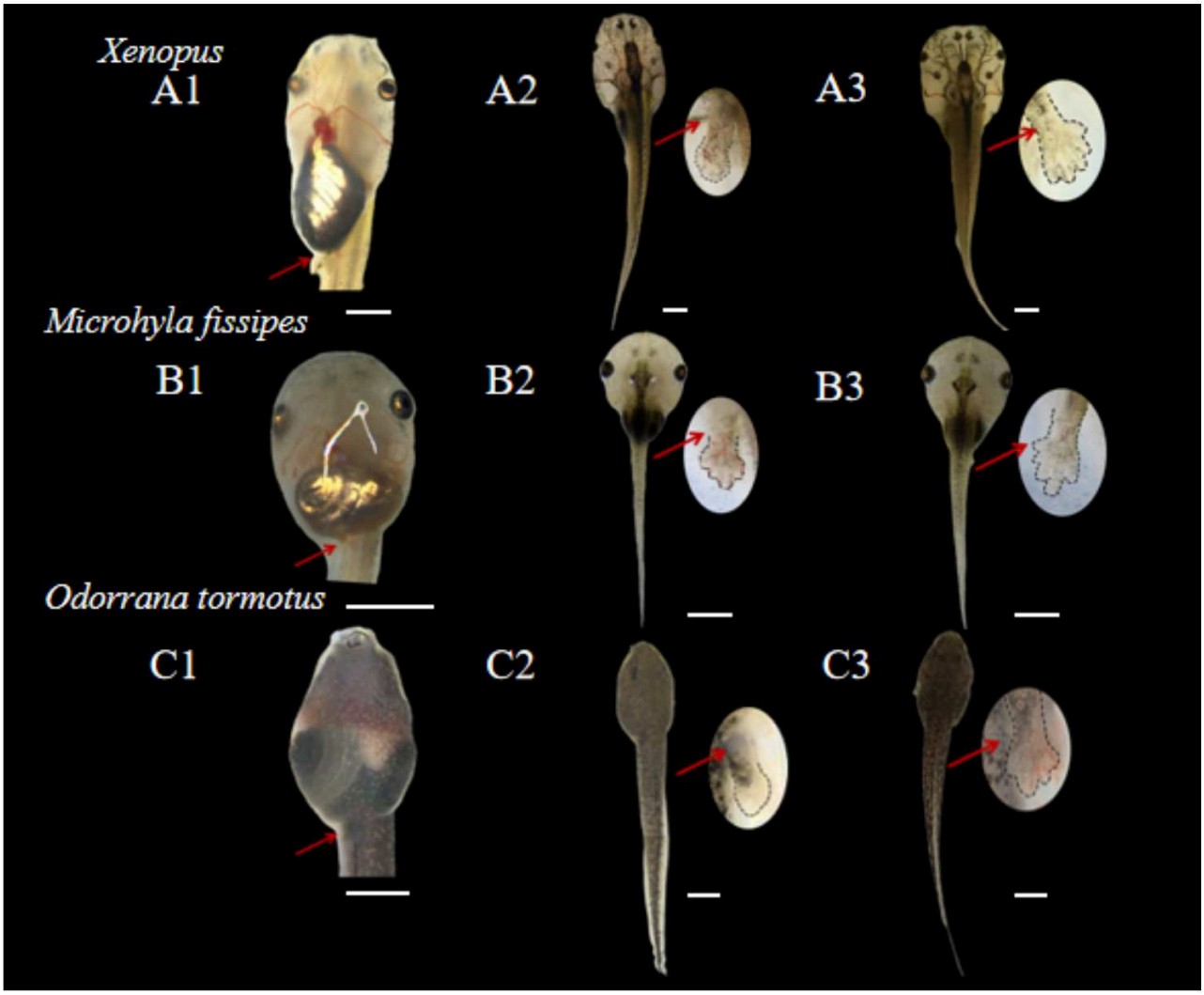

Figure 2

he comparison analysis of representative limb bud developmental stages across 3 species: (A) Xenopus, (B) Microhyla fissipes, and (C) Odorrana tormotus; (A1-C1) Appearance of limb bud; (A2-C2) Development of limb bud; (A3-C3) Toe differentiation. Red arrows show the magnification of limb buds or differentiated toes. Scale bars are 2 mm.

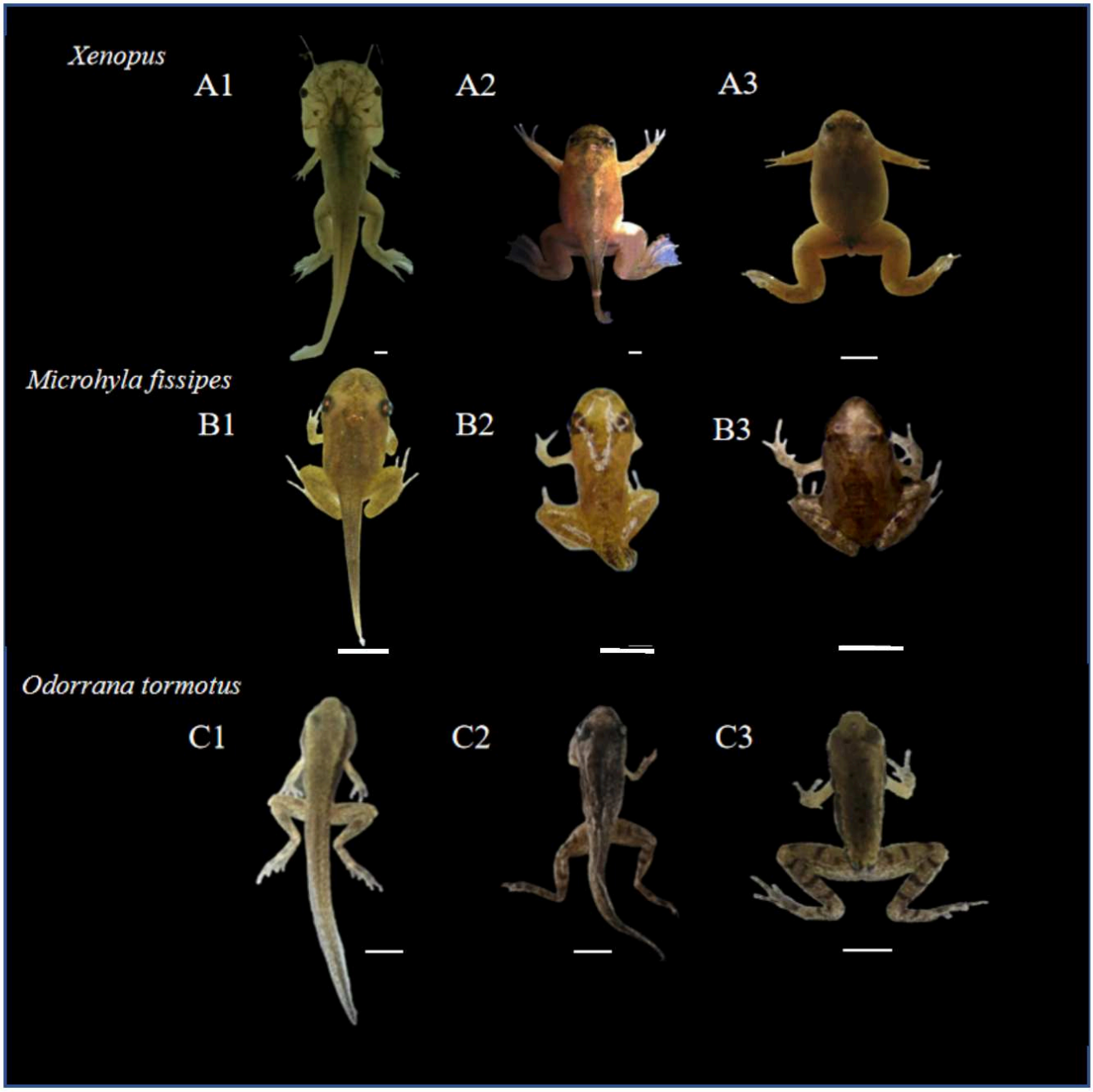

Figure 3

Tail resorption stages in Xenopus (A), Microhyla fissipes (B), and Odorrana tormotus(C). (A1-C1) Eruption of forelimbs (dorsal view); (A2-C2) Tail resorptions at metamorphosis climax (dorsal view); (A3-C3) Completion of metamorphosis (dorsal view). Scale bars are 2 mm.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.pdf
- Table2.pdf
- FigS1.pdf