# Hemipenial microornamentation in *Algyroides marchi* VALVERDE, 1958

(Squamata: Sauria: Lacertidae)

Hemipenis-Oberflächenfeinstrukturen bei *Algyroides marchi* VALVERDE, 1958 (Squamata: Sauria: Lacertidae)

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## ABSTRACT

The hemipenial microornamentation of *Algyroides marchi* VALVERDE, 1958 is described. It consists of crown-shaped tubercles, as in *A. fitzingeri*, but different from the spiniform tubercles of the Balkan species *A. moreoticus* and *A. nigropunctatus*. Possible phylogenies and intrageneric relationships are discussed.

## KURZFASSUNG

Die Oberflächenfeinstrukturen des Hemipenis von Algyroides marchi VALVERDE, 1958 werden beschrieben. Sie bestehen aus kronenförmigen Tuberkeln wie bei A. fitzingeri und unterscheiden sich von den stachelförmigen Tuberkeln der Balkan-Arten A. moreoticus und A. nigropunctatus. Mögliche Phylogenien und Beziehungen innerhalb der Gattung werden diskutiert.

#### KEY WORDS

Reptilia: Squamata: Sauria: Lacertidae: Algyroides, Algyroides marchi, hemipenis microornamentation, phylogeny, systematics, taxonomy, morphology, Spain

# INTRODUCTION

Gross morphology of the everted hemipenis is comparatively homogeneous in lacertid lizards, but the study of the microornamentation of the hemipenial epithelium during the reproductive period reveals the existence of some small microscopic tubercles, which can differ in form among the different species. The development of this epithelium is controlled by hormonal changes during the reproductive cycle, as a result of which tiny protuberances develop. Each protuberance is a unique epithelial cell (BÖHME 1971, 1993) that can have the form of a thorn, more or less rounded or curved (called finger-shaped, hooked, or spiniform microornamentation), fork (forked microornamentation), or small tubercle with minuscule spiniform prolongations on the apex (crown-shaped microornamentation) (KLEM-MER 1957; BÖHME 1971; ARNOLD 1973, 1986, 1989; Arribas 1994, 2001). Moreover, it was shown that these epithelia are shed every day during the reproductive cycle (IN DEN BOSCH 2001). The use of hemipenial microornamentation for taxonomic and systematic purposes in lacertids dates back to KLEMMER (1957) and BÖHME (1971). The latter author reviewed the microornamentations of a great number of species. Subsequently, other authors such as ARNOLD (1973, 1986, 1989), ARRIBAS (1994, 2001), BÖHME (1993), and LEPTIEN & BÖHME (1994) used this information for taxonomic purposes.

Regarding the Mediterranean genus Algyroides Bibron & Bory DE Saint-Vin-CENT, 1833, BÖHME (1971) described the microornamentation of the Balkan species A. moreoticus Bibron & Bory de Saint-Vin-CENT, 1833 and A. nigropunctatus (DUMÉRIL & BIBRON, 1839), both with thorn-shaped tubercles, and the Tyrrhenian species A. fitzingeri (WIEGMANN, 1834), with crownshaped tubercles. However, comparative information lacks for A. marchi VALVERDE, 1958 (see e. g., HARRIS et al. 1999). In addition to results presented in a general account of Algyroides (ARNOLD et al. 2007), the author describes the hemipenial microornamentation of A. marchi in comparison with the structures found in its congeners.

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## MATERIALS AND METHODS

Two specimens of *Algyroides marchi* fixed with everted hemipenes were studied. The specimens were captured on April 21, 1989 in Calar del Mundo, Riopar, Albacete Province (Spain) at 1100 m a.s.l.

For the study of the microornamentation, the procedure described in ARRIBAS (2001) was followed. The hemipenes were dehydrated in a series of increasing ethanol concentrations (70%, 90%, 100%, 12 hours in total). After this, the material was transferred to xylene for two to six hours, then to

paraffined xylene for the same period and finally immersed and soaked in melted paraffin for eight hours for embedding.

The solidified blocks were cut with a hand microtome; slices (as thin as possible) were deparaffined with xylene (15 min), and rehydrated with ethanol of decreasing concentration (100%, 90%, 70%) and finally distilled water (five to ten min), prior to microscopic observation at a magnification of 1000 times. No staining.

# RESULTS AND DISCUSSION

The hemipenis microornamentation of *Algyroides marchi* can be classified crownshaped. Thus, apart from some immature tubercles similar to hook or finger shaped protuberances, the majority was ampulliform (bottle-shaped), fairly elongate with truncate tips and spiny prolongations in their fine apexes (Fig. 1).

It is difficult to decide whether this constitutes a primitive or derived character The hook or finger-shaped type (which is very common in the Eremiadini) is considered primitive by the ontogenetic criterion and its lesser complexity. Among Lacertini, however, both states (crownshaped and finger-shaped) coexist in parallel and some of the latter seem to be secondary regressions (ARRIBAS 2001). Moreover, both types of microornamentation appear in closely related species, like Omanosaura cyanura (ARNOLD, 1972) versus Omanosaura jayakari (BOULENGER, 1887), the species of Algyroides, or Iberolacerta (*Pyrenesaura*). Prudence is required in the use of this character for phylogenetic reconstruction, apart from the mere species diagnoses.

The hemipenial microornamentations of *A. marchi* and *A. fitzingeri* are very similar to each other, which coincides with the position of both species in the most recent phylogenetic reconstructions of the genus (Harris et al. 1999; Pavlicev & Mayer 2009). According to Harris et al. (1999), the relationships of the species within the genus, based on a combination of morpho-

logical and molecular (mitochondrial 12S and 16S rRNA sequences) information are (((marchi, fitzingeri) moreoticus) nigropunctatus), without distinct groupings. PAV-LICEV & MAYER (2009) who obtained their results from a combination of nuclear and mitochondrial genes (cyt b, 12S rRNA, 16S rRNA, rag1, and c-mos; 3600 bp in total) presented an interesting topological detail in their phylogeny of the subfamily Lacertinae: Although not fully supported, especially for nuclear genes, close relationship between Dinarolacerta [D. mosorensis (Ko-LOMBATOVIĆ, 1886) and D. montenegrina Ljubisavljević, Arribas, Džukić & Car-RANZA 2007] with Algyroides was suggested. Of the two trees in Pavlicev & Mayer (2009), the tree derived from all studied genes together, had the topology ((marchi, fitzingeri) (mosorensis, montenegrina)) (moreoticus, nigropunctatus), suggesting that Algyroides was paraphyletic, whereas the analysis of nuclear genes alone supported monophyly of Algyroides. However, a tree topology alternative to a monophyletic Algyroides, could not be rejected (PAVLICEV & MAYER 2009).

These authors suggested to investigate the relationship between *Dinarolacerta* and *Algyroides*, although the results were not sufficient to conclude paraphyly of *Algyroides*. These studies must include, from the current author's point of view, different approaches and all the species involved. Also *Dinarolacerta mosorensis* has crown-shaped protuberances (BÖHME 1971).



Algyroides marchi



Algyroides nigropunctatus



Algyroides moreoticus

Fig. 1: Hemipenial microornamentation of *Algyroides* species: *Algyroides marchi* Valverde, 1958 (original), *Algyroides fitzingeri* (Wiegmann, 1834), *A. nigropunctatus* (Duméril & Bibron, 1839) and *A. moreoticus*Bibron & Bory, 1833 (the last three redrawn from Böhme 1971).

The author's preferred hypothesis is Dinarolacerta being the sister group of all Algyroides. In the hypothetical case that Algyroides is paraphyletic, two procedures are possible: 1) Dinarolacerta is synonymized with Algyroides, which is problematic as both would not share any of the characters diagnostic of Algyroides (see below); 2) Algyroides is split into two entities. If this latter approach is adopted, the East Mediterranean (Balkan) group should retain the name Algyroides s. str. (type species: Algyroides moreoticus BIBRON & BORY DE SAINT-VINCENT, 1833), and for the West Mediterranean (Ibero-Tyrrhenian) group, a new name had to be chosen after a careful study of synonymies.

It is important to bear in mind that the nominal genus Algyroides was polyphyletic until the systematic review by ARNOLD (1973), and included both European and African *Algyroides*-like species. ARNOLD (1973) clearly showed that there were enough osteological, hemipenial and morphological differences to place the African species into a separate genus, Adolfus STERN-FELD, 1912, but retained the European species in the somewhat heterogeneous genus Algyroides (ARNOLD et al. 2007). Recently, a new genus was splitted from Adolfus: Congolacerta Greenbaum, Villanueva, Kusamba, Aristote & Branch, 2011 (type species: Lacerta vauereselli Tornier, 1902) including C. asukului Greenbaum, VILLA-

NUEVA, KUSAMBA, ARISTOTE & BRANCH, 2011).

European Algyroides have some characteristics in common: M-NORs (Mediumsized Nucleolus Organizer Regions) in the karyotype, more or less enlarged dorsal scales and a characteristic scale microorna-"raised pustulate mentation showing swellings projecting among the upturned posterior edges of the strap-shaped cell surfaces that constitute the oberhautchen" (HARRIS et al. 1999: ARNOLD 2002: ARNOLD et al. 2007). The M-NORs character seems to have developed independently (by chromosomal fragment rearrangement) in different groups among Lacertini: It is shared by Hellenolacerta graeca (Bedriaga, 1886), a pair of Central Spanish *Iberolacerta*, namely I. cyreni (Müller & Hellmich, 1937) and I. martinezricai (ARRIBAS, 1996), all Podarcis species and Timon lepidus (DAUDIN, 1802), but not by Dinarolacerta. The characteristic above mentioned pustulate swellings of the "oberhautchen" appear only in one of the African Algyroides-like Adolfus viz. A. africanus (BOULENGER, 1906) but not in *Dinarolacerta* nor in other Lacertini species apart from Algyroides (ARNOLD 2002). About the enlarged and keeled scales present in *Algyroides*, there is a lot of literature that suggests that this character is habitat-linked (see for instance ARNOLD 1973 and ARNOLD et al. 2007). On the other hand, morphological variability within *Algyroides* is very high, the different species presenting different numbers of vertebrae and premaxillary teeth, marginated or emarginated clavicles, and spiny hemipenial ornamentations or crown-shaped tubercles (Fig. 1).

If *Dinarolacerta* was included in *Algyroides*, the group would lose all its main diagnostic characteristics mentioned above (enlarged scales, M-NOR and oberhautchen). On the contrary, if *Algyroides* was split in two groups, there would be several diagnostic characters for each entity: *Algyroides* s. str. (Balkan species) would have (usually) pterygoid teeth, supraocular osteoderms that are complete in adults, and

hook-shaped hemipenial tubercles; the Ibero-Tyrrhenian group (in the sense expressed here, including the species *fitzingeri* and *marchi*) on the other hand, would lack pterygoid teeth, have the supraocular osteoderms incomplete (fenestrated) in adults, and crown-shaped hemipenial tubercles.

Whereas the above considerations are open to further discussion, it seems rather clear at the moment that there is a close relationship between *Algyroides* and *Dinarolacerta*. New phylogenetic studies, including more genes and analyses are needed in order to clarify the relationships between *Algyroides* and *Dinarolacerta* and among the species of *Algyroides*.

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