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First Report of Hemiclitores in Females of South American Liolaemid Lizards

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Abstract.—**Studies about copulatory organs in Squamata were restricted to the morphology of hemipenes until Böhme reported homologous paired structures in females of a species of *Varanus*, which he called hemiclitores. We report the presence of hemiclitores in females of *Phymaturus* and of two species of *Liolaemus* and describe observations on the interspecific variation in hemipenis morphology in *Phymaturus*. *Phymaturus* and *Liolaemus* belong to Liolaemidae, a species-rich family of lizards; research about hemipenis morphology is scarce and limited to a few species of those genera. We found the retractor clitoridis magnus in all of the species analyzed; however, the transverse penis was not present in all of them. The general structure of hemiclitores of *Phymaturus* and *Liolaemus* females resembled that described for other squamate species; they were smaller than hemipenes and exhibited a sulcus spermaticus. The variation found in different features of these organs (shape, size, pigmentation), as well as the general morphology of hemipenes, should be studied more extensively in more species. These results contribute to the description of structures that are still poorly known in the large groups of Squamata and support the idea that hemiclitores should be considered an apomorphy of Squamata.**

Resumen.—**Los estudios de los órganos copuladores de Squamata estuvieron restringidos a la morfología de los hemipenes hasta que Böhme reportó la presencia de estructuras pareadas homologas a la de los machos, a las que llamó hemiclitoris. Nosotros reportamos la presencia de hemiclitoris en las hembras del género *Phymaturus* y en dos especies del género *Liolaemus* así como observaciones sobre la variación interespecífica en la morfología de los hemipenes de *Phymaturus*. *Phymaturus* y *Liolaemus* pertenecen a Liolaemidae, una familia de lagartijas muy diversa, para los cuales las investigaciones en la morfología de los hemipenes son escasas y limitadas a unas pocas especies. En todas las especies revisadas encontramos el músculo retractor clitoridis magnus pero no el transversus penis. La estructura general de los hemiclitoris de las hembras de *Phymaturus* y *Liolaemus*, se asemeja a la descrita para otras especies de Squamata; son más pequeñas que los hemipenes y exhiben un sulcus spermaticus. La variación encontrada en diferentes características de estos órganos (forma, tamaño, pigmentación), así como la morfología general de hemipenes, debe ser estudiada más extensamente incluyendo un mayor número de especies. Estos resultados contribuyen con el reporte y la descripción de estructuras que son aún poco conocidas en los grandes grupos de Squamata, y soporta la idea que los hemiclitoris deben ser consideramos una apomorfia de Squamata.**

During an embryological study of *Phymaturus*, we found paired structures in the cloaca of females that have not been described previously. Hence, we revisited the cloaca in adult specimens and confirmed that these structures were constant in these lizards and, thus, merit description.

The morphology of the hemipenes is of great taxonomic interest and an excellent source of information for the study of phylogenetic relationships (e.g., Arnold, 1986a,b). These copulatory organs have been studied since the 19th century (Lereboullet, 1851; Cope, 1896), and numerous works focusing on several lizard and snake families and amphisbaenians have been published (e.g., Presch, 1978; Böhme, 1989; Guo and Zhang, 2001). The comprehensive anatomical study of musculature of the hemipenis region (Arnold, 1984) is a useful guide to all structures related to these organs. Böhme (1988) revised the hemipenis morphology in representatives of all lizard families and provided original terminology for the structures and ornamentation. Hemiclitores were first described by Böhme (1995) for a species of *Varanus* as structures present in squamate females homologous to hemipenes; however, the presence of a structure similar to hemipenes in the cloaca of female lizards and snakes was mentioned previously but without giving them any relevance or even providing names (Gadow, 1887; Minton and Minton, 1973; Honegger, 1978; Gasc and Renous, 1979; Arnold, 1984; Davis and Phillips, 1991; King and Green, 1993). The occurrence of hemiclitores seems to be common across different lizard and snakes families (e.g., Ziegler and Böhme, 1997) and amphisbaenids (Kasperoviczus et al., 2011), but the structure and variation in shape and size are poorly known, except for varanids, helodermatids, and lanthanotids (Ziegler and Böhme, 1997). Recent investigations, aiming to address the functional reproductive morphology of the hemiclitores, have analyzed the hormonal influence and the neuromuscular complex as a whole in species of *Anolis* and *Eublepharis* (Lovern et al., 2004; Holmes et al., 2005). The variation found in these studies indicates that muscles associated with hemipenes and hemiclitores can be present in both sexes of *Eublepharis* (Holmes et al., 2005) or restricted only to males in *Anolis carolinensis* (Lovern et al., 2004).

The family Liolaemidae contains three genera of lizards, *Liolaemus*, *Ctenoblepharys*, and *Phymaturus*. The last is an endemic genus inhabiting the arid southwestern region of South America that includes at least 40 species (Lobo et al., 2012). The systematics (e. g., Lobo et al., 2012), reproductive biology (e.g., Cruz and Ramirez Pinilla, 1996), and behavior and thermoregulation (e. g., Labra et al., 2007; Valdecantos et al., 2013) have been studied in *Phymaturus* and *Liolaemus*. However, there are only two studies describing hemipeneal morphology, the description of *Phymaturus palluma* by Böhme (1988) and the report of Lobo (2000) of 18 species of *Liolaemus* and *Phymaturus dorsimaculatus*, who provided a preliminary overview of the diversity in this clade. We report the presence of hemiclitores in females of both *Phymaturus* and *Liolaemus* and report observations on the interspecific variation found in hemipenis morphology within *Phymaturus*.

Materials and Methods

The cloacal region was observed in females and embryos of 20 species of *Phymaturus* belonging to both groups: *palluma* and *patagonicus* (Lobo et al., 2012). To standardize the observations, only embryos belonging to stages 34 and 35, according to Lemus et al. (1981), and hatchlings were included. Embryos and hatchlings were sexed by direct observation of gonads.

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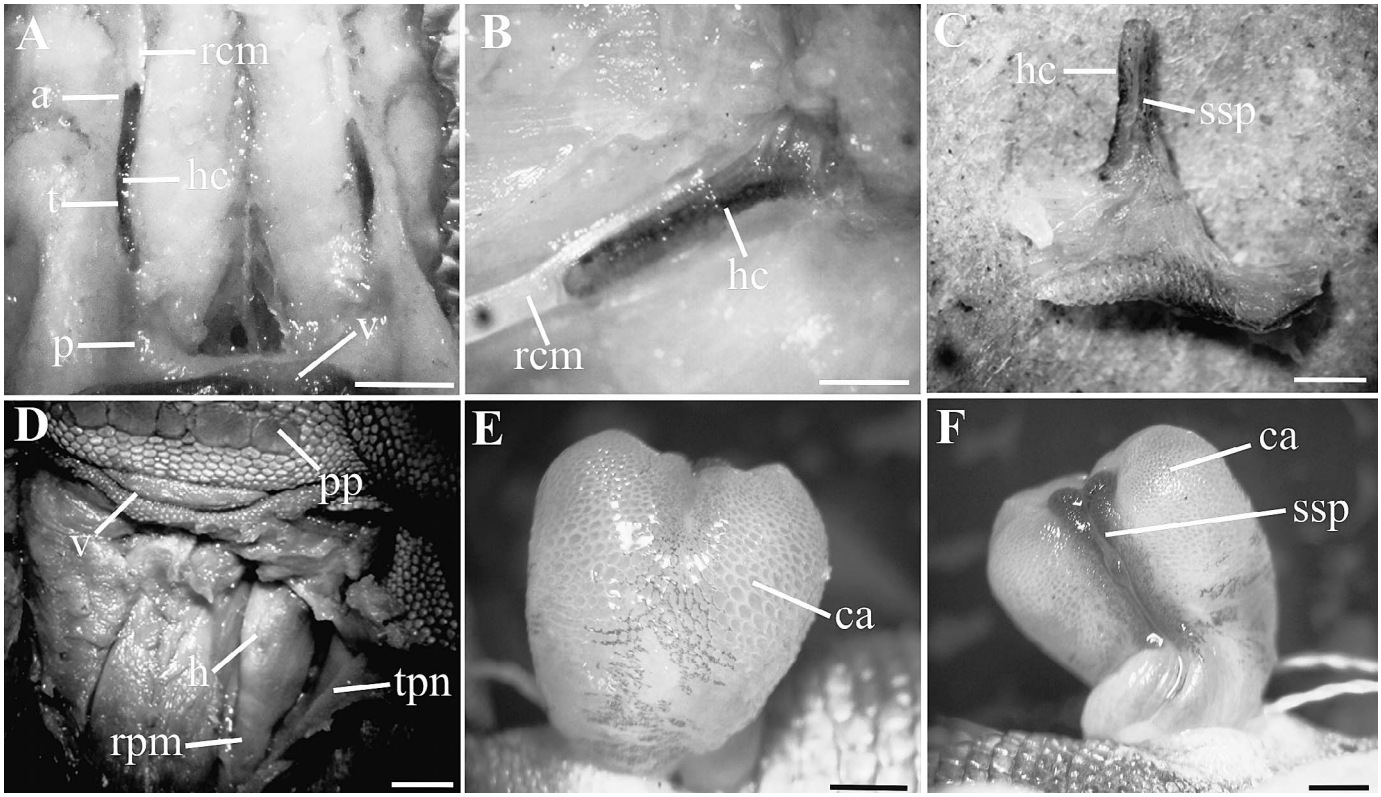

FIG. 1. (A) Adult female of *Phymaturus ceii* (MCN 910); view of postcloacal region showing hemiclitores (skin removed) (scale = 2 mm). (B) Adult female of *Phymaturus patagonicus* (MCN 3273); view of postcloacal region showing hemiclitoris (skin removed) (scale = 1 mm). (C) Hemiclitoris of adult female of *P. patagonicus* (MCN 3273) removed from its natural position and everted (scale = 2 mm). (D) Adult male of *Phymaturus dorsimaculatus* (MCN 3728); view of postcloacal region showing hemipenis position (skin removed) (scale = 5 mm). (E) Adult male of *Phymaturus roigorum* (MCN 1963) showing the opposite side of sulcus of the everted hemipenis (scale = 2mm). (F) Same hemipenis of sulcus side (scale = 2mm). Abbreviations: a, apex; ca, calices; hc, hemiclitoris; h, hemipenis, p, pedicel; pp, precloacal pores; rcm, retractor clitoridis magnus; rpm, retractor penis magnus; ssp, sulcus spermaticus; t, truncus; tpn, transverses penis; v, cloacal opening.

Also, we reviewed the hemipenes of 18 other species representative of both groups (Appendix 1). All descriptions were made following the nomenclature of Arnold (1984) and Ziegler and Böhme (1997). We also observed one male and one female of two species of *Liolaemus*, *Liolaemus irregularis* and *Liolaemus umbrifer*, each belonging to the two subgenera, to check the hemiclitoris presence in *Liolaemus*, the sister taxon of *Phymaturus*. In almost all male embryos, we detected hemipenes with the naked eye because they were already everted, but in a few cases, we everted them by manually pressing behind the posterior border of the cloaca. Hemiclitores in the females are in the same position as the hemipenes in males, also everted, and in most cases are associated with the same musculature.

RESULTS AND DISCUSSION

Description of Hemiclitores in Adult Females of Phymaturus.—Retracted hemiclitores lay behind the vent, in the same position as that of the hemipenes (Fig. 1A). By contrast, the hemiclitores were superficial and not always covered by the transversus penis. The retractor lateral posterior was not observed in all species, whereas the retractor clitoridis magnus was always present, as in males (Figs. 1A,B).

The general morphology of the hemiclitoris was similar to that described by Ziegler and Böhme (1997); it was composed of an apex, a truncus having a sulcus spermaticus, and a basal region called pedicel. In *Phymaturus patagonicus*, the hemiclitoris (Figs. 1C,D) was pigmented all along its truncus and only barely over the apex, similarly to other 11 species of the genus, whereas the remaining eight species lacked any kind of pigmentation. We did not observe any kind of ornamentation in hemiclitores. Only in *Phymaturus ceii*, female did the hemiclitoris structure show the apex divided into lobes (Fig. 1A). This character exhibited a remarkable variation between embryo and adult, because the adult female of *Phymaturus verdugo* showed no bilobed apex, but it was bilobed in the embryo (Fig. 2C).

Observations of Embryos and Hatchlings of Phymaturus.—The embryos studied (stages 34 and 35) exhibited everted hemipenes or hemiclitores (Figs. 2A,C). Because hemiclitores were smaller than hemipenes, they were observed mostly by opening the vent with tweezers. Hatchlings did not have everted copulatory organs; they were sexed by looking at gonads and in the case of males, hemipenes were everted by manually pressing behind the posterior border of the cloaca. Everting the hemiclitores was much more difficult than everting the hemipenes because of their reduced size. In both male embryos and hatchlings, two structures, testicles and mesonephros (the epididymis), were well developed (Fig. 2B), whereas in females, three structures were well developed (ovary, mesonephros, and oviduct) (Fig. 2D). Male and female embryos of *P. verdugo* had hemipenes and hemiclitores with bilobed apex, although hemiclitores were clearly smaller than hemipenes of males at the same stage (Figs. 2A,C). Hemiclitores of this species showed ontogenetic variation; in adult females, this structure lacked this bilobed shape.

Description of Hemipenes of Adult Males of Phymaturus.—In males, the retracted hemipenes were located behind the vent and were observed forming cloacal diverticula. In ventral view, the

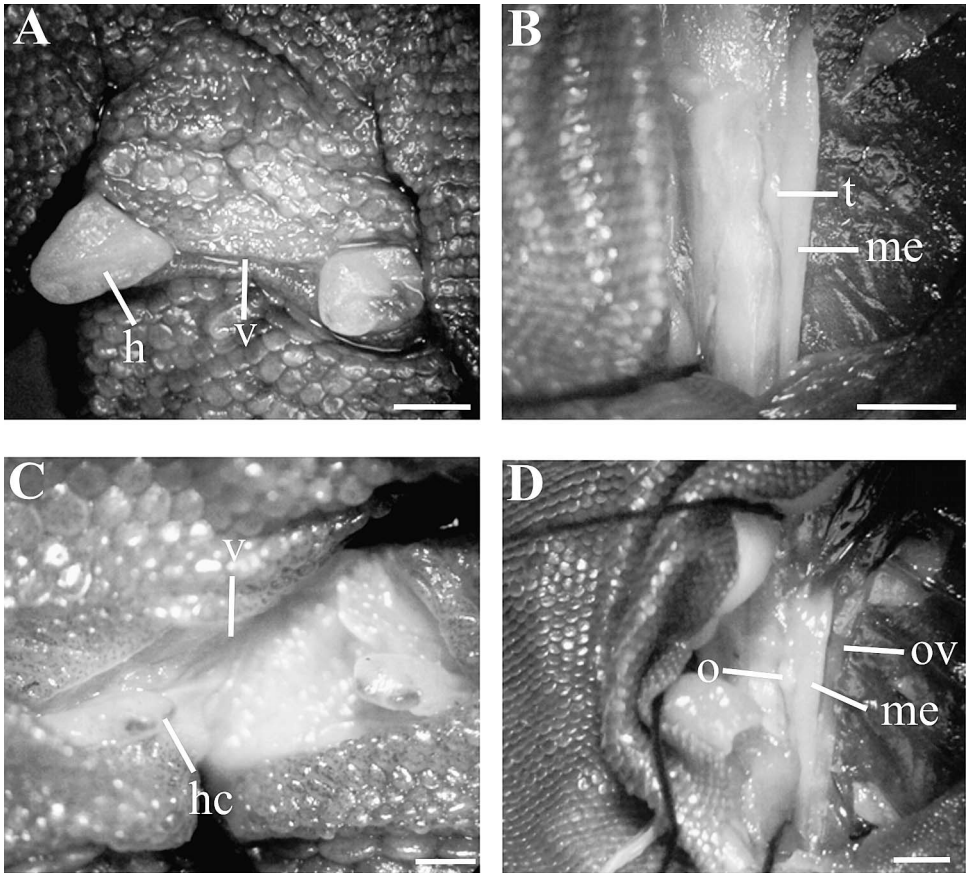

FIG. 2. (A) *Phymaturus verdugo* embryo male with protruded hemipenes (MCN 1958) (scale = 1 mm); (B) same embryo dissected (scale = 5 mm). (C) *P. verdugo* embryo female with conspicuous hemiclitores at cloacal opening (MCN 1958) (scale = 0,5mm); (D) same embryo dissected (scale = 2mm). Abbreviations: h, hemipenis; hc, hemiclitoris; o, ovary; t, testis; me, mesonephros; ov, oviduct; v, cloacal opening.

hemipenis was covered by conspicuous transverses penis muscle (cut and displaced in Fig. 1D). Both retractor muscles of the hemipenis were present (retractor penis magnus and retractor lateralis posterior). This condition is similar to that described by Arnold (1984) for *P. palluma*, indicating a widespread condition for iguanids but pointing out a difference: "*Phymaturus* approaches this but the retractors laterals are closely applied and, in addition to its attachment at the dorsal confluence of the hemipenis and cloaca, the retractor lateralis posterior has a well defined fleshy insertion on the side of the basis of the hemipenis (instead of one that is less clearly defined, tendinous or absent)." Everted hemipenes in adults of *Phymaturus* species exhibited very limited variation (as was described for *Liolaemus*, Lobo, 2000) and were similar to the main characteristics described by Böhme (1988) for *P.* "*palluma*" from Chile. The hemipenis had a wide trunk and a bilobed apex. The only kind of ornamentation found was limited to calices spread out on both sides of the organ (Figs. 1E,F) and was restricted to the distal half of its body; basal calices were larger and became smaller to the top of apices. The sulcus spermaticus (Fig. 1F) had its basal borders conspicuously widened, similar to *Liolaemus* (Lobo, 2000). The sulcus was pigmented along its entire length in *P. dorsimaculatus, P. verdugo* and *Phymaturus roigorum*. In species belonging to the puna clade (*Phymaturus denotatus*, *Phymaturus laurenti*, and *Phymaturus punae*), this pigmentation was extended over apical surfaces of lobes, which may be an additional apomorphy for this subclade (Lobo et al., 2012). The everted hemipenis of *P. patagonicus* and other representatives of the *patagonicus* group were completely white, lacking any kind of pigmentation on its sulcus and lobes.

Observations on Liolaemus *Species*.—The presence of hemiclitores in *L. irregularis* and *L. umbrifer* females was confirmed. Variation in size and pigmentation between them was evident, and the morphology of this organ within the genus deserves further investigation. The transversus penis was not found in either species of *Liolaemus* examined, although the retractor clitoridis magnus was present. Arnold (1984) found a difference between *Phymaturus* and the related genera *Liolaemus* and *Ctenoblepharys*, with the latter two exhibiting insertion of retractor lateralis posterior more strongly developed than in *Phymaturus*. Additionally, the retractor lateralis anterior did not extend backward beyond the level of the vent in *Phymaturus* and originated from a fascia below the anterior caudofemoralis muscle.

Final Considerations.—In hemipenes and hemiclitores we did not observe hemibacula as described for varanids (Böhme, 1988; Card and Kluge, 1995). Variation in the degree of development of muscles associated with the hemiclitoris was reported by Arnold (1984). This author emphasized that muscle arrangement behind the vent "is often surprisingly like that found in males." He clearly described the variation found in those "diverticula" and associated muscles among several genera of lizards, reporting differences among males and females in lacetids, varanids, and teiids. These diverticula reported by Arnold (1984) were formally recognized as homologous to hemipenes in females by Böhme (1995) and Ziegler and Böhme (1997). In the case of *Liolaemus*, we confirmed the presence of hemiclitores and associated muscles only in two species, but this genus is one the most diverse within Iguania (more than 250 species). The present research poses several questions that deserve further investigation. For example,

is there the same kind of variation in the presence of hemiclitores within liolaemids, similar to the variation reported *Bothrops* (Marques et al., 2002)? Is there the same variation of the whole associated musculature? How does reduction in certain muscles affect the function of evertion in hemiclitores given the variation found among adult females of different liolaemid species?

In conclusion, the anatomy of the hemipenes/hemiclitores complex is one of the most striking apomorphies of Squamata and deserves deeper analysis because of its phylogenetic and functional implications.

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Appendix 1

Specimens Examined

Phymaturus adult females: *Phymaturus aguanegra* (MCN 972); *Phymaturus* cf. *antofagastensis* (MCN 2123); *Phymaturus calcogaster* (MCN 4302); *Phymaturus castillensis* (MCN 3961); *Phymaturus ceii* (MCN 910, 915, 3938); *Phymaturus* cf. *ceii* (MCN 3894); *Phymaturus dorsimaculatus* (MCN 3735); *Phymaturus felixi* (MCN 3984, 3985); *Phymaturus excelsus* (MCN 1589); *Phymaturus indistinctus* (MCN 3955); *Phymaturus nevadoi* (MCN 3654); *Phymaturus querque* (MCN 3859); *Phymaturus laurenti* (MCN 314); *Phymaturus* cf. *palluma* (MCN 2111); *Phymaturus palluma* (MCN 1975); *Phymaturus patagonicus* (MCN 3273); *Phymaturus roigorum* (MCN 2098); *Phymaturus* cf. *roigorum* (MCN 3718); *Phymaturus verdugo* (MCN 1961); *Phymaturus zapalensis* (MCN 3848, 3849).

Phymaturus embryos: *Phymaturus aguanegra* (MCN 972, 976, 981, 986); *P.* cf. *antofagastensis* (MCN 2123); *Phymaturus bibroni* (SSUC Re 0429) *P. dorsimaculatus* (MCN 3732, MCN 3740, MCN 3735, MCN 3736); *P. calcogaster* (MCN 4302, 4304, 4298); *P. castillensis* (MCN 3960, 3964); *P.* cf. *ceii* (MCN 3935, 3897, 3898, 3896); *P. felixi* (MCN 3985); *P. excelsus* (MCN 1589); *P. indistinctus* (MCN 3559); *P. laurenti* (MCN 325); *P. nevadoi* (MCN 3656, 3654, 3660); *P.* cf. *palluma* (MCN 3623); *P. palluma* (MCN 3621, 3628, 3128, 3129); *P. patagonicus* (IBA 789–5, 789–2, 789–6); *P. querque* (MCN 3854, 3855, 3858, 3862); *P. roigorum* (MCN 1962, IBA 733–4); *P.* cf. *roigorum* (MCN 3713); *P. verdugo* (MCN 1973, 1974, 1958); *P. zapalensis* (MCN 3848, 3853).

Phymaturus adult males: *Phymaturus aguanegra* (MCN 3284, 3285); *P. antofagastensis* (MCN 1436); *P. calcogaster* (MCN 4295); *P. castillensis* (MCN 3968); *Phymaturus denotatus* (MCN 3159); *P. dorsimaculatus* (MCN 3728, 3779); *Phymaturus etheridgei* (MCN 4305); *P. felixi* (MCN 3979); *P. indistinctus* (MCN 3954); *P. laurenti* (MCN 320); *Phymaturus manuelae* (MCN 3933); *P. nevadoi* (MCN 3653); *P. palluma* (MCN 1977); *P. patagonicus* (MCN 1255); *Phymaturus payuniae* (MCN 3666); *Phymaturus punae* (MCN 3124); *P. roigorum* (MCN 1963); *Phymaturus somuncurensis* (MCN 4550).

Liolaemus adult females: *Liolaemus irregularis* (MCN 2369); *Liolaemus umbrifer* (MCN 4360).