



New Records of Interspecific Amplexus Between Three Anuran Species in Shanghai, China

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Interspecific amplexus occurs frequently in a wide range of amphibian species in various natural ecosystems with diverse evolutionary consequences, such as interspecific hybridization or waste of reproductive resources (Kraus 2015). However, when such behavior is observed in urban ecosystems, the underlying mechanisms of disturbance from human activities needs further investigation. Producing an

effective urban management plan of biodiversity conservation requires comprehensive knowledge of target species at multiple levels (Lambert and Donihue 2020). Therefore, exploring the causes of these behaviors and how they are connected to anthropogenic activities may provide us with novel perspectives and insights for improvement to urban wildlife conservation and urban planning.

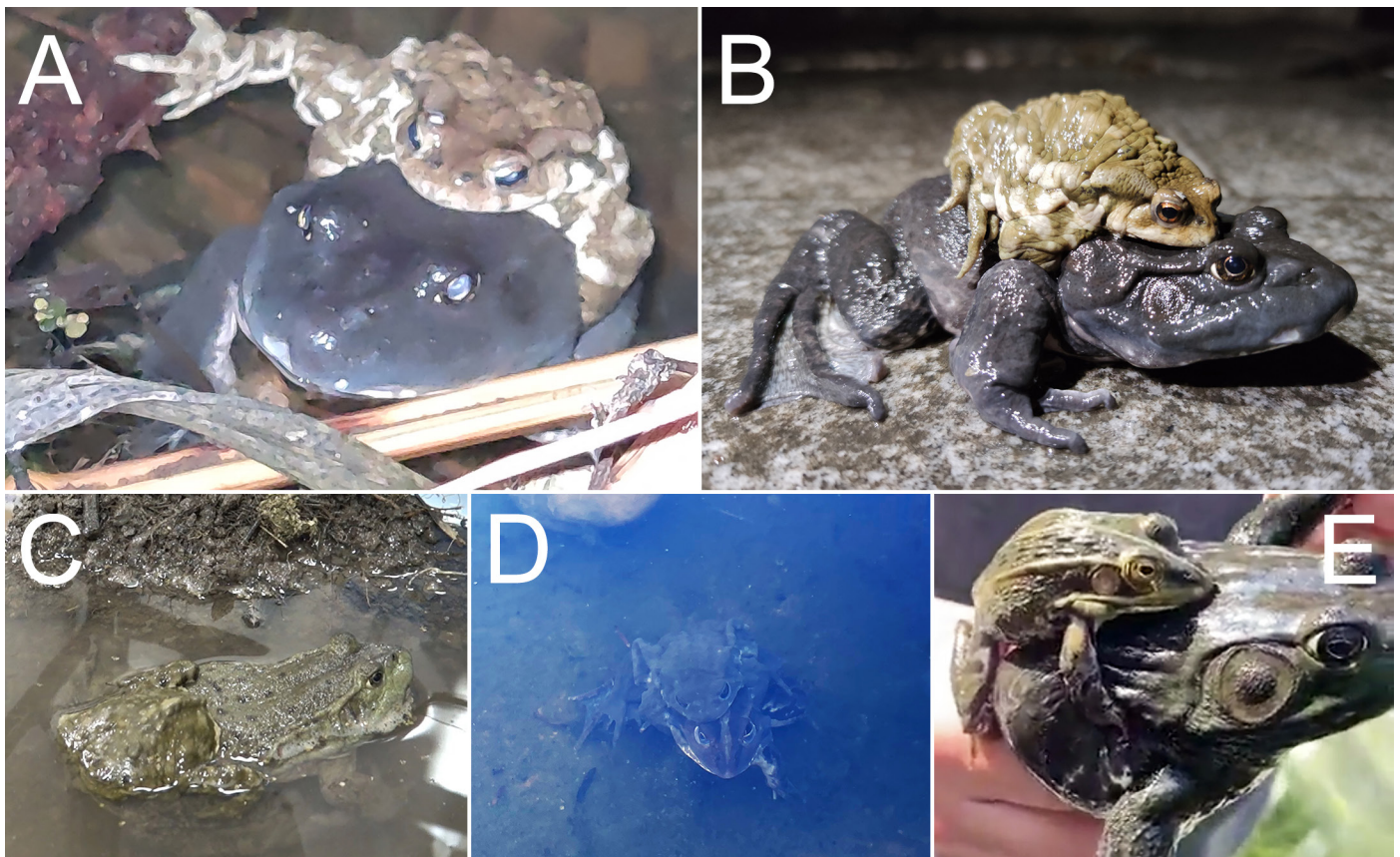


Fig. 1. Interspecific amplexus between an Asiatic Toad and an American Bullfrog: in situ (A), after capture (B), and a reverse inguinal position during a laboratory observation (C); interspecific amplexus between an Asiatic Toad and a Black-spotted Frog (D); a Black-spotted Frog claspings an American Bullfrog after capture (E). Photographs by Zhenghuan Wang (A, B), Xu Wei (C), Jianjun Gu (D), and Junfu Wang (E).

On the night of 1 February 2021, we observed an American Bullfrog (*Lithobates catesbeiana*) carrying an Asiatic Toad (*Bufo gargarizans*; Fig. 1A) in a pond on the campus of East China Normal University, Shanghai, China (31.027394°N, 121.447353°E). The pair was captured (Fig. 1B) and placed in a glass terrarium in the lab for further observation. The next morning, the toad was found to have changed its axillary amplexus to a backward inguinal position on the back of the American Bullfrog (Fig. 1C), and it remained in this posture for two weeks (14 days) until the toad released the bullfrog by itself. During this period, the toad clasped the bullfrog tightly, and the bullfrog was observed making multiple failed attempts to separate itself from the toad. However, after the amplexus, the toad always tried to avoid the bullfrog by digging in the mud or hiding itself in the moss in the terrarium. Both toad and bullfrog were euthanized later by keeping them in a -18°C refrigerator. Sexes of the two anuran individuals were thus double checked and confirmed by necropsy. Although no eggs were released during the amplexus, well-developed eggs in the ovary of the bullfrog could be identified (Fig. 2).

On 9 February 2021 (1100 h), another interspecific amplexus event between a male Asiatic Toad and a female Black-spotted Frog (*Pelophylax nigromaculatus*; Fig. 1D) was observed in a pond in a residential area of Minhang District, Shanghai, China (31.104592°N, 121.385472°E). The Black-spotted Frog was still hibernating when it was dug out and tightly embraced by the Asiatic Toad (Gu 2021). The Black-spotted Frog usually hibernates in the deep substrate at the bottom of ponds. As an artificial waterbody, the bottom of the pond in the residential area in Shanghai is paved with cement to prevent leaking, and is only covered by a shallow layer of sediment, which results in anurans being discovered much more readily during their hibernation.

The Black-spotted Frog normally enters its breeding season in late March (Fei et al. 2012). On 20 April 2021 (0800 h), we observed a male Black-spotted Frog clasped to the back of an American Bullfrog (Fig. 1E) in the artificial pond of Jing'an

Sculpture Park (31.237183°N, 121.459403°E). The gender of the Black-spotted Frog was identified by its nuptial pads.

Although anuran species richness has decreased rapidly during the past 40 years of fast urbanization in Shanghai City, there are still five native anuran species living in local habitats of the city. They are: Asiatic Toad, Hong Kong Rice-paddy Frog (*Fejervarya multistriata*), Ornamented Pygmy Frog (*Microhyla fissipes*), Eastern Golden Frog (*P. plancyi*), and Black-spotted Frog (Zhang et al. 2016). Among these five species, the Asian toad is the earliest to stop hibernating and begin to breed as early as February each year, thus avoiding interspecific amplexus with other native anuran species. However, since the introduction of the bullfrog farming industry in the 1990s, spillover of individuals from farms has resulted in the bullfrog becoming a successful invasive species in Shanghai. Although exact information about the breeding behavior of the bullfrog is still unclear in East China, our observation and dissection indicated that the biological rhythm between bullfrogs and Asian Toads may largely overlap. Therefore, although reproduction interference is one of the impacts of the American Bullfrog on native amphibian species (Kraus 2015), and American Bullfrogs have been reported to have interspecific amplexus with other *Rana* species (e.g., *R. draytonii*; D'Amore et al. 2009), this is the first report of amplexus with *Bufo* species, which are far more genetically distinctive from *Lithobates* species than *Rana* species. As to the understanding of ecological consequences of invasive bullfrogs on native species in China, advantages in competition and predation are well known. Our report of the interspecific amplexus behavior reminds us that we need to remain vigilant regarding the impacts of American Bullfrogs on the reproduction of local native anuran species. And such impacts may be even stronger in heavily urbanized areas where the wildlife communities can be more fragile than those in natural ecosystems.

To become a more ecologically friendly city, various kinds of green spaces have been constructed since the late 1990s in Shanghai City. To enhance the ecological functions

Table 1. Morphological measurements of the Asiatic Toad (*Bufo gargarizans*) and American Bullfrog (*Lithobates catesbeiana*) reported in this study. Bilateral measurements given as left/right.

Specimen Voucher	ECNU21002BG	ECNU21001LC
Species	<i>Bufo gargarizans</i>	<i>Lithobates catesbeiana</i>
Gender	Male	Female
Snout-vent length (mm)	71.88	133.47
Body mass (g)	39.95	179.71
Length of lower arm and hand (mm)	34.43/33.76	54.14/55.15
Femoral length (mm)	27.84/27.46	54.86/56.73
Tibial length (mm)	27.20/27.30	50.08/49.98

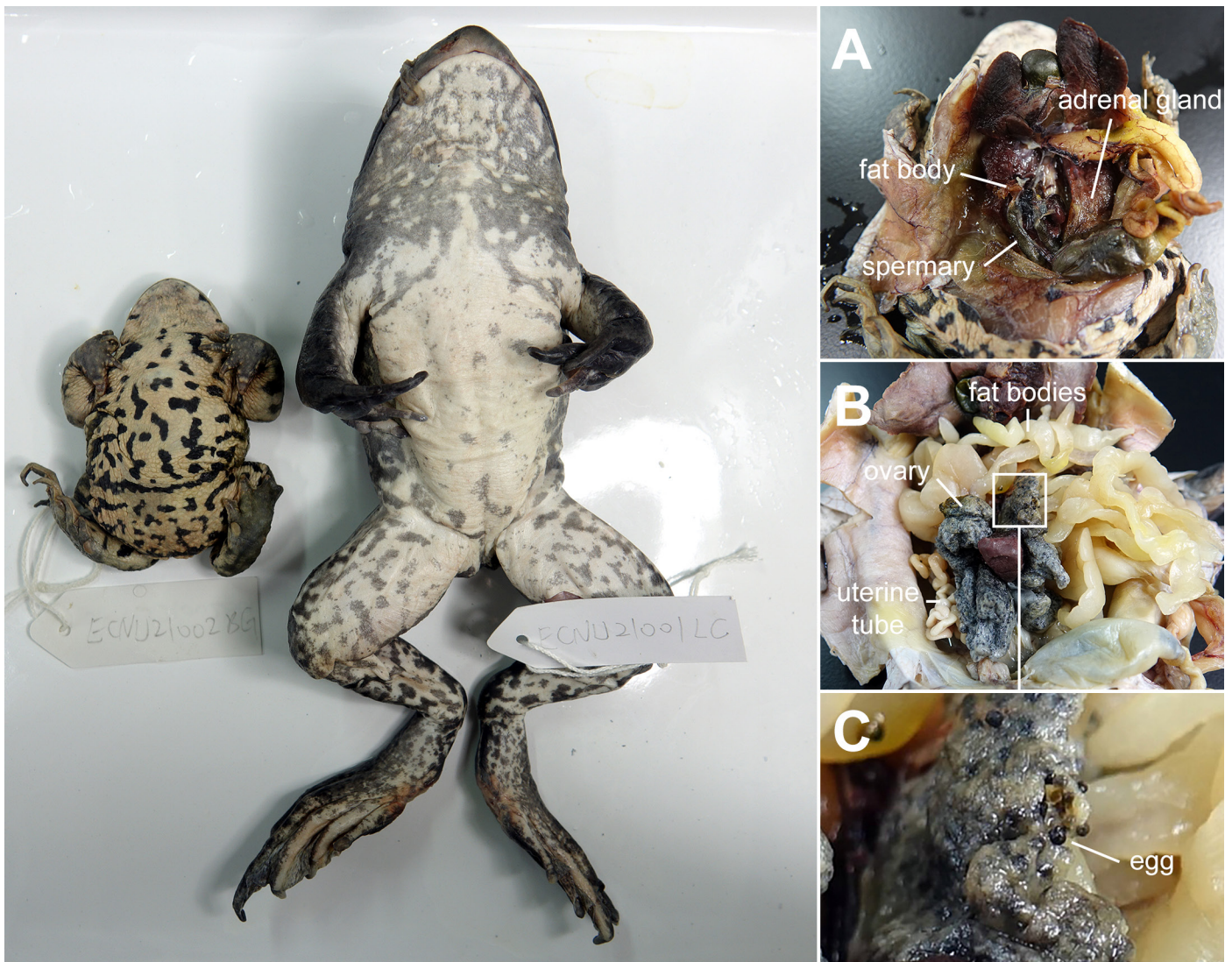


Fig. 2. Sex determination and body condition of an Asiatic Toad (*Bufo gargarizans*) (A) and an American Bullfrog (*Lithobates catesbeiana*) (B); and eggs in the ovary of the American Bullfrog (C). Photographs by Xu Wei.

of these green spaces, artificial waterbodies are usually crucial aspects, and become important habitats for anuran species in heavily urbanized areas of the city. Although the aquatic vegetation planting and natural banks with better edge effect consideration (e.g., Boissinot et al. 2019) are characteristics of the design of modern artificial waterbodies, a substrate of sufficient depth is usually neglected, especially for artificial ponds scattered in residential areas. The breeding and feeding habitat functions of these ponds for anurans usually receive more attention than the hibernation habitat function (i.e., suitable substrate with adequate depth). Consequently, in the long term, this type of artificial pond will actually act as an ecological trap rather than good habitat for native urban anurans.

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