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Conservation Breeding of the Laos Warty Newt (*Laotriton laoensis*) Until the F_3-Generation

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Abstract

Since 2008 the commercial trade of *Laotriton laoensis* has been prohibited in Laos. This is to be welcomed although protection in practice is hardly to be monitored. According to IUCN the species is listed as "Endangered EN". The species is still threatened by local people by poisonous fishing. The newts are used for food in Laos. Even more problematic is the use in traditional medicine, which can become a serious threat in the medium term despite the trade ban. They are used for example as drugs against respiratory diseases and arthritis. The importance for the international animal trade was repeatedly discussed. Due to the diurnal activity the newts are easily captured. Its size and attractiveness make the newts to a desirable species for the pet trade. However, the report presented here shows that coordinated offspring management is a good way to counter the threats. All possible keepers who are interested in keeping this species should refrain from acquiring wild caught animals.

Keywords: Laos Warty Newt; *Laotriton laoensis*; Conservation breeding; F3-generation

Laotriton laoensis is a large newt with a total length up to 23 cm, about half of which represents the tail (Bachhausen, 2007). The tail of the female is longer than that of males. The body appears strong. The head is broad and dull. The back shows intense sulfur yellow white-brown to orange streaks on black ground. These bright coloring patterns are arranged in three more or less regular strips or it can cover the entire back. This color is individually designed for each animal and makes its identification possible. On the back are two dorsolateral warts and a medial ridge. The paratoids are prominent. The warts are laterally less strong developed. The warts are missing on the belly. The coloring of the belly consists of large, orange spots on a black ground. Adult males show a silvery stripe made by several large spots on both sides of the tail which is particularly intense during the reproductive phase. At

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mating season and time of oviposition the female's cloaca develops into a long sidenarrowed ovipositor. It is broad and hemispherical in the male newt. In contrast to the species of the genus *Paramesotriton* the iris of *L. laoensis* is monochrome black. *L. laoensis* is the only warty newt with light color patterns on the back. Subspecies of *L. laoensis* have not been described yet (Bachhausen, 2013) (Figs. 1 and 2).

First Description

L. laoensis was first described by Stuart and Papenfuss (2002). Dubois and Raffaelli (2009) placed the species into the new genus *Laotriton*.

Distribution

Terra typica: Laos, Saysamboun Special Zone, in larger excavations of the Houay Sang Kat Creek (18° 52′49 N 103° 06′32 E, 1160 m) at the foot of Phou Sang Kat Mountain, Xiang Khouang Province. Further occurrences were found in Houay Pa Tin and in Phoukhoudt, Xieng Khouang province near Ban Nyot in the north of the country (1450–1500 m asl) (Bachhausen & Espallargas, 2008). According to the latest data, the distribution covers an area of approximately 4800 km^2 (Phimmachak, Stuart, & Sivongxay, 2012).

Fig. 1. *Laotriton laoensis*, adult female, first generation.

Fig. 2. Belly pattern of an adult male, first generation.

Habitat

The habitat of Laos Warty Newt are the streams of the mountainous regions of Northern Laos on elevations from 1100 to 1500 m asl. According to the best of our current knowledge, *L. laoensis* can only be found in parts of the provinces Vientiane, Xieng Khuang and Luang Prabang. Adult and semiadult newts were preferably found in deeper outcrops of streams with a width of 1–10 m with muddy, pebbly or rocky subsoil. The surrounding area consists of evergreen forests, shrubs, meadows and rice fields. According to Phimmachak et al. (2012) the water temperature is between 13 and 25 °C (usually 18–21 °C). The water is slightly acid with a pH from 5 to 6. The newts use a wide range of food, which includes mayfly larvaes, other insects, earthworms, spiders, centipedes, snails, fresh water crabs, eggs of their own species and eggs of frogs.

Keeping and Breeding

In February 2006, I had the opportunity to get a couple from a private import. The locality was Laos, Xiang Khouang Province, Phoukout District. At this time the Laos Warty newt was considered in lovers circles as almost impossible to breed. Already in winter of 2006/07, however, the first breeding of the species succeeded with about 240

metamorphored newts. The very difficult rearing of the larvae is described in Bachhausen (2008). A second breeding with approximately 80 and a third offspring period with approx. 110 young newts succeeded in the two following years. The incubation period until hatching was 5–6 weeks (Bachhausen, 2009). During winter of 2010/11 the first F_2 offspring was managed. About 80 young newts were raised. Much more would have been possible Bachhausen (2012). F_1 and F_2 offspring also took place in the following years. At the same time a F_2-group of young newts was raised, which developed into a group of one male and three female newts. This group reproduced successfully for the first time in winter 2014/15. These F_3-offspring also developed well. Inbreeding was not established (Figs. 11 and 12).

Overview reproduction success:

Year	Successful breeding until transmission	Breeding groups	Filial generation	Remarks
2007	240	1	F1	Maximum possible rearing success with very great effort
2008	80	1	F1	Maximum possible breeding success with reasonable effort
2009	110	1	F1	Maximum possible breeding success with reasonable effort
2010	–	1		Mating behavior, but no eggs
2011	78	3	F1, F2	Many fertile eggs, only small aliquots for incubation
2012	29	3	F1, F2	Many fertile eggs, only small aliquots for incubation
2013	57	3	F1, F2	Many fertile eggs, only small aliquots for incubation
2014	41	3	F1, F2	Many fertile eggs, only small aliquots for incubation
2015	45	4	F1, F2, F3	Many fertile eggs, only small aliquots for incubation
2016	53	3	F1, F2, F3	Many fertile eggs, only small aliquots for incubation

Rearing During Growth Period

The rearing of the young newts up to maturity succeeded – with the F_1 as well as the F_2 group – from metamorphosis until adult newts without loss of newts. At first they were kept at 20 °C on gravel with daily irrigation. After about four months they could easily be accustomed back into water. At the age of two years the sex was determined on the basis of the form of the cloaca. With two and a half years males developed the first pair coloration during autumn and occasional courtship was observed with approximately three

years during winter. The females remained uninvolved. In the following year the newts were nearly aged four and mating was intensified. In both sexes the cloaca was formed typical and the first F2- (in 2011) (Fig. 8) and F_3-breedings (in 2015) happened. At this time females had a total length up to 20 cm and the males had a size 17 cm.

Influence of Husbandry Conditions on Reproduction

During winter 2010/11 three breeding groups were kept under slightly different temperature conditions in order to gain further knowledge about the demands of the species (Bachhausen, 2012). On average, the temperatures were kept about 2 °C higher or lower than for the original group. In all three groups successful reproduction was carried out by the offspring female, but with clear differences in the rate of fertilization. This variation in the storage conditions revealed that temperatures below 15 °C are not useful. It appears to be reasonable if the temperature is not kept constant, but is subject to slight fluctuations. Successful mating was triggered by abrupt lowering of the temperature by changing water from approx. 18/19 °C to 15/16 °C in the late autumn. If no mating followed, the temperature was slowly increased again and the reduction was repeated after about three weeks. During summer temperatures of approximately 24 °C to short-timed

Fig. 3. Female laying eggs on leaf.

Fig. 4. Eggs just laid.

26 °C are optimal. Phimmachak et al. (2012) found terrestrial newts a few meters from the stream at air temperatures between 25 and 29.6 °C and a humidity of 98 and 89% during. The mating and oviposition behavior was comparable in all three groups. In the offspring newts, too, the mating of the female was often caused by a bite of the male near the spermatophores. This behavior seems to occur in undisturbed pairs (Fig. 5). In nature there is often a strong current in the water that could make a successful sperm intake difficult. Long-term fixation of the females in low flowing areas should counteract. During all years the eggs were only laid between leaves of large leaved plants (living and plastic plants). Foliage on the ground was preferably assumed so far as the desired structure was present. Investigations by Phimmachak et al. (2012) confirmed that this corresponds with the behavior in habitat, where the eggs are deposited in leaf litter at the bottom of potholes. The eggs have an inner diameter of 2.5–3 mm, with 3.5–5 mm jelly (Figs. 3 and 4 and 6 and 7).

Influence of Husbandry Conditions on Larval Development

Studies on the influence of temperature and other conditions have shown that the best rearing rates have been achieved in the larvae group, which were kept at a

Fig. 5. Male fixing the female after successful mating, F1 breed.

constant temperature of 19 °C with a full water change every second day. These larvae showed a very good growth and were almost without loss. Larvae occasionally kept at lower temperatures showed significantly poorer breeding rates. The same happened with larvae kept in a separate container inside the aquarium containing the adults. An "acidification" of the water by infructescence of alder or oak leaves brought positive results.

Conditions after Metamorphosis

After metamorphosis the offspring were kept on humid gravel, sprayed daily, for about 3 months. During this time young newts were fed with oven fish, aphids, white woudlouses, sludge worms, enchytraeen, small earthworms and frozen mosquito larvae (Bachhausen, 2012). For a readaptation of the newts to aquatic conditions a slight movement of the water is beneficial. A fully aquatic rearing started from metamorphosis was also possible in well-drained, shallow water (about 2–3 cm depth) (Figs. 9 and 10).

Further development during the whole period there have been no losses in breeding groups. The Laos Warty Newt is easy to keep and lives long in captivity until the husbandry conditions are suitable. In 2015 all breeding groups were tested for chytridiomycosis

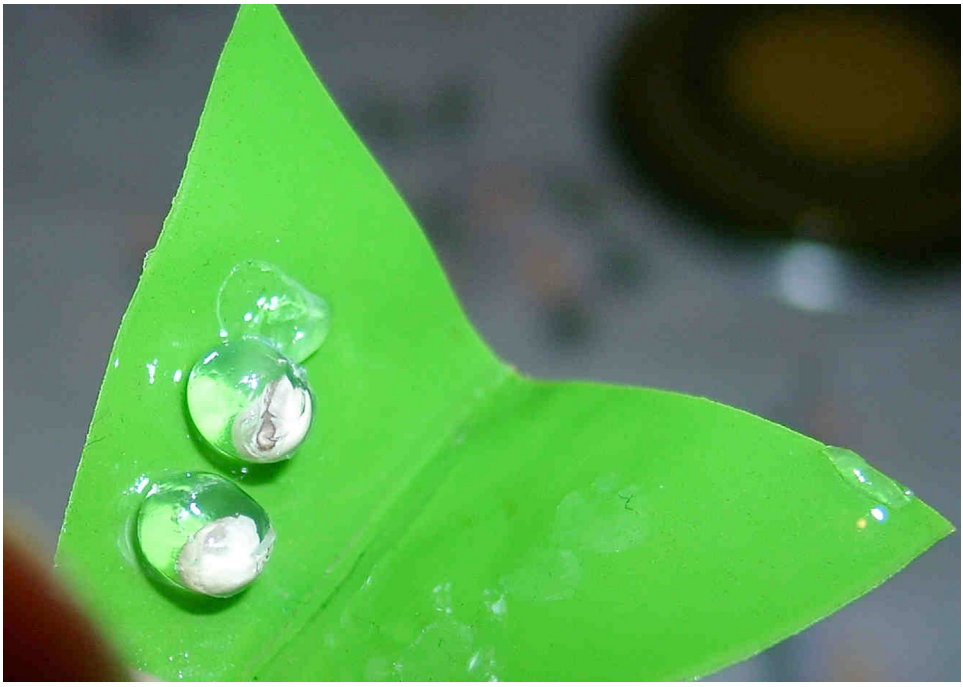

Fig. 6. Developing eggs.

(Bachhausen, 2016). Similar results were brought for the offspring from 2015 and 2016 after testing. All results showed a negative result of *Batrachochytrium dendrobatidis* (Bd) and *Batrachochytrium salamandrivorans* (Bsal).

Overview of the husbandry conditions (Bachhausen, 2008)

- Oxygen-rich, clean water with aquarium pump
- Annual temperature of adult animals of:
  - 20–24 (short-term to 26) °C in summer
  - 18–20 °C in spring and autumn
  - Under 18–15 (short term to 13 possible) °C in winter
  - 16–18 °C for egg laying
- Water change of a larger quantity of water and filling with cooler water: spontaneous decrease of the temperature from approx. 18 to approx. 16 °C e.g. in November
- Large-leaved robust, but flexible plants for oviposition
- Rearing of eggs at temperatures of 17–18 °C, towards the end at 19 °C
- Larvae rearing at 18–20 °C, avoid temperature fluctuation
- Young newts rearing at 20–24 °C, no permanent temperatures below 18 °C
- High hygienic standard during rearing of eggs and raising of young newts
- Very high effort in larval rearing with daily cleaning and frequent water changes

Fig. 7. Larvae in eggs, some days bevor slip.

Fig. 8. First F2-breeding, metamorphosis.

Fig. 9. F1-breeding three months after metamorphosis.

- Recovery of the young animals into the water, approx. 3 months after the metamorphosis, completely fully aquatic conditions also possible during metamorphosis
- Diversified and balanced nutrition
- High food supply for the entire growth phase

Fig. 10. Juvenile F1 breeding six months after metamorphosis.

Fig. 11. F2-breeding, one year old.

- Avoiding infections by other amphibian species (no plants, furniture or cleaning objects from other newts or other amphibian species)
- Avoiding infections by food (e.g., no live red mosquito larvae from unsafe sources)
- Investigate animals on Bd and Bsal, or purchase only examined animals

Newt Register

At the end of 2006 a newt register was established for the Laos Warty Newt by AG Urodela (group of newt and salamander enthusiasts of the DGHT). The sponsorship of the register was taken over by Gustavo Espallargas, Spain. The participation in the register was agreed for all captive bred offspring. All relevant information could be passed on at short-term. In addition all findings gained in captive husbandry have been made generally accessible by articles in professional journals. So it was quickly possible that other keepers from different countries could achieve a succession of offspring. This approach has been so successful that there has been a sufficient supply of offspring of the species in Europe for several years now. Further wildlife harvesting could thus be minimized. The breeding success in the second and third generation shows that the continuously keeping of the Laos Warty Newt is possible in our terrariums without further imported specimens. It would be a good concern, when zoological institutions would participate in the captive breeding of this species (Figs. 13 and 14).

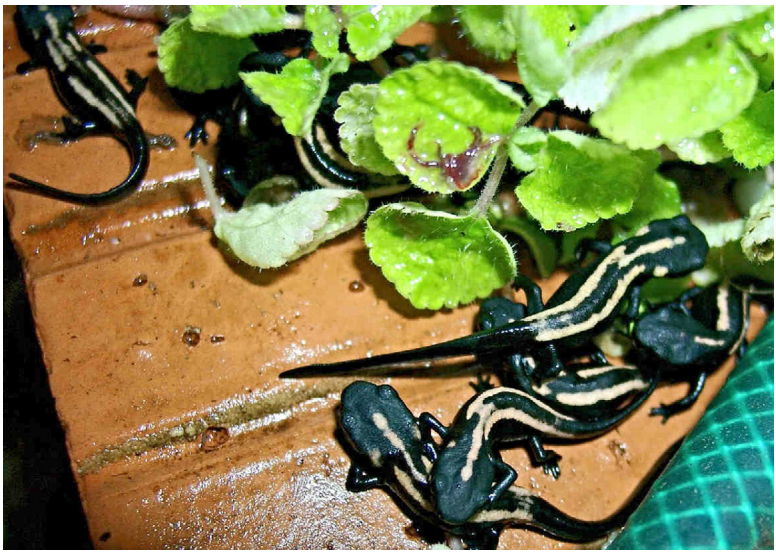

Fig. 12. F3-breeding, one month after metamorphosis.

Threat and Protection

Since 2008 the commercial trade in these newts has been prohibited in Laos (Category I Art in Lao Wildlife and Aquatic Law). This is to be welcomed although protection in practice

Fig. 13. Adult male with red colour on back, because of feeding. Bachhausen (2010).

Fig. 14. Adult male, F1 breeding.

is hardly to be monitored. According to IUCN the species is listed as "endangered", also recommanded are ex situ conservation measures (specifically conservation breeding). The species is still threatened by local people by poisonous fishing. The newts are used for food in Laos. Even more problematic is the use in traditional medicine, which can become a serious threat in the medium term despite the trade ban. They are used for example as drugs against respiratory diseases and arthritis. The importance for the international animal trade was repeatedly discussed. Due to the diurnal activity the newts are easily captured. Its size and attractiveness make the newts to a desirable species for the pet trade. However, the report presented here shows that coordinated offspring management is a good way to counter the threats. All possible keepers who are interested in keeping this species should refrain from acquiring wild caught animals.

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