# Proceedings

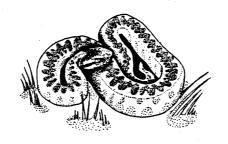
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### Seasonal independent sex determinant feature of the yellow-bellied toad (Bombina variegata L., 1758)

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The past literature is limited to the statements of the sex distinguishable features in the free living male yellow-bellied toad. These are the presence or absence of nuptial excrescences of the medial surface of the forelimbs in adult males (BIRKENMEIER 1954) (Fig. 1).

The excrescences are dark, pigmented and thickened, and are reformed after sloughing. During the spawning season, only weakly pigmented nuptial excrescences could be observed. SEIDEL (1988) does not exclude successful mating, although the pigmentation is less intensive. Another typical discriminatory feature in males compared to females are the little nuptial pads on the digits of the hind limbs as described by MERTENS (1928) (Fig. 1). These are only seen during the spawning season.

#### Material and methods

There is no reliable season-independent method of sex determination. This complicates the determination of sex in small animals (borderline cases) during field studies in spring and late summer. FUHN (1960) mentioned briefly the presence of keratin protuberances on the upper body surface, which are more distinct in males than females.

Our observations show, that in addition to nuptial excrescences, the intensity of the keratinization of the epidermal protuberances can be considered as a further sex character to distinguish between males and females. The heavily keratinized and pointed tips at the end of the warts are much more apparent in males. They cover the whole dorsal surface, and are very easily and clearly observed on the lower hindlimb, especially on the outer edge (Fig. 2). In females, these black keratin protuberances are blunt and flattened (Fig. 3).

To verify this observation, dead animals (n=12) from our breeding stock were necropsied to determine their sex. There was complete agreement with the external, sex-phenotypical appearance of the keratin protuberances. This sex determination is unequivocally possible even when the nuptial excrescences are not yet, or no longer, developed (young males, spring, fall).

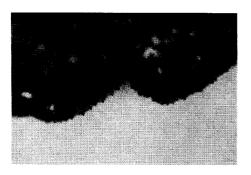
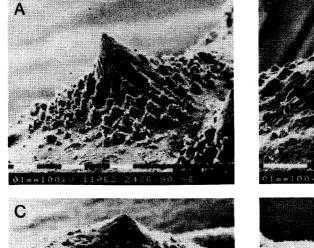


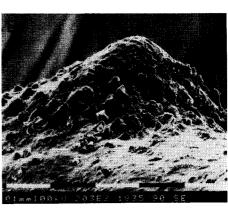
Fig. 2. Males, pointy keratin protuberances (visible on the outer edge of the lower hindlimbs)



Fig. 3. Females, blunt keratin protuberances (visible on the outer edge of the lower hindlimbs)



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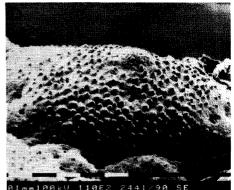


Fig. 4. Scanning electronmicrographs (SEM) of surface structure of keratin protuberances: Hindlimb of a male (A) and female (B) during mating season. SEM preparations of a male (C) and female (D) made in early October (nuptial excrescences visible, keratin structures still intact)

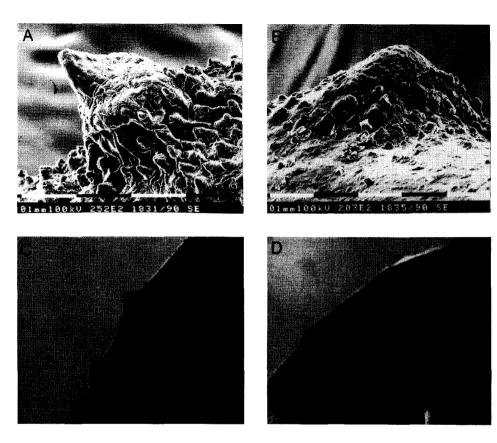


Fig. 5. Comparison between scanning electronmicrographs (A and B) and close-up photographs (C and D) distinguishing the morphological differences of keratin protuberances between the two sexes. A and C show the surface strucure on the outer edge of the lower hindlimb of a male, B and D of a female. The close-up photograph is from a one year old toad of 28 mm snout-vent length (magnification 50x). The SEM pictures are of adult toads. There is a certain spherical aberration so that the height cannot be measured with the 0.1 mm scale bar

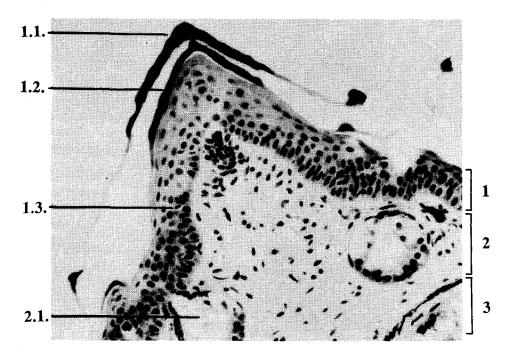


Fig. 6. Photomicrograph of a cross-section of a male keratin protuberance. 1=epidermis: 1.1=stratum corneum (before sloughing), 1.2=stratum corneum, 1.3=stratum germinativum, 2=stratum laxum: 2.1=mucous gland, 3=stratum compactum