Canadian Journal of Zoology
Revue canadienne de zoologie

HEMIPENIAL MORPHOLOGY AND DIVERSITY IN SOUTH AMERICAN ANOLES (SQUAMATA: DACTYLOIDAE)

Journal:	<i>Canadian Journal of Zoology</i>
Manuscript ID	cjz-2015-0194.R1
Manuscript Type:	Article
Date Submitted by the Author:	10-Jan-2016
Complete List of Authors:	D'Angiolella, Annelise; Universidade da Amazonia - UNAMA, Urban Development and environment; Programa de Pós-Graduação em Zoologia UFPA/MPEG, Herpetology Klaczko, Julia; Universidade Estadual de Campinas, Biologia Animal Rodrigues, Miguel; Universidade de São Paulo, Zoologia Avila Pires, Teresa Cristina; Museu Paraense Emilio Goeldi, Herpetologia
Keyword:	Hemipenis, Anolis, Norops, Dactyloa, South America, Male genitalia

SCHOLARONE™
Manuscripts

HEMIPENIAL MORPHOLOGY AND DIVERSITY IN SOUTH AMERICAN
ANOLES (SQUAMATA: DACTYLOIDAE)

A. B. D'Angiolella, J. Klaczko, M. T. Rodrigues, T. C. S. Avila-Pires

Author for correspondence: A.B.D'Angiolella: Programa de Pós-Graduação em Zoologia UFPA/MPEG, Caixa Postal 399, CEP 66017-970; Belém, PA, Brazil. annelise.dangiolella@gmail.com. Current affiliation and address: Universidade da Amazônia – UNAMA, Programa de Pós-Graduação em Desenvolvimento e Meio Ambiente Urbano – PPDMU. Av. Alcindo Cacela, 287, CEP 66060902; Belém, PA, Brazil. Phone number: 55 91 983008722;

J. Klaczko: Departamento de Biologia Animal, Instituto de Biologia, Universidade Estadual de Campinas, CEP 13.083-862, Campinas, Brazil. jklaczko@gmail.com

M.T. Rodrigues: Universidade de São Paulo, Instituto de Biociências, Departamento de Zoologia, Caixa Postal 11.461, CEP 05508-090, São Paulo, SP, Brazil. mturodri@usp.br.

T.C.S. Avila-Pires: Museu Paraense Emilio Goeldi, Belém, Pará, Brazil; Caixa Postal 399, CEP 66017-970; Belém, PA, Brazil. avilapires@museu-goeldi.br.

ABSTRACT

Hemipenial morphology has provided useful characters to improve species identification and phylogenetic relationships in squamates. Here we provide hemipenial description and illustration of thirteen South American anoles. At generic and specific levels, differences are mainly related to shape and ornamentation; intraspecific variation is low. An asulcate process, present in the hemipenis of most anole species studied, was highly variable among species and may be a useful taxonomic character in hemipenial morphology of this group.

Key words: *Norops*, *Dactyloa*, South America, Hemipenis, male genitalia.

Draft

INTRODUCTION

Morphology of male genitalia provide helpful taxonomic characters in many groups of vertebrates and invertebrates (Keogh 1999; Lüpold et al. 2004; Yoshizawa and Jonhson 2006; Köhler et al. 2007; Song and Bucheli 2010; Köhler 2010). Genitalia can be particularly useful in differentiating closely related species when overall body morphology is highly conserved. (Keogh 1999). In squamates, male genitalia consist of a pair of blind-ended tubular structures known as hemipenes. Among different species, hemipenes may vary in size, shape and aspects of ornamentation (Dowling and Savage 1960). In some groups hemipenial morphology is conservative (Klaver and Böhme 1986; Keogh 1999), while in others it displays significant intraspecific variation (Zaher and Prudente 1999; Köhler 2009; Nunes et al. 2012). The study of hemipenial morphology has provided important insights in the systematics of squamate reptiles, elucidating questions about species identification and phylogenetic relationships (Dowling and Savage 1960; Böhme 1991; Böhme and Ziegler 1997; Keogh 1999; Köhler et al. 2012). In addition, as it is directly involved in the copulation process, modifications in its morphology may result in reproductive isolation (Arnold 1986; Köhler and Sunyer 2008; Köhler 2009; Nunes et al. 2012). Among lizards, hemipenial characters are most studied for members of the families Agamidae, Chamaleonidae, Dactyloidae, Gymnophthalmidae, Lacertidae, and Varanidae (Arnold 1973, 1986; Köhler and Veselý 2007; Böhme and Ziegler 2008; Rodrigues et al. 2008; Myers et al. 2009, Nunes et al. 2012; Maduwage and Silva 2012; Nunes et al. 2014). In the last decade, anole hemipenes have gained more attention, thanks to the work of G. Köhler and colleagues with mainland Central American species. These authors found several cases of geographically separated populations of putative closely related species with very distinct hemipenial morphology — usually a large and bilobate organ in one population

contrasting with a smaller and unilobate one in the other population (Köhler et al. 2010; Köhler et al. 2012). Some of these studies revealed the importance of hemipenis in identifying cryptic diversity and helping to elucidate the taxonomy at species level (e.g., Köhler and Sunyer 2008; Köhler and Veselý 2010). Klaczko et al. (2015) showed that anole hemipenial morphology evolved six times faster than other non-genital morphological traits, and therefore can be directly related to reproductive isolation and the speciation process. On the other hand, in spite of such high variability between closely related species, Böhme and Ziegler (2008) showed that, at least for some groups of lizards, genital morphological characters are more informative than external characters for recovering phylogenetic relationships, once it is subject only to sexual selection.

Since there is much convergence in external features of anoline lizards, the description of hemipenial morphology is especially useful as an effort to provide additional phylogenetic characters for investigating their relationships. With that in view, we present a detailed description of hemipenial morphology of three *Dactyloa* and ten *Norops* species occurring in South America.

MATERIAL AND METHODS

We examined the hemipenes of 13 anole species occurring in South America: *Dactyloa punctata* (Daudin 1802), *D. phyllorhina* (Myers and Carvalho 1945), *D. transversalis* (Duméril 1851), *Norops auratus* (Daudin 1802), *N. brasiliensis* (Vanzolini and Williams 1970), *N. chrysolepis* (Duméril and Bibron 1837), *N. fuscoauratus* (Duméril and Bibron 1837), *N. meridionalis* (Boettger 1885), *N. ortonii* (Cope 1868), *N. planiceps* (Troschel 1848), *N. scypheus* (Cope 1864), *N. tandai* (Avila-Pires 1995), and *N. trachyderma* (Cope 1875). We prepared from three to ten hemipenes per species, mostly four (Table 1), except for *D. phyllorhina*, of which description and comparisons

were based on photos of a hemipenis prepared by the late Gabriel Skuk. For hemipenis preparation, we followed a combination of techniques described in Pesantes (1994), Keogh (1999), Myers and Cadle (2003), and Zaher and Prudente (2003). To minimize possible variation caused by the eversion process, only partially or totally everted hemipenes were prepared, irrespective of being the right or left hemipenis. Once the preserved specimen was selected, one of the hemipenes was removed through a small incision at the base of the tail. In general, anole hemipenes have a very thin skin and to avoid possible damages, KOH 2% was used only when the specimen was too old or when the lobes could not be totally everted with hot water. To allow a better visualization of ornamentation structures, the fully everted hemipenis was filled with colored vaseline with the aid of a syringe with a sanded tip needle, to avoid damages to the hemipenis skin, and was subsequently conserved in 70% ethanol. We obtained digital images of hemipenes in sulcate, asulcate, and lateral views. For the smallest hemipenes we used a JVC camera KYF75U attached to a stereomicroscope, with the aid of Auto-Montage Pro 5.02; all other hemipenes were photographed using a Nikon D90, macro lens of 105 mm.

Basic hemipenial terminology follows Klaver and Böhme (1986). We refer to a 'nude disk' when the lobes terminate in a flat and naked disk delimited by raised sulcal lips (Keogh, 1999), while if a 'naked area' is mentioned, no raised sulcal lips are present. We adopt the term 'flounces' in reference to widely spaced, irregular and non-overlapping folds (Savage, 1997). Descriptions of hemipenes of some anole species mention the presence of an asulcate process (e.g., Köhler et al. 2007; Sunyer et al. 2008; Köhler and Smith, 2008; Köhler and Sunyer, 2008). In the present study, we differentiate two structures that may be present on the asulcate surface: a fleshy

projection that expands outward from the lobular crotch or bends toward the truncus; and a skin ridge (sensu Keogh, 1999) formed by a series of raised fleshy tissue.

RESULTS

All hemipenes of South American anoles are bilobed, with globular or tubular calyculated lobes showing a naked area or naked disk, and present a deep and bifurcated sulcus spermaticus. No mineralized structures are present (Figure 1). Whereas several differences are observed at the generic and specific levels, intraspecific variation is relatively low.

In *Dactyloa*, hemipenes of the three species examined show globular lobes, with a wide and elongate naked area in the sulcate surface. In contrast, lobes of most *Norops* tend to be proportionally smaller and the naked area form a round disk (except in *N. ortonii*, where an elongate naked area is present). Hemipenes of *D. transversalis* and *D. phyllorhina* are more similar to each other than to that of *D. punctata*. Both show a longer truncus, a skin ridge on the asulcate surface, and no triangular flap covered by folds between lobes, whereas in *D. punctata* the hemipenial truncus is short, the lobes occupy almost the entire hemipenial extension, and a triangular flap is present between lobes on the asulcate surface.

Among the species of the *Norops chrysolepis* group (sensu D'Angiolella et al. 2011) the hemipenes of *N. meridionalis*, *N. tandai*, and *N. chrysolepis* are very similar, presenting thick sulcal lips, a wide and subdivided fleshy projection between the globular lobes, and a thin skin pleat demarcating the asulcate and sulcate surfaces on truncus. *N. planiceps* and *N. brasiliensis* have both tubular-shaped lobes; naked disk in terminal position on lobes, delimited by a raised edge of skin; and a medial skin ridge on the asulcate surface of truncus. The hemipenis of *N. scypheus* is similar to those of *N.*

meridonalis, *N. tandai*, and *N. chrysolepis* in having globular lobes and naked disks on lobes that extends from their base to almost the apex. However, it is also similar to those of *N. brasiliensis* and *N. planiceps* in lacking a fleshy projection between lobes, presenting instead a small skin lump medially on asulcate surface of truncus.

The hemipenis of *N. trachyderma* is similar to those of *N. brasiliensis* and *N. planiceps* in having tubular lobes, a terminal naked disk, and a medial skin ridge on the asulcate surface, extending from the midway on the truncus to the crotch. This later characteristic is also present in *N. fuscoauratus*. Hemipenes of *N. ortonii* and *N. auratus* are similar to each other in presenting globular lobes, a finger-shaped process projecting from the crotch on the asulcate surface, and truncus covered by well-delimited transverse folds. Besides, *N. ortonii* presents the naked area on lobes elongate, not forming a definite disk as in the other *Norops* examined, reminding to a certain extent those of *Dactyloa*.

Below we present the hemipenial description of all species studied (Figure 2).

DACTYLOA SPECIES

Dactyloa punctata (Figure 2A)

Hemipenis bilobed, with globular lobes representing more than half of the hemipenis length. Sulcus spermaticus deep and bifurcated, with well-developed sulcal lips. It opens into a wide and elongate naked area extending laterally from the base of the lobes to the apex. Lobes densely calyculate outside this naked area. A triangular flap, covered by enlarged and irregular folds, present in the asulcate surface, initiating midway on the lobes and pointing toward the base of the hemipenis. Truncus short, wrinkled on both sulcate and asulcate surfaces, with a short and smooth base.

Dactyloa phyllorhina (Figure 2B)

Hemipenis bilobed, with globular lobes representing about half of the hemipenis length. Sulcus spermaticus deep and bifurcated, with well-developed sulcal lips. It opens into a wide and elongate naked area extending laterally from the base of the lobes to the apex. Lobes densely calyculate outside this naked area. A skin ridge present in the asulcate surface, initiating midway on the lobes, where it is surrounded by enlarged calyces, and reaching the median region of truncus, where it is covered by enlarged and irregular transverse folds. Truncus wrinkled on both sulcate, and asulcate surfaces.

Dactyloa transversalis (Figure 2C)

Hemipenis bilobed, with globular lobes representing at least half of the hemipenis length. Sulcus spermaticus deep and bifurcated, with well-developed sulcal lips. It opens into a wide and elongate naked area extending laterally from the base of the lobes to the apex. Lobes densely calyculate outside this naked area. Enlarged and irregular calyces cover the asulcate surface between lobes. Truncus with a shallow medial skin ridge surrounded by transverse folds.

NOROPS SPECIES:

Norops auratus (Figure 2D)

Hemipenis bilobed, with globular lobes representing about half of the hemipenis length. Sulcus spermaticus deep and bifurcated, with poorly developed sulcal lips. It opens into a round naked disk extending laterally from the base of the lobes to the apex. Lobes densely calyculate outside the naked disk. A fleshy finger-shaped process projects from the crotch on the asulcate surface, surrounded by enlarged calyces. Asulcate and lateral surfaces of truncus covered with well-delimited transverse folds; oblique or longitudinal wrinkles on the sulcate surface.

Norops brasiliensis (Figure 2E)

Hemipenis bilobed, with tubular lobes representing about half of the hemipenis length. Sulcus spermaticus deep and bifurcated, with well-developed sulcal lips. It opens into a flat and naked disk on terminal region of each lobe. Lobes densely calyculate outside naked disk. A shallow medial skin ridge is present on the asulcate surface, from halfway the truncus to the crotch. Truncus covered with calyces distally and with flounces basally, both on sulcate and asulcate surfaces.

Norops chrysolepis (Figure 2F)

Hemipenis bilobed, with globular lobes representing about half of the hemipenis length. Sulcus spermaticus deep and bifurcated, with well-developed sulcal lips becoming thicker at the lobes. It opens into a round naked disk that extends laterally from the base of the lobes to almost the apex. Lobes densely calyculate outside the naked disk; separated by a large, naked, triangular expansion that pushes them to the sides, giving the hemipenis a 'T' shape. An extension of this expansion, with wrinkled surface, projects shortly towards the truncus and is followed by a medial and shallow skin ridge that extends towards the base of the hemipenis. A thin skin pleat extends from the basal region of the hemipenis to the base of the lobes laterally, demarcating the asulcate and sulcate surfaces. Truncus covered with flounces on asulcate and sulcate surfaces.

Norops fuscoauratus (Figure 2G and 2H)

Hemipenis bilobed, with globular lobes representing less than half of the hemipenis length. Sulcus spermaticus deep and bifurcated, with poorly developed sulcal lips. It opens into a round naked disk that extends laterally from the base of the lobes to the apex. Lobes densely calyculate outside the naked disk. A medial skin ridge from the distal part of truncus to the crotch on asulcate surface. Truncus covered with flounces on asulcate and sulcate surfaces. Some of the hemipenes show small pigmented spots on truncus, however the amount of pigmentation varies among specimens and localities,

and in a same locality there are specimens with pigmented and specimens with non-pigmented hemipenis (e.g. Guyana: Potaro-Siparuni and Brazil: Pacoti-CE and Porto Velho-RO).

Norops meridionalis (Figure 2I)

Hemipenis bilobed, with globular lobes representing about half of the hemipenis length. Sulcus spermaticus deep and bifurcated, with well-developed sulcal lips that become thicker at the lobes. It opens into a round naked disk that extends laterally from the base of the lobes to almost the apex. Lobes densely calyculate outside the naked disk. A fleshy projection between lobes on asulcate surface, that becomes a triangular pleat toward the truncus. A thin skin pleat extends from the hemipenial base to the base of the lobes laterally, demarcating the asulcate and sulcate surfaces. Truncus shows a naked region below the triangular pleat projection on asulcate surface, and longitudinal wrinkles on sulcate surface.

Norops ortonii (Figure 2J)

Hemipenis bilobed, with globular lobes representing more than half of the hemipenis length. Sulcus spermaticus deep and bifurcated with well-developed sulcal lips, which become shallow halfway the lobes. It opens into an elongate naked area, extending laterally from the base of the lobes to the apex. Lobes densely calyculate outside the naked area. A finger-shaped process projects from the crotch on the asulcate surface, surrounded by enlarged calyces. Asulcate and lateral surfaces of truncus covered with transverse folds.

Norops planiceps (Figure 2K)

Hemipenis bilobed, with tubular lobes representing about half of the hemipenis length. Sulcus spermaticus deep and bifurcated, with well-developed sulcal lips. It opens into a flat and naked disk on terminal region of each lobe. Lobes strongly calyculate outside

naked disk. A fleshy skin ridge on asulcate surface, from halfway the truncus to the crotch. Truncus covered with calyces distally and with flounces basally, both on sulcate and asulcate surfaces.

Norops scypheus (Figure 2L)

Hemipenis bilobed, with globular lobes representing about half of the hemipenis length. Sulcus spermaticus deep and bifurcated, with well-developed sulcal lips. It opens into a round naked disk that extends laterally from the base of the lobes to almost the apex. Lobes densely calyculate outside the naked disk. A delicate soft skin lump present medially on truncus asulcate surface. Truncus covered with flounces.

Norops tandai (Figure 2M)

Hemipenis bilobed, with globular lobes representing about half of the hemipenis length. Sulcus spermaticus deep and bifurcated, with well-developed sulcal lips that become thicker at the lobes. It opens into a round naked disk that extends laterally from the base of the lobes to almost the apex. Lobes densely calyculate outside the naked disk; separated by a large, naked, triangular expansion that pushes them to the sides, giving the hemipenes a 'T' shape. An extension of this expansion, with wrinkled surface, projects shortly towards the truncus and is followed by a medial and shallow skin ridge that extends towards the hemipenial base. A thin skin pleat extends from the basal region of the hemipenes to the base of the lobes laterally, demarcating the asulcate and sulcate surfaces. Truncus covered with flounces.

Norops trachyderma (Figure 2N)

Hemipenis bilobed, with tubular lobes representing about half of the hemipenis length. Sulcus spermaticus deep and bifurcated, with poorly developed sulcal lips. It opens into

a naked disk on the terminal region of each lobe. Lobes densely calyculate outside the naked disk. A fleshy medial skin ridge on asulcate surface, from about midway on the truncus to the crotch. Truncus distally ornamented with flounces on asulcate surface, surrounding the skin ridge, and shallow wrinkles on sulcate surface; base naked.

DISCUSSION

The use of hemipenial characters in systematic studies of anoles has increased substantially in the past years (Köhler et al. 2012 and references therein). This sudden interest is due mostly to the discovery of a large number of potential informative characters, helping to diagnose morphologically similar species (Köhler et al. 2007; Köhler and Veselý, 2010). In a variety of studies, divergent hemipenial morphology was the starting point for systematic revisions and/or species description (Köhler 2009, 2010, 2011). In the present work, we show that intergeneric and interspecific hemipenial variation in South American anoles is high, with considerable differences in hemipenial shape and ornamentation.

South American anoles do not form a monophyletic group and the same applies to the South American radiation of *Norops*, which has their closest relatives in Central America / West Indies (Nicholson et al. 2012). An exception is the *Norops chrysolepis* species group, which evolved in South America. D'Angiolella et al. (2011) revisited the taxonomy of the group, using both morphology and molecular characters. The hemipenial morphology variation is concordant with their recovered molecular phylogeny for this group, with closely related species presenting similar hemipenes. In addition, *N. meridionalis* shares with *N. tandai* and *N. chrysolepis* the presence of a thin skin pleat demarcating the asulcate and sulcate surfaces on truncus. Vanzolini and Williams (1970), on basis of morphology and habitat use (respectively grassland and forest), considered *N. meridionalis* as only superficially similar to the *chrysolepis*

group. Molecular studies, however, recovered *N. meridionalis* within this group, sister to *N. tandai* and *N. chrysolepis* (Nicholson et al. 2006; D'Angiolella et al. 2011). Even though this relationship received weak support in both studies, our results corroborate it. Böhme and Ziegler (2008) found high levels of coincidence between molecular-genetic phylogenies and phylogenetic hypotheses proposed on basis of hemipenial morphology, and attributed this to the fact that genital morphology seems to be only subject to sexual selection, not being affected by environmental selective pressures. In the case of *N. meridionalis*, its divergent morphology might be related to its ecological adaptation as a grassland dweller, while hemipenial characters were not affected by such environmental selection, retaining the phylogenetic signal.

With the present study, we were able to observe that the most conspicuous feature of the anole hemipenes studied is the presence of asulcate processes, either as fleshy projections or as a skin ridge. This structure varied among the species in relation to form, extension, development and ornamentation, suggesting it may be an important character for systematic and taxonomic future analyses.

ACKNOWLEDGMENTS

ABD thanks Marcelia Bastos and Darlan Feitosa for providing initial help with hemipenes preparation; Adriano O. Maciel for taking the pictures of the hemipenes, Ana Prudente for suggestions about preparation and nomenclature. We thank Hussam Zaher, David Kizirian, Jonathan Losos, and Ana Prudente for giving permission for hemipenial preparation in MZUSP, AMNH, MCZ and MPEG, respectively. CNPq provided fellowships to ABD, TCSAP and MTR. FAPESP provided fellowships to JK and funding to MTR.

REFERENCES

- Arnold, E.N. 1973. Relationships of the Palearctic lizards assigned to the genera *Lacerta*, *Algyroides* and *Psammodromus* (Reptilia: Lacertidae). Bull. Br. Mus. (Nat. Hist.), Zool. **25**: 289–366.
- Arnold, E.N. 1986. Why copulatory organs provide so many useful taxonomic characters: the origin and maintenance of hemipenial differences in lacertid lizards (Reptilia: Lacertidae). Biol. J. Linn. Soc. **29**: 263–281.
- W. Böhme. 1991. New findings on the hemipenial morphology of monitor lizards and their systematic implications. In *Advances in Monitor Research. Edited by W. Böhme and H.G. Horn*. Mertensiella Deutsche Gesellschaft für Herpetologie und Terrarienkunde e.V., Rheinbach. pp. 42–49.
- Böhme, W., and Ziegler, T. 1997. A taxonomic review of the *Varanus* (*Polydaedalus*) *niloticus* (Linnaeus, 1766) species complex. Herpetol. J. **7**(4): 155–162.
- Böhme, W., and Ziegler, T. 2008. A review of iguanian and anguimorph lizard genitalia (Squamata: Chamaeleonidae, Varanoidea, Shinisauridae, Xenosauridae, Anguidae) and their phylogenetic significance: comparisons with molecular data sets. J. Zool. Syst. Evol. Res. **47**(2): 189–202.
- D'Angiolella, A.B., Gamble, T., Avila-Pires, T.C.S., Colli, G.R., Noonan, B.P., and Vitt, L.J. 2011. *Anolis chrysolepis* Duméril and Bibron, 1837 (Squamata: Iguanidae), revisited: molecular phylogeny and taxonomy of the *Anolis chrysolepis* species group. Bull. Mus. Comp. Zool. **160**(2): 35–63.
- Dowling, H.G., and Savage, J.M. 1960. A guide to the Snake hemipenis: a survey of basic structure and systematic characteristics. Zoologica, **45**: 17–28.

- Klaczko, J., Ingram, T., and Losos, J. 2015. Genitals evolve faster than other traits in *Anolis* lizards. *J. Zool. (Lond.)* **295**(1): 44–48.
- Klaver, C., and Böhme, W. 1986. Phylogeny and classification of the Chamaeleonidae (Sauria) with special reference to hemipenis morphology. *Bonn Zoological Monograph*, **22**: 1–64.
- Keogh, S. 1999. Evolutionary implications of hemipenial morphology in the terrestrial Australian elapid snakes. *Zool. J. Linn. Soc.* **125**: 239–278.
- Köhler, G., and Veselý, M. 2007. The hemipenis of *Opipeuter xestus* (Squamata: Gymnophthalmidae). *Salamandra*, **43**(1): 1–3.
- Köhler, G., Ponce, M., Sunyer, J., and Batista, A. 2007. Four new species of anoles (genus *Anolis*) from the Serranía de Tabasará, west-central Panama (Squamata: Polychrotidae). *Herpetologica*, **63**(3): 375–391.
- Köhler, G., and Smith, E.N. 2008. A new species of anole of the *Norops schiedei* group from western Guatemala (Squamata: Polychrotidae). *Herpetologica*, **64**(2): 216–223.
- Köhler, G., and Sunyer, J. 2008. Two new species of anoles formerly referred to as *Anolis limifrons* (Squamata: Polychrotidae). *Herpetologica*, **64**(1): 92–108.
- Köhler, G. 2009. New species of *Anolis* formerly referred to as *Anolis altae* from Monteverde, Costa Rica (Squamata: Polychrotidae). *J. Herpetol.* **43**(1): 11–20.
- Köhler, G. 2010. A revision of the Central American species related to *Anolis pentapryon* with the resurrection of *A. beckeri* and the description of a new species (Squamata: Polychrotidae). *Zootaxa*, **2354**: 1–18.
- Köhler, G., and Veselý, M. 2010. A revision of the *Anolis sericeus* complex with the resurrection of *A. wellbornae* and the description of a new species (Squamata: Polychrotidae). *Herpetologica*, **66**(2): 207–228.

- Köhler, G., Dehling, D.M., and Köhler, J. 2010. Cryptic species and hybridization in the *Anolis polylepis* complex, with the description of a new species from the Osa Peninsula, Costa Rica (Squamata: Polychrotidae). *Zootaxa*, **2718**: 23–38.
- Köhler, G. 2011. A new species of anole related to *Anolis altae* from Volcán Tenorio, Costa Rica (Reptilia, Squamata, Polychrotidae). *Zootaxa*, **3120**: 29–42.
- Köhler, J., Hahn, M., and Köhler, G. 2012. Divergent evolution of hemipenial morphology in two cryptic species of mainland anoles related to *Anolis polylepis*. *Salamandra*, **48**(1): 1–11.
- Köhler, G., Batista, A., Veselý, M., Ponce, M., Carrizo, A., and Lotzkat, S. 2012. Evidence for the recognition of two species of *Anolis* formerly referred to as *A. tropidogaster* (Squamata: Dactyloidae). *Zootaxa*, **3348**: 1–23.
- Lüpold, S., McElligott, A.G., and Hosken, D.J. 2004. Bat genitalia: allometry, variation and good genes. *Biol. J. Linn. Soc.* **83**(4): 497–507.
- Maduwage, K., and Silva, A. 2012. Hemipeneal Morphology of Sri Lankan Dragon Lizards (Sauria: Agamidae). *Ceylon Journal of Science (Biological Sciences)*, **41**(2): 111–123.
- Myers, C.W., and Cadle, J.E. 2003. Points of view. *Herpetol. Rev.* **34**(4): 295–302.
- Myers, C.W., Fuenmayor, G.R., and Jadin, R.C. 2009. New species of lizards from Auyantepui and La Escalera in the Venezuelan Guayana, with notes on “microteiid” hemipenes (Squamata: Gymnophthalmidae). *Am. Mus. Novit. No.* **3660**: 1–31.
- Nicholson, K.E., Mijares-Urrutia, A., and Larson, A. 2006. Molecular phylogenetics of the *Anolis onca* series: a case history in retrograde evolution revisited. *J. Exp. Zool. B* **306**(5): 450–459.

- Nicholson, K.E., Crother, B.I., Guyer, C., and Savage, J.M. 2012. It is time for a new classification of anoles (Squamata: Dactyloidae). *Zootaxa*, **3477**: 1–108.
- Nunes, P.M.S., Fouquet, A., Curcio, F.F., Kok, P., and Rodrigues, M.T. 2012. Cryptic species in *Iphisa elegans* Gray, 1851 (Squamata: Gymnophthalmidae) revealed by hemipenial morphology and molecular data. *Zool. J. Linn. Soc.* **166**(2): 361–376.
- Nunes, P.M.S., Curcio, F.F., Roscito, J.G., and Rodrigues, M.T. 2014. Are Hemipenial Spines Related to Limb Reduction? A Spiny Discussion Focused on Gymnophthalmid Lizards (Squamata: Gymnophthalmidae). *Anat. Rec.* **297**(3): 482–495.
- Pesantes, O.S. 1994. A method for preparing the hemipenis of preserved snakes. *J. Herpetol.* **28**: 93–95.
- Rodrigues, M.T., Camacho, A., Nunes, P.M.S., Recoder, R.S., Teixeira, M., Jr., Valdujo, P.H., Ghellere, J.M.B., Mott, T, and Nogueira, C. 2008. A new species of the lizard genus *Bachia* (Squamata: Gymnophthalmidae) from the Cerrados of Central Brazil. *Zootaxa*, **1875**: 39–50.
- Savage, J.M. 1997. On terminology for the description of the hemipenes of squamate reptiles. *J. Herpetol.* **7**: 23–25.
- Song, H. and Bucheli S.R. 2010. The utility of insect genitalia in morphological analyses. *Cladistics*, **26**(1): 23–35.
- Sunyer, J., Veselý, M., and Kohler, G. 2008. Morphological variation in *Anolis wermuthi* (Köhler and Obermeier, 1998), a species endemic to the highlands of north-central Nicaragua. *Senckenbergiana Biologica*, **88**(2): 335–343.
- Vanzolini, P.E., and Williams, E.E. 1970. South American anoles: the geographic differentiation and evolution of the *Anolis chrysolepis* species group (Sauria, Iguanidae). *Arquivos de Zoologia*, **19**(1-2): 1–176.

Yoshizawa, K., and Johnson, K.P. 2006. Morphology of male genitalia in lice and their relatives and phylogenetic implications. *Syst. Entomol.* **31**(2): 350–361.

Zaher H., and Prudente A.L.C. 1999. Intraspecific variation of the hemipenes in *Siphlophis* and *Tripanurgos*. *J. Herpetol.* **33**(4): 698–702.

Zaher, H., and Prudente, A.L.C. 2003. Hemipenes of *Siphlophis* (Serpentes, Xenodontinae) and techniques of hemipenial preparation in snakes: a response to Dowling. *Herpetol. Rev.* **34**(4): 302–307.

Draft

Table 1: Museum numbers and collecting locality of *Norops* and *Dactyloa* specimens whose hemipenes were prepared for the present study.

Identification Number	Genus	Species	Locality	Country
MPEG28904	<i>Dactyloa</i>	<i>punctata</i>	Floresta Nacional de Caxiuanã, PA	Brazil
MPEG16011	<i>Dactyloa</i>	<i>punctata</i>	29 km North of Rio Branco, AC	Brazil
MPEG28866	<i>Dactyloa</i>	<i>punctata</i>	Floresta Nacional de Caxiuanã, PA	Brazil
MPEG21575	<i>Dactyloa</i>	<i>transversalis</i>	Floresta Nacional Serra da Cotia, RO	Brazil
MPEG21576	<i>Dactyloa</i>	<i>transversalis</i>	Floresta Nacional Serra da Cotia, RO	Brazil
MPEG4106	<i>Norops</i>	<i>auratus</i>	Boa Vista, RR	Brazil
MPEG4068	<i>Norops</i>	<i>auratus</i>	Boa Vista, RR	Brazil
MPEG18236	<i>Norops</i>	<i>brasiliensis</i>	Minacu, GO	Brazil
MPEG12383	<i>Norops</i>	<i>brasiliensis</i>	Arari, Maranhão	Brazil
MPEG24491	<i>Norops</i>	<i>brasiliensis</i>	Tanguro, MT	Brazil
MPEG29707	<i>Norops</i>	<i>chrysolepis</i>	Laranjal do Jari, AP	Brazil
MPEG27374	<i>Norops</i>	<i>chrysolepis</i>	Serra do Acari, PA	Brazil
MPEG22197	<i>Norops</i>	<i>chrysolepis</i>	Porto Trombetas, Oriximiná, PA	Brazil
MPEG27367	<i>Norops</i>	<i>chrysolepis</i>	Urucará, PA	Brazil
MSH12316	<i>Norops</i>	<i>fuscoauratus</i>	Santa Isabel do Rio Negro, AM	Brazil
MPEGVXG14	<i>Norops</i>	<i>fuscoauratus</i>	Vitoria do Xingu, PA	Brazil
MPEGBP02	<i>Norops</i>	<i>fuscoauratus</i>	Bacia Rio Capim, Paragominas, PA	Brazil
MPEG29607	<i>Norops</i>	<i>fuscoauratus</i>	Afuá, Ilha do Marajó, PA	Brazil
MPEG20590	<i>Norops</i>	<i>fuscoauratus</i>	P. Walter, Juruá, AC	Brazil
MPEG28863	<i>Norops</i>	<i>fuscoauratus</i>	Floresta Nacional Caxiuanã, PA	Brazil

AMNHR-115871	<i>Norops fuscoauratus</i>	Napo	Ecuador
AMNHR-133697	<i>Norops fuscoauratus</i>	Amazonas	Venezuela
MZUSP27091	<i>Norops fuscoauratus</i>	Napo, Limoncocha	Ecuador
MZUSP65666	<i>Norops fuscoauratus</i>	Estação Ecológica de Saltinho, PE	Brazil
MZUSP81620	<i>Norops fuscoauratus</i>	Apiacás, MT	Brazil
MZUSP16182	<i>Norops fuscoauratus</i>	Canavieiras, BA	Brazil
AMNH151836	<i>Norops fuscoauratus</i>	Guyana	Guyana
MBS024	<i>Norops meridionalis</i>	Mina Guaporé, MT	Brazil
MBS010	<i>Norops meridionalis</i>	Mina Guaporé, MT	Brazil
MPEG21781	<i>Norops ortonii</i>	Floresta Nacional de Caxiuanã, PA	Brazil
MPEG26941	<i>Norops ortonii</i>	Porto Urucu, AM	Brazil
MPEG31113	<i>Norops ortonii</i>	Taracauá, AC	Brazil
MPEG22225	<i>Norops planiceps</i>	São João Lucas, RR	Brazil
MPEG15814	<i>Norops planiceps</i>	Inpa, Manaus, AM	Brazil
MPEG3922	<i>Norops planiceps</i>	Coronel Mota, RR	Brazil
MSH12327	<i>Norops planiceps</i>	Santa Isabel do Rio Negro, AM	Brazil
MPEG16765	<i>Norops planiceps</i>	Reserva Duke, Manaus, AM	Brazil
MPEG15277	<i>Norops scypheus</i>	Maraã, AM	Brazil
MCZ110284	<i>Norops scypheus</i>	Sarayacu: Pastaza	Ecuador
MPEG27674	<i>Norops tandai</i>	Maués, AM	Brazil
MPEG26931	<i>Norops tandai</i>	Porto Urucu, AM	Brazil
MPEG27082	<i>Norops tandai</i>	Mutum, Juruti, PA	Brazil
MPEG27673	<i>Norops tandai</i>	Maués, AM	Brazil

MPEG29414	<i>Norops</i>	<i>tandai</i>	Itaituba, PA	Brazil
MPEG 18924	<i>Norops</i>	<i>tandai</i>	Careiro da Varzea, AM	Brazil
MPEG30038	<i>Norops</i>	<i>trachyderma</i>	Itaituba, PA	Brazil
MPEG31114	<i>Norops</i>	<i>trachyderma</i>	Taracauá, AC	Brazil
MPEG30332	<i>Norops</i>	<i>trachyderma</i>	Rio Juruá, AC/AM	Brazil
MPEG20737	<i>Norops</i>	<i>trachyderma</i>	Porto Walter, Rio Juruá, AC	Brazil
MPEG17586	<i>Norops</i>	<i>trachyderma</i>	Santarém, PA	Brazil

Draft

Figure 1. Sulcate view of the hemipenis of *Norops trachyderma*, with indication of the main features used in hemipenial descriptions.

Figure 2: Hemipenes of *Dactyloa* and *Norops* species studied in sulcate, lateral and asulcate views. A) *Dactyloa punctata*, B) *D. phyllorhina*, C) *D. transversalis*, D) *Norops auratus*, E) *N. brasiliensis*, F) *N. chrysolepis*, G) *N. fuscoauratus*, H) *N. fuscoauratus* with pigmentation, I) *N. meridionalis*, J) *N. ortonii*, K) *N. planiceps*, L) *N. scypheus*, M) *N. tandai* and N) *N. trachyderma*.

Draft

Figure 1. Sulcate view of the hemipenis of *Norops trachyderma*, with indication of the main features used in hemipenial descriptions.
86x69mm (300 x 300 DPI)

Figure 2: Hemipenes of *Dactyloa* and *Norops* species studied in sulcate, lateral and asulcate views. A) *Dactyloa punctata*, B) *D. phyllorhina*, C) *D. transversalis*, D) *Norops auratus*, E) *N. brasiliensis*, F) *N. chrysolepis*, G) *N. fuscoauratus*, H) *N. fuscoauratus* with pigmentation, I) *N. meridionalis*, J) *N. ortonii*, K) *N. planiceps*, L) *N. scypheus*, M) *N. tandai* and N) *N. trachyderma*.
167x236mm (300 x 300 DPI)