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# Divergent evolution of hemipenial morphology in two cryptic species of mainland anoles related to *Anolis polylepis*

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**Abstract**. Divergent evolution of hemipenial morphology between closely related forms appears to be a common phenomenon in mainland anoles. We discuss possible evolutionary scenarios for this finding, based on different lines of evidence from our work on *Anolis polylepis* and *A. osa* from southern Costa Rica. Molecular analysis suggests a very recent separation of these two forms and the occurrence of hybridisation in their contact zone. Evidence from crossbreeding experiments supports these assumptions. The results of an examination of female cloacal morphology indicate co-evolution of male and female genital morphologies and thereby contradict functional neutrality of hemipenial morphology.

Key words. Squamata, Polychrotidae, *Anolis osa*, *Anolis polylepis*, genital morphology, hybrid zone.

## Introduction

Male genital morphology is an important diagnostic character to discriminate among closely related species in many different groups of organisms. This is due to a general trend of male genitalia to evolve more rapidly and more divergently than other morphological characters (Eberhard 1985). In squamate reptiles, the morphology of the male intromittent organs (the hemipenes) has been shown to provide valuable information, both for species identification and the reconstruction of phylogenies (Dowling & Savage 1960, Arnold 1986, Böhme 1988, 1991, Ziegler & Böhme 1997). However, applicability of hemipenial morphology to the latter point appears to be limited in the organisms studied here due to an interesting phenomenon. In Central American mainland anoles, comprehensive studies of morphological characters (including hemipenial morphology) revealed at least ten cases in which extreme differences in hemipenial morphology occur between anoles that are otherwise morphologically very similar or even undistinguishable (e.g., Köhler & Kreutz 1999, Köhler & Sunyer 2008, Köhler & Vesely 2010). In all cases, hemipenial morphologies are strictly correlated with geographical ranges. Within the geographic area covered by each hemipenial morph, phenotypes are very stable, and ontogenetic differences concern only the size of copulatory organs but not their shapes. Interestingly, in eight of the ten cases, a larger bilobate hemipenis in one population contrasts with a smaller unilobate hemipenis in the other. In some cases, preliminary data suggest that female cloaca shape corresponds to hemipenial morphology (J. Köhler 2007). Because of the external similarity, most of these forms have traditionally been considered conspecific, but some have recently been described as distinct species, assuming that differences in hemipenial morphology (and some other characters) are a result of separate phylogenetic histories (Köhler & Kreutz 1999, Köhler et al. 2003, 2007, Köhler & Sunyer 2008, Köhler 2009). Likewise, the two forms discussed here have been taxonomically separated by the description of *A. osa* (Köhler et al. 2010). This species resembles *A. polylepis* Peters, 1973 in its external morphology, but the relatively small hemipenes of males are unilobate with an unbranched sulcus spermaticus running towards a simple apex, whereas the hemipenes of male *A. polylepis* are larger and the apex is medially divided into two lobes, each one with a separate branch of the sulcus spermaticus. *Anolis polylepis* is distributed along the Pacific slopes of southeastern Costa Rica and adjacent northwestern Panama and is replaced by *A. osa* on the Península de Osa in Pacific southern Costa Rica. On the neck of this peninsula, in the vicinity of the village of Rincón de Osa, the two forms meet in an apparently very narrow (about 1 km wide) contact zone, in which individuals with an intermediate hemipenial mor-

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phology are found (Fig. 1) (Köhler et al. 2010). Here we present several lines of evidence that facilitate a cautious discussion of possible evolutionary scenarios to explain the apparently very rapid divergent evolution of genital morphology between these closely related forms. We conducted a phylogenetic analysis based on a 306 bp fragment of the cytochrome *b* gene to gain a general idea about the evolutionary history of the situation (Hahn 2009). Crossbreeding experiments were initiated to try and address questions about functional differences between hemipenial morphs and hybrid fertility. Finally, we dissected several females to obtain comparative data on female cloacal structure.

## Material and methods

### Sampling and laboratory protocols

All samples were taken from collected voucher specimens directly after fixation. Tissue samples (forelimbs or tail tips) were stored in absolute ethanol and frozen at -20°C after return from the field. DNA extraction was carried out either by phenol-chloroform protocols or by Quiagen Dneasy-Blood and Tissue Kit. DNA concentration was checked photometrically and electrophoretically. Amplification of a 306 bp fragment of the mitochondrial cyt *b* gene was carried out using primers and a PCR program as published by Kocher et al. (1989) and Stenson et al. (2004). PCR products were purified using the QIAquick Purification Kit and sequenced by a commercial provider with an ABI 3730 sequencer.

### Phylogenetic analyses

All sequences were aligned with MEGA 4.1 software (Tamura et al. 2004, Tamura et al. 2007) and the alignment obtained was checked manually for gaps and stop codons. The data matrix included 30 samples with an aligned sequence length of 306 base pairs. A Bayesian analysis was carried out using Mr. Bayes v3.1.2. (Ronquist & Huelsenbeck 2003). The Nucleotid substitution model was selected with Modeltest 3.7 (Posada & Crandall 1998), applying AIC and hlRT criteria. Four Markov chains were run twice for 2,000,000 generations. Every 100$^{th}$ generation was sampled. The first 1000 generations were discarded as "burn in". Additionally, a parsimony analysis was conducted with a data matrix from which six very similar samples (H2, H10, H16, H17, Os7, Os14) were excluded to alleviate the calculations. Parsimony analysis was conducted with PAUP* v4b10 (Swofford 2002) with 1000 bootstrap replicates to infer branch support.

### Female cloacal morphology

Twenty-six preserved female specimens from 17 localities were dissected to investigate cloacal morphology. The body cavity was opened with a longitudinal ventral cut from the cloacal slit to the level of the forelimbs. The pelvis was cut longitudinally and the intestine was cut to uncover the cloaca. The tube-like extensions (termed vaginal tubi here), that connect the lumen of the cloaca with the very thin and membranous tissue of the oviducts were measured to the nearest 0.01 mm from the anteriormost insertion into the cloaca to their distal ends, using the ocular micrometer of a stereomicroscope (Leica MZ 12). To minimize measuring errors, each vaginal tubus of each specimen was measured five times. As no external characters are known to distinguish females of the two sibling species studied here, the specimens were assigned to the corresponding hemipenial morphs on a geographical basis. Individuals from localities 1 to 8 (as given in Fig. 6 and Tab. 3) were assigned to *Anolis polylepis* and individuals from localities 9 to 17 to *A. osa*. The mean values of the two groups were compared applying an unpaired *t*-test.

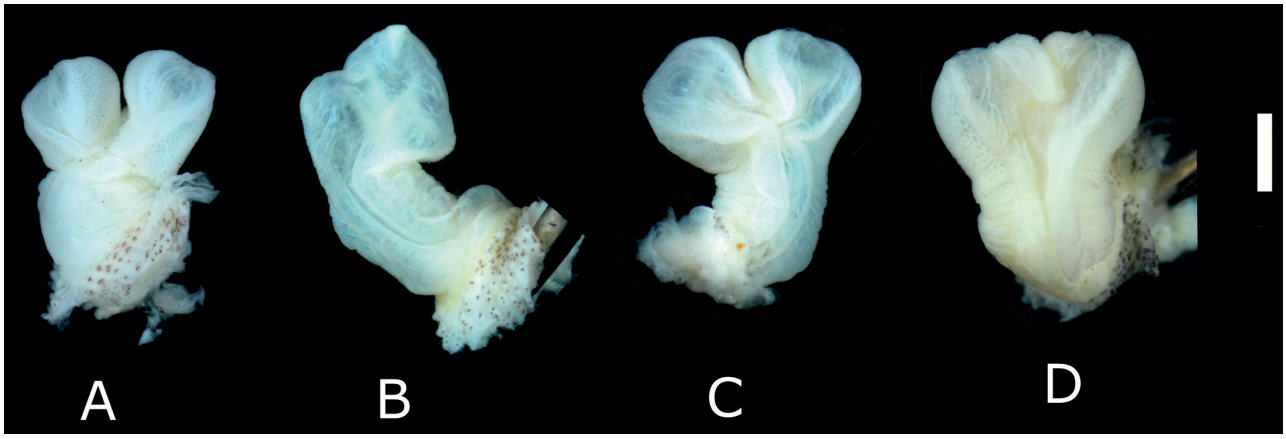


Figure 1. Sulcal sides of hemipenes of the species discussed. A) Hemipenis of *Anolis polylepis* (SMF 89627). B) Hemipenis of *Anolis osa* (SMF 89216). C) Hemipenis of a male from the hybrid zone (SMF 89241). D) Hemipenis of captive-bred hybrid with an *A. osa* mother (not preserved yet) and an *A. polylepis* father (SMF 93589). Scale bar: 1 mm.

### Crossbreeding experiments

Eighteen individuals were taken alive to the laboratory to conduct hybridisation experiments. *Anolis polylepis* were collected from a locality about 21 km northeast of the contact zone, and *A. osa* were taken from two localities 15 and 16 km southwest of the contact zone, respectively. They were housed individually in glass vivaria measuring 300 × 300 × 400 mm and placed together in an experimental tank of 300 × 200 × 300 mm for periods of up to 12 hours. When mating occurred, the experiment was terminated after copulation was completed and the individuals were separated. All these encounters were video-recorded. Some couples were housed together in larger tanks (700 × 400 × 600 mm) for periods of 21 days. As females of both forms are able to store sperm, a female was considered uninseminated and used for crossbreeding experiments only after laying at least five unfertilized eggs consecutively. Unfertilized eggs do not have a fully developed shell and are not hidden by the female, but are laid seemingly at random on the floor, the decoration, or even in the water bowl.

## Results

### Phylogenetics

The Bayesian analysis of the data set resulted in the tree shown in Fig. 2. Cytochrome *b* genotypes are highly differentiated between geographically distant populations (Fig. 3 and Fig. 4). *Anolis osa* forms a weakly supported clade within the southeastern populations of *A. polylepis*. Two specimens that would be assignable to *A. osa* from evidence of hemipenial morphology, (Os7 & Os14) from the vicinity (3 km) of the contact zone, are not included in this

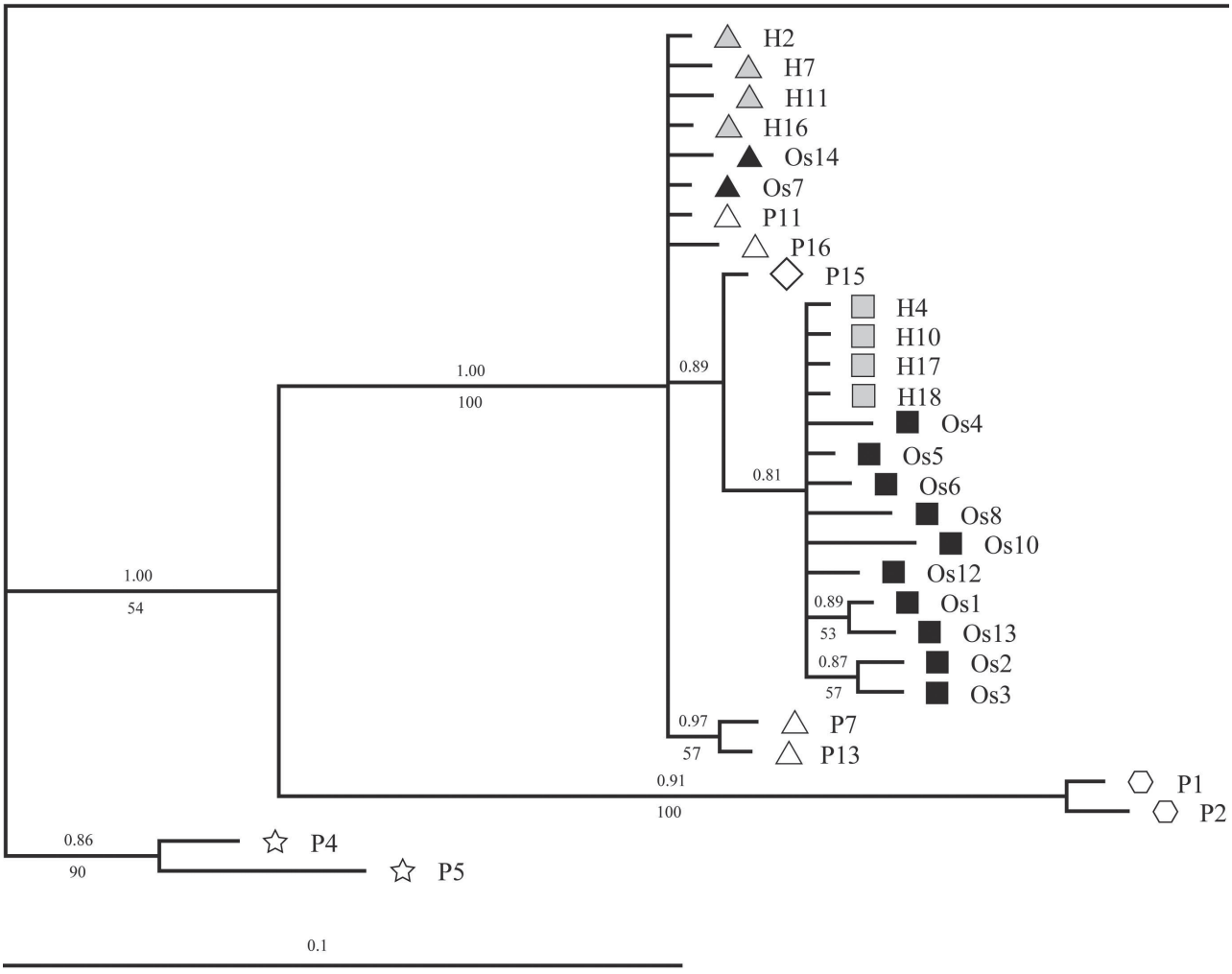


Figure 2. Bayesian phylogram based on sequences of the mitochondrial cytochrome *b* gene (306 bp). Posterior probabilities are shown above branches, parsimony bootstrap values > 50 are shown below branches. Shapes correspond to clades found in the phylogram, colours correspond to hemipenis morphology: White: bilobate; Black: unilobate; Grey: intermediate. Outgroup: *Anolis oculatus* (GenBank accession number: AF 426913).

clade (Fig. 2 and Fig. 4). The specimens considered being hybrids due to their intermediate hemipenial morphology are scattered between the *A. osa* clade and southeastern *A. polylepis*.

## Crossbreeding experiments

In a total of 33 video-surveyed staged encounters, male display behaviour was observed only in 19 cases and only six of these encounters led to copulation (Tab. 1). Male *Anolis polylepis* performed display behaviour only in 12 of 25 encounters and no mating was observed in any of these trials. Only one male *A. polylepis* that was housed with a female *A. osa* for 21 days in one of the larger tanks mated and sired offspring. Male *A. osa* displayed and mated more readily under the conditions provided in the staged encounter experiments: In a total of eight encounters, display behaviour was observed in six and mating occurred in four trials.

$F_1$ hybrids produced by an *Anolis polylepis* male and an *A. osa* female reproduced in the large tanks. In two staged encounter trials, both hybrid males displayed, but only one trial lead to an unsuccessful copulation, after which the hybrid female did not produce any fertile eggs. The maximum number of fertile eggs laid after separation was produced by a female *A. osa* bred with an *A. polylepis* male in the large tank. This female laid sixteen fertile eggs over a period of 141 days (for the other numbers of fertile eggs laid see Tab. 1). A wild-caught adult female *A. osa* that was kept individually after capture laid 20 fertile eggs over a period of 214 days, whereas the maximum number of eggs obtained from an *A. polylepis* female under the same conditions was ten eggs in 91 days. Although the conclusions that can be drawn from these experiments are limited, two statements can be made with confidence (see Tab. 1). Firstly, each hemipenial morph is generally able to successfully inseminate the sperm-storing structures of a female of the other morph. Male *A. osa* successfully inseminated female *A. polylepis* with only one copulation. Secondly, at least hybrids of the first generation are not sterile. Furthermore, genital morphology of male hybrids is intermediate, similar to the morphology of supposed hybrids collected in the field (Fig. 1).

## Female cloacal morphology

Females from the mainland north and northeast of the contact zone (assigned to *A. polylepis*) have significantly ($P < 0.0001$) longer vaginal tubi than females from the Osa Peninsula (assigned to *A. osa*) (Figs. 5–7), although the differences do not seem to be as unambiguous as those found in male genital morphology. Three females that would be assignable to *A. osa* on a geographical basis (9, 11c, and 15)

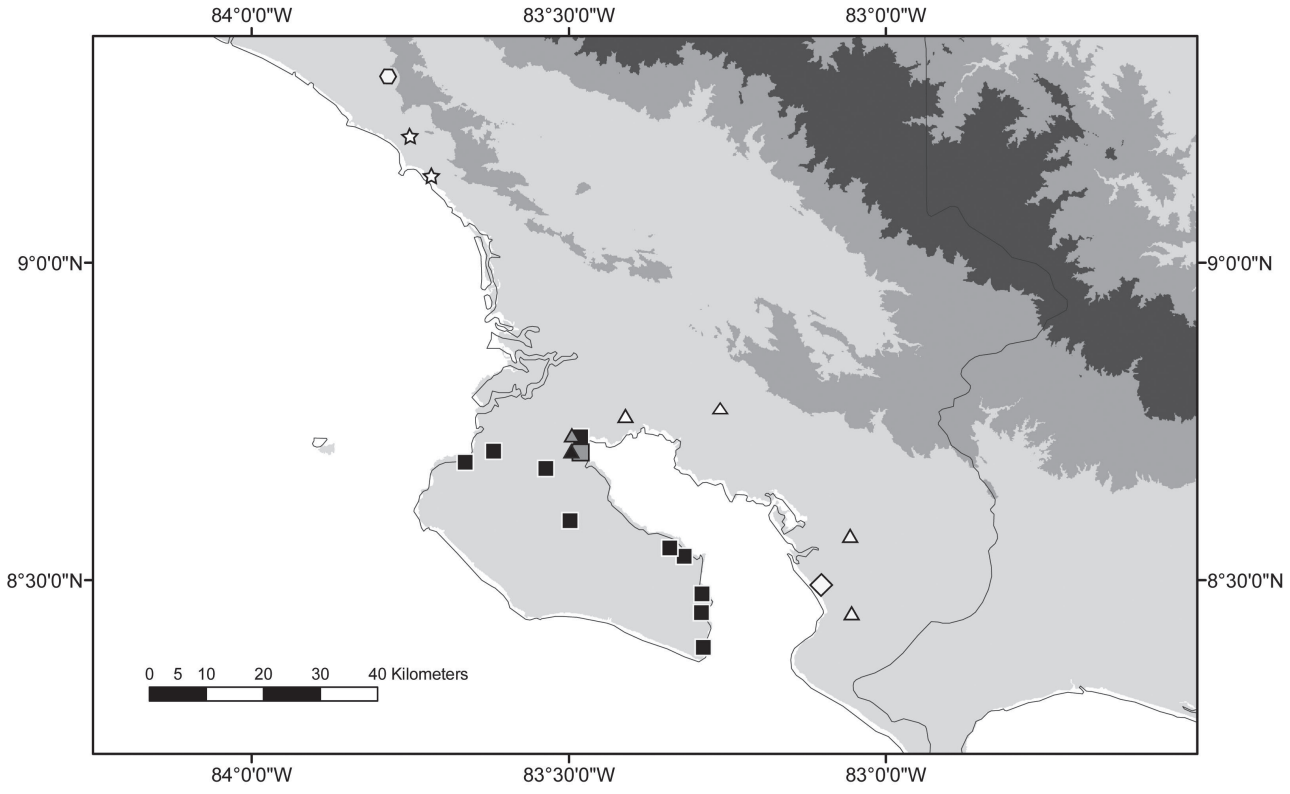


Figure 3. Geographical distribution of specimens used in the molecular analysis. Shapes of symbols correspond to clades found in phylogram (Fig. 2). Colours correspond to hemipenis morphology: White: bilobate; Black: unilobate; Grey: intermediate.

Table 1. Results of the crossbreeding experiments.

| ♂ | × | ♀ | Nr. of experiments | ♂ display behaviour | matings | fertilized eggs laid |
|---|---|---|---|---|---|---|
| Experiments performed in video surveilled experimental tank, individuals separated after one mating or 12 hours | | | | | | |
| *A. osa* | × | *A. osa* | 1 | 1 | 1 | 12 |
| *A. polylepis* | × | *A. polylepis* | 6 | 3 | 0 | 0 |
| *A. osa* | × | *A. polylepis* | 7 | 5 | 4 | 4/13/0/4 |
| *A. polylepis* | × | *A. osa* | 17 | 8 | 0 | 0 |
| Hybrid | × | Hybrid | 2 | 2 | 1 | 0 |
| Couples housed together for 21 days without permanent surveillance | | | | | | |
| *A. polylepis* | × | *A. osa* | 1 | ? | ? | 16 |
| Hybrid | × | Hybrid | 2 | ? | ? | 0/12 |

have notably longer vaginal tubi than would be expected for a female from the peninsula. However, locality 9 lies within the known contact zone and locality 11 only about 3.5 km south of it. The two females of *A. polylepis* with the shortest vaginal tubi (4 and 5) are small individuals (44 and 42 mm SVL, resp.) and the only ones without developing eggs in their oviducts.

## Discussion

Evidence from the phylogenetic analysis suggests a very recent split of the *Anolis osa* clade from southeastern populations of *A. polylepis* and generally indicates a very limited or non-existing geneflow along maternal lines between geographically different populations. Similar conditions of

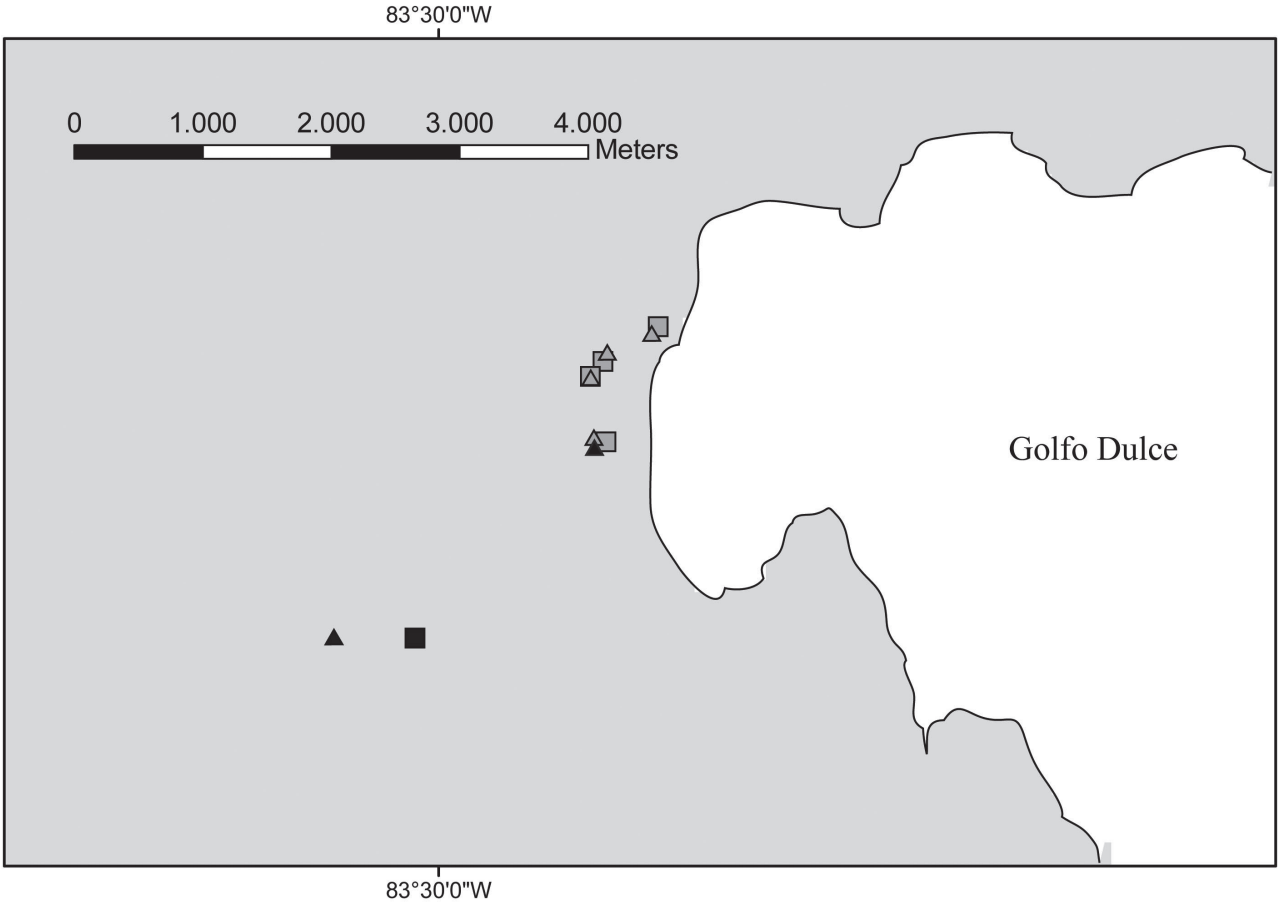


Figure 4. Geographical distribution within the contact zone of specimens used in the molecular analysis. Shapes of symbols correspond to clades found in the phylogram (Fig.2). Colours correspond to hemipenis morphology: White: bilobate; Black: unilobate; Grey: intermediate.

Table 2. Voucher specimens, sample ID, locality information and GenBank accession numbers of the specimens used in genetic analysis.

| Sample ID | Voucher specimen | Accesion nr. | Species | Locality | Cordinates |
|---|---|---|---|---|---|
| h2 | SMF 89648 | HQ641714 | *Anolis* cf. *polylepis* | ca. 1.5 km N Rincón de Osa | 8°42'46"N, 83°29'5.1"W |
| h4 | SMF 89243 | HQ641715 | *Anolis* cf. *polylepis* | ca. 1.5 km N Rincón de Osa | 8°42'46.9"N, 83°29'3.8"W |
| h7 | SMF 89246 | HQ641716 | *Anolis* cf. *polylepis* | ca. 1.5 km N Rincón de Osa | 8°42'41.4"N, 83°29'17.1"W |
| h10 | SMF 89652 | HQ641717 | *Anolis* cf. *polylepis* | ca. 1.5 km N Rincón de Osa | 8°42'18.1"N, 83°29'17.7"W |
| h11 | SMF 89653 | HQ641718 | *Anolis* cf. *polylepis* | ca. 1.5 km N Rincón de Osa | 8°42'19.1"N, 83°29'20"W |
| h16 | SMF 89658 | HQ641719 | *Anolis* cf. *polylepis* | ca. 1.5 km N Rincón de Osa | 8°42'34.8"N, 83°29'21.3"W |
| h17 | SMF 89659 | HQ641720 | *Anolis* cf. *polylepis* | ca. 1.5 km N Rincón de Osa | 8°42'34.8"N, 83°29'21.3"W |
| h18 | SMF 89247 | HQ641721 | *Anolis* cf. *polylepis* | ca. 1.5 km N Rincón de Osa | 8°42'38.3"N, 83°29'17.7"W |
| os1 | SMF 89189 | HQ641722 | *Anolis osa* | dirt road to Ranger Station "Los Patos" | 8°35'43.2"N, 83°29'52.6"W |
| os2 | SMF 89618 | HQ641723 | *Anolis osa* | 4 km W Puerto Jiménez | 8°33'5.6"N, 83°20'24.8"W |
| os3 | UCR 20732 | HQ641724 | *Anolis osa* | Puerto Jiménez | 8°32'17.5"N, 83°19'1.6"W |
| os4 | SMF 89207 | HQ641725 | *Anolis osa* | 8.5 km SW Puerto Jiménez, 25 m after branch to Playa la Colorada | 8°28'21"N, 83°16'52.1"W |
| os5 | SMF 89208 | HQ641726 | *Anolis osa* | road 11 km SW Puerto Jiménez | 8°26'44.4"N, 83°17'6.7"W |
| os6 | SMF 89209 | HQ641727 | *Anolis osa* | 16.5 km S Puert Jiménez | 8°23'42.8"N, 83°17'23"W |
| os7 | SMF 91702 | HQ641728 | *Anolis osa* | 2 km W Rincón de Osa | 8°41'27"N, 83°30'26.1"W |
| os8 | SMF 89226 | HQ641729 | *Anolis osa* | S Rincón de osa, 1 km after branch of road to Drake | 8°41'28.9"N, 83°30'5.9"W |
| os10 | SMF 91703 | HQ641730 | *Anolis osa* | road 6 km SW Rincón de Osa | 8°40'36.7"N, 83°32'7.9"W |
| os12 | SMF 89624 | HQ641731 | *Anolis osa* | Bahía Drake, Agujitas | 8°41'19"N, 83°39'50.6"W |
| os13 | SMF 89625 | HQ641732 | *Anolis osa* | Bahá Drake, 3–4 km W Drake | 8°42'9.4"N, 83°37'8.9"W |
| os14 | SMF 89236 | HQ641733 | *Anolis osa* | Rincón de Osa | 8°42'16.5"N, 83°29'20"W |
| p1 | SMF 89660 | HQ641734 | *Anolis polylepis* | 2.5 km N Platanillo | 9°17'36.8"N, 83°47'4.9"W |
| p2 | SMF 89661 | HQ641735 | *Anolis polylepis* | 2.5 km N Platanillo | 9°17'36.8"N, 83°47'4.9"W |
| p4 | SMF 89605 | HQ641736 | *Anolis polylepis* | N Uvita, Reserva oro verde | 9°12'16"N, 83°45'39"W |
| p5 | SMF 91701 | HQ641737 | *Anolis polylepis* | Uvita, La Cusinga rainforest lodge | 9°8'9.3"N, 83°43'2.8"W |
| p7 | SMF 89611 | HQ641738 | *Anolis polylepis* | 2–3 km after branch of road to Rincon de Osa | 8°46'13.1"N, 83°15'45.7"W |
| p11 | SMF 89643 | HQ641739 | *Anolis polylepis* | W Los Mogos | 8°45'29.2"N, 83°24'33.8"W |
| p13 | SMF 89632 | HQ641740 | *Anolis polylepis* | 5 km W Conte | 8°26'57.1"N, 83°3'24.4"W |
| p15 | SMF 89157 | HQ641741 | *Anolis polylepis* | 9 km S Zancudo | 8°29'32.4"N, 83°6'7.5"W |
| p16 | SMF 89637 | HQ641742 | *Anolis polylepis* | near Trenzas | 8°34'16.9"N, 83°3'54.9"W |

highly structured mitochondrial genotypes have been observed in other anole species (Losos 2009). Our molecular data suggest that *A. osa* is most closely related to representatives of *A. polylepis* occurring on the mainland near the Peninsula de Osa. This phenomenon is to be expected when a subset of populations (i.e., satellite subpopulations) of an otherwise widespread species becomes isolated from the main species' range and evolves divergently into a distinct species, thereby rendering the parental species paraphyletic. Several examples of such a phylogenetic situation have been reported from anoles (Schneider et al. 2001, Jackman et al. 2002, Thorpe & Stenson 2003). Ongoing geneflow within the parental species may continue to wash out any persisting evidence of paraphyly, making this phenomenon more difficult to detect in cases with ancient speciation events. The occurrence of specimens Os7 and Os14, that were assigned to *A. osa* based on the hemipenial morphology of males from the same locality outside the *A. osa* clade, shows that genomic introgression expands the very narrow hybrid zone assumed from hemipenial morphotypes to a minimum width of about 3 km (see Fig. 4). The general structure of the cladogram suggests that the bilobate hemipenial morphology of *A. polylepis* is the ancestral morphology state. We assume a most plausible scenario in which divergent evolution of genital morphology took place in allopatry, as geological data suggest that the Osa Peninsula has been a full island during most of its geological history (Malzer 2001, Coates & Obando 1996).

The close relationship of *Anolis osa* to *A. polylepis* that is evident from our phylogenetic analysis, as well as the absence of any known distinguishing external characters, illustrate the surprising rapidity of the evolutionary change of genital morphology in this case. In a more recent review of lizard hemipenial morphology, Böhme & Zieg-

ler (2008) observed an interesting tendency: They found hemipenial morphology to be more conservative between taxa that are reproductively separated by visible, sexually dimorphic, epigamic characters, compared to taxa without such ornaments. This trend seems to occur between higher taxonomical divisions (e.g., Varanidae and Iguanidae) as well as between more closely related species (e.g., within the Chamaeleonidae). Clearly, anoles in general do not fit this pattern well in that they show considerably variable hemipenial morphologies and highly developed visual courtship displays. However, in the case of the pair of species discussed here, no distinguishing differences in dewlap colour or size could be defined (Köhler et al. 2010). No distinct differences could be identified by preliminary and superficial comparison of courtship behaviour either. It should however be considered here that the differences in anole hemipenial morphology usually concern the presence and length of the lobes, whereas other hemipenial characters (e.g., ornamentation) are quite uniform. Several sets of hypotheses have been suggested to explain rapid divergent evolution of genital morphology. They are comprehensively discussed in Eberhard (1985), and for squamate reptiles in particular in Böhme (1988) and Ziegler & Böhme (1997), and will be mentioned only briefly in the following to discuss their applicability to the case of mainland anoles in general, and the case of *A. polylepis* and *A. osa* in particular. According to Eberhard (1985), the "lock and key" or "genital recognition" hypothesis is the oldest and most often invoked. It supposes divergent evolution of genitalia as a morphological hybridisation barrier. However, this does not appear to provide a convincing explanation in the case presented here. Firstly, there is no evidence of the genital differences being more pronounced in the contact zone than in other areas as would be predicted. Secondly, the occurrence of assumed hybrids in the field contradicts a fully functional reproductive barrier. Finally, copulation seems to be a suboptimal moment for mate discrimination (Kraus 1968, Arnold 1986, Böhme 1988) especially in anoles, with their highly elaborate (and often species-specific) courtship behaviour. According to the pleiotropism hypothesis as proposed by Mayr (1963) and modified by Arnold (1973), the genital differences observed are not a product of a selective pressure to avoid hybridisation, but are more likely caused by pleiotropic effects from genetic alteration that influence other characters that are subject to selection. It assumes that the actual shape of the intromittent organ is largely neutral by means of functionality. The observed correlation between hemipenial and cloacal morphology contradicts this assumption. Similar relationships of male and female genitals have been found in Southeast Asian pit vipers (Pope 1941). Böhme & Sieling (1993) provided evidence for function-

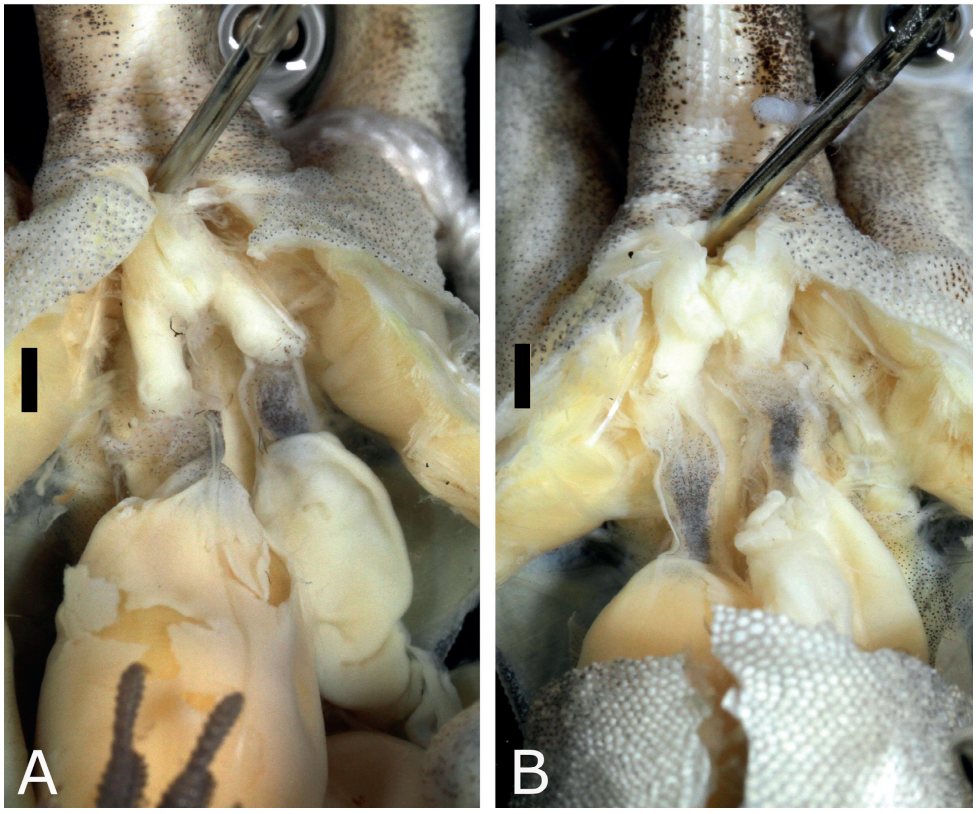


Figure 5. Ventral view of female cloaca and oviducts. Intestines removed. A) *Anolis polylepis* IDNr. 8a (SMF 89179). B) *Anolis osa* IDNr. 13a (SMF 89622). Scale bars: 1 mm

Table 3. Voucher specimens and locality information of specimens used for investigation of female cloacal morphology.

| Sample ID | Voucher specimen | Assigned to species | Locality | Coordinates |
|---|---|---|---|---|
| 1 | SMF 91696 | *Anolis polylepis* | Dominical | 9°18'43.6"N, 83°46'18.7"W |
| 2 | SMF 91697 | *Anolis polylepis* | Uvita | 9°8'13.6"N, 83°42'16.7"W |
| 3 | SMF 89162 | *Anolis polylepis* | San Buenaventura | 9°1'17"N, 83°35'19.9"W |
| 4 | SMF 89163 | *Anolis polylepis* | Balzar | 8°59'20.9"N, 83°31'16.8"W |
| 5 | SMF 89170 | *Anolis polylepis* | branch of road to Sierpe | 8°54'32.7"N, 83°24'51.1"W |
| 6 | SMF 89640 | *Anolis polylepis* | 2 km W Villa Colón | 8°52'49.7"N, 83°21'43.3"W |
| 7a | SMF 89614 | *Anolis polylepis* | 2–3 km after branch of road to Rincon de Osa | 8°46'13.1"N, 83°15'45.7"W |
| 7b | SMF 89615 | *Anolis polylepis* | 2–3 km after branch of road to Rincon de Osa | 8°46'13.1"N, 83°15'45.7"W |
| 8a | SMF 89179 | *Anolis polylepis* | 3 km E Santa Cecilia | 8°45'7.3"N, 83°17'59.7"W |
| 8b | SMF 89180 | *Anolis polylepis* | 3 km E Santa Cecilia | 8°45'7.3"N, 83°17'59.7"W |
| 9 | SMF 89186 | *Anolis polylepis* | 2 km N Rincón de Osa, Restaurant Ventanas al Golfo | 8°42'54.1"N, 83°29'7"W |
| 10 | SMF 89226 | *Anolis osa* | S Rincón de osa, 1 km after branch of road to Drake | 8°41'28.9"N, 83°30'5.9"W |
| 11a | SMF 91702 | *Anolis osa* | 2 km W Rincón de Osa | 8°42'27"N, 83°30'26.1"W |
| 11b | SMF 89218 | *Anolis osa* | 2 km W Rincón de Osa | 8°42'27"N, 83°30'26.1"W |
| 11c | SMF 89219 | *Anolis osa* | 2 km W Rincón de Osa | 8°42'27"N, 83°30'26.1"W |
| 11d | SMF 89220 | *Anolis osa* | 2 km W Rincón de Osa | 8°42'27"N, 83°30'26.1"W |
| 11e | SMF 89221 | *Anolis osa* | 2 km W Rincón de Osa | 8°42'27"N, 83°30'26.1"W |
| 12a | SMF 89230 | *Anolis osa* | road 6 km SW Rincón de Osa | 8°40'36.7"N, 83°32'7.9"W |
| 12b | SMF 89232 | *Anolis osa* | road 6 km SW Rincón de Osa | 8°40'36.7"N, 83°32'7.9"W |
| 13a | SMF 89622 | *Anolis osa* | 9.5 km E Agujitas, Rancho Quemado | 8°41'27.2"N, 83°34'29.6"W |
| 13b | SMF 89623 | *Anolis osa* | 9.5 km E Agujitas, Rancho Quemado | 8°41'27.2"N, 83°34'29.6"W |
| 14a | SMF 91698 | *Anolis osa* | Drake Bay | 8°42'9.4"N, 83°37'8.9"W |
| 14b | SMF 91699 | *Anolis osa* | Drake Bay | 8°42'9.4"N, 83°37'8.9"W |
| 15 | SMF 89192 | *Anolis osa* | dirt road to Ranger Station "Los Patos" | 8°35'43.2"N, 83°29'52.6"W |
| 16 | SMF 89618 | *Anolis osa* | 4 km W Puerto Jiménez | 8°33'5.6"N, 83°20'24.8"W |
| 17 | SMF 89621 | *Anolis osa* | 8.5 km SW Puerto Jiménez, 25 m after branch to Playa la Colorada | 8°28'21"N, 83°16'52.1"W |

al differences between unilobate versus bilobate hemipenes in relation to copulatory behaviour in boid snakes. In this example, the species with unilobate hemipenes needed to perform two consecutive penetrations with an alternating use of the hemipenes in order to inseminate both oviducts. Several studies of anole copulatory behaviour have revealed a strong tendency for alteration of hemipenis use in subsequent mating events (Crews 1978, Tokarz 1988, Tokarz & Slowinski 1990). However, these findings are most likely explained by means of increased sperm transfer when intercopulatory intervals are relatively short. Furthermore, functional neutrality would predict polymorphisms in this trait that do not seem to occur. It appears quite plausible to assume that the constitution of genital morphology is ecologically largely neutral in squamates, because their genitals are internal structures that are usually not exposed to the environment (Böhme 1988, Ziegler & Böhme 1997). Under this assumption, an aberrant genital morphology would not notably influence the ability to survive, but the ability to successfully reproduce, depending on genital or sensorial constitution of the opposite sex, i.e., the genital morphology would be of selective importance for sexual encounters. The "sexual selection hypothesis" is developed and discussed in detail in Eberhard (1985) and refined in Eberhard (1996), with special emphasis on sexual selection by female choice. In anoles, males are known to be highly territorial and the access to females appears to be the major determinant of territory size and topology (Stamps 1977, Losos 2009), i.e., males compete for females by competing for territories. Although territory holders father most of the offspring, evidence from several species shows that female anoles regularly mate and reproduce with more than one male, which proves the general concept of female choice (Trivers 1976, Hicks & Trivers 1983, Passek 2002). Considering the available evidence, we assume that sexual selection by female choice (Eberhard 1985, Eberhard 1996) is the most plausible explanation of the peculiarly rapid divergent evolution of genital morphology between *A. polylepis* and *A. osa*. Assuming that females are able to discriminate between mates with different hemipenial morphologies and that insemination success varies depending on female preference, any male performing a superior stimulus would benefit from advantages in reproductive success. Females that prefer mates performing this stimulus in turn would be favoured by producing favourable male offspring. Small initial changes in female

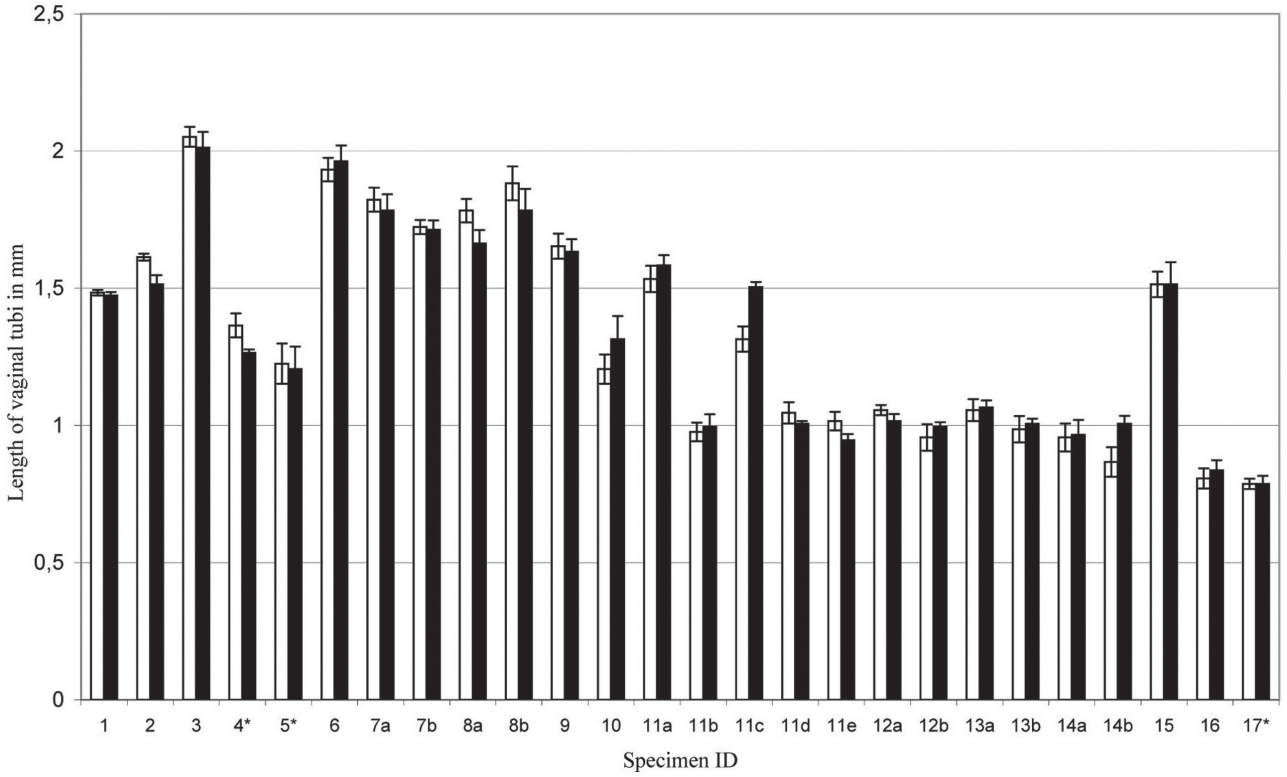


Figure 6. Mean and SE of 5 measurements of length of vaginal tubi of 26 females. White columns: left vaginal tubus; Black columns: right vaginal tubus. Numbers correspond to localities as given in Fig. 7 and Tab. 3. Individuals from the same locality are distinguished by letters (see Tab. 3). Marked columns are values from juvenile females, without eggs in the oviducts.

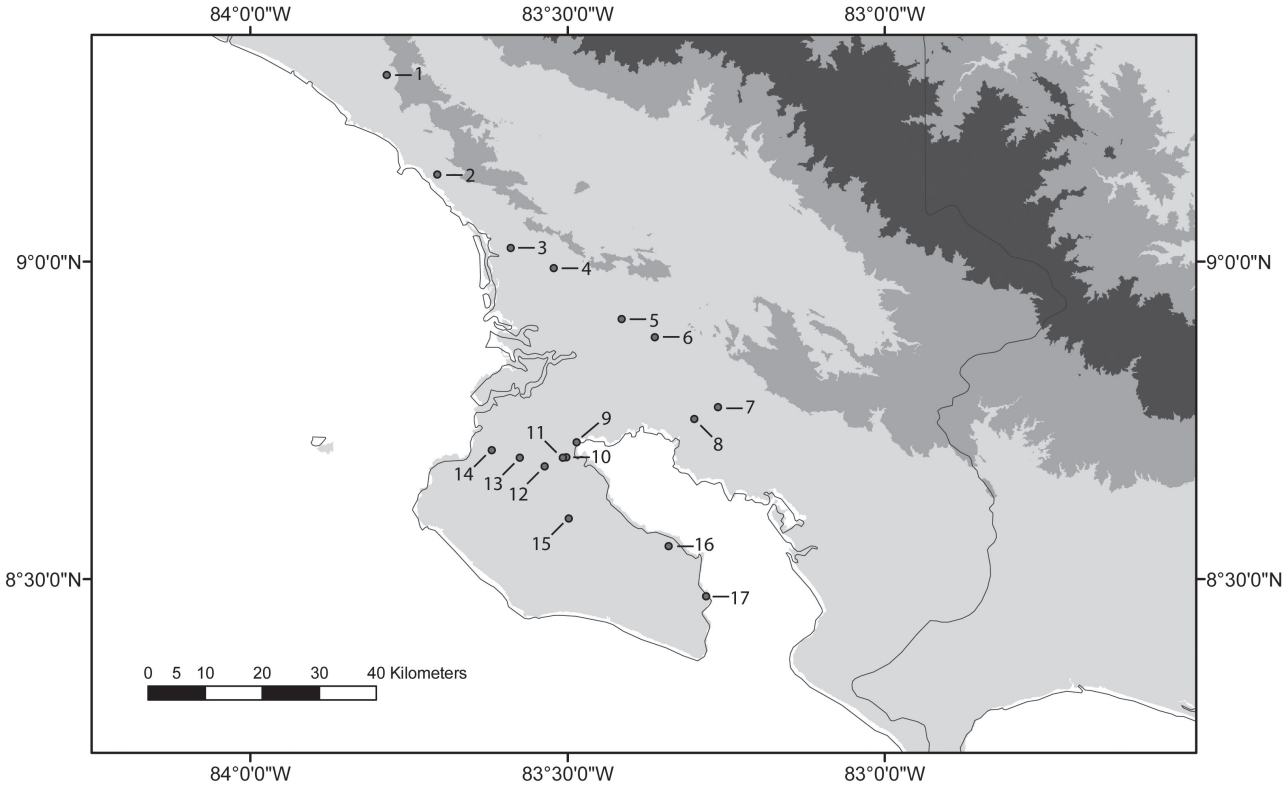


Figure 7. Geographical distribution of female cloacal morphology. Locality numbers correspond to Fig. 6.

preference of hemipenial morphology thereby could give rise to a runaway process as proposed by Fisher (1958). However, in what is presumably a secondary contact zone, "mismatched" mating events occur due to the absence of effective premating isolating mechanisms and the reproductive success of such mating events is apparently greater than zero. Differences in the functionality of hemipenial morphology would affect reproductive success of males mainly or only in situations of direct competition for female gametes, and this functionality would be dependent on female genital or sensorial conditions. If females tend to be philopatric (which is implied by the structure found in the sequences of mitochondrial DNA), a male entering the contact zone would predominantly encounter females that prefer the other hemipenial morph and be disadvantaged with regard to their reproductive success. Depending on how strong this sexual selection works, it could even maintain the geographical integrity of genital morphology against homogenising effects from a male-biased gene flow (Stenson et al. 2002).

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### References

Arnold, E. N. (1973): Relationships of the palaearctic lizards assigned to the genera *Lacerta*, *Algyroides* and *Psammodromus* (Reptilia: Lacertidae). – Bulletin of the British Museum of Natural Nistory (Zoology), **25**: 291–366.

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). – Biological Journal of the Linnean Society, **29**: 263–281.

Böhme, W. (1988): Zur Genitalmorphologie der Sauria: Funktionelle und stammesgeschichtliche Aspekte. – Bonner zoologische Monografien, **27**: 1–176.

Böhme, W. (1991): New findings on the hemipenial morphology of monitor lizards and their systematic implications. – pp. 42–49 in Böhme, W. & H. G. Horn (eds.): Advances in monitor research. – Mertensiella, **2**.

Böhme, W. & U. Sieling (1993): Zum Zusammenhang zwischen Genitalstruktur, Paarungsverhalten und Fortpflanzungserfolg bei squamaten Reptilien: erste Ergebnisse. – Herpetofauna, Weinstadt, **15**(82): 15–23.

Böhme, W. & T. Ziegler (2008): A review of iguanian and anguimorph lizard genitalia (Squamata: Chamaeleonidae; Varanoidea, Shinisauridae, Xenosauridae, Anguidae) and their phylogenetic significance: comparisons with molecular data sets. – Journal of Zoological Systematics and Evolutionary Research, **47**: 189–202.

Coates, A. G. & J. A. Obando (1996): The geological evolution of the Central American isthmus. – in Jackson, B. C., A. F. Budd & A. G. Coates (eds.): Evolution and environment in tropical America. – Chicago & London (The University of Chicago Press): 425 pp.

Crews, D. (1978): Hemipenile preference: stimulus control of male mounting behavior in the lizard *Anolis carolinensis.* – Science, **199**: 195–196.

Dowling, H. G. & J. M. Savage (1960): A guide to the snake hemipenis: a survey of basic structure and systematic characteristics. – Zoologica, **45**: 17–28.

Eberhard, W. G. (1985): Sexual selection and animal genitalia. – Cambridge, Massachusetts (Harvard University Press), 244 pp.

Eberhard, W. G. (1996): Female control: sexual selection by cryptic female choice. – Princton, New Jersey (Princeton University Press), 501 pp.

Fisher, R. A. (1958): The genetical theory of natural selection. – New York: (Dover Publications), 318 pp.

Hahn, M. (2009): Untersuchung einer Kontaktzone kryptischer Saumfingerechsen (Polychrotidae) aus Costa Rica mittels Analyse mitochondrialer DNA. – Diplomarbeit, Johannes Gutenberg Universität Mainz and Forschungsinstitut Senckenberg, Frankfurt am Main, 102 pp. [unpublished]

Hicks, R. A. & R. L. Trivers (1983): The social behavior of *Anolis valencienni.* Advances in Herpetology and Evolutionary Biology. – pp. 570–595 in Rhodin, A. G. J. & K. Miyata (eds.): Essays in Honor of Ernest E. Williams. – Cambridge, Massachusetts (Museum of Comparative Zoology).

Jackman, T. R., D. J. Irschick, K. De Queiroz, J. B. Losos & A. Larson (2002): Molecular phylogenetic perspective on evolution of lizards of the *Anolis grahami* series. – Journal of Experimental Zoology **294**: 1–16.

Kocher, T. D., W. K. Thomas, A. Meyer, S. V. Edwards, S. Pääbo, F. X. Villablanca & A. C. Wilson (1989): Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. – Proceedings of the National Academy of Sciences of the U.S.A., **86**: 6196–6200.

Köhler, G. (2009): A new species of *Anolis* formerly referred to as *Anolis altae* from Monteverde, Costa Rica (Squamata: Polychrotidae). – Journal of Herpetology, **43**: 11–20.

Köhler, G., D. M. Dehling & J. Köhler (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. & J. Kreutz (1999): *Norops macrophallus* (Werner, 1917), a valid species of anole from Guatemala and El Salvador (Squamata: Sauria: Iguanidae). – Herpetozoa, **12**: 57–65.

Köhler, G., J. R. McCranie, K. E. Nicholson & J. Kreutz (2003): Geographic variation of hemipenial morphology in *Norops humilis* (Peters, 1863) and the systematic status of *Norops quaggulus* (Cope, 1885) (Reptilia: Squamata: Polychrotidae). – Senckenbergiana biologica, **82**: 213–222.

Köhler, G., M. Ponce, J. Sunyer & A. Batista (2007): Four new species of anoles (genus *Anolis*) from the Serranía de Tabasará, west-central Panama (Squamata: Polychrotidae). – Herpetologica, **63**: 375–391.

Köhler, G. & J. Sunyer (2008): Two new species of anoles formerly referred to as *Anolis limifrons* (Squamata: Polychrotidae). – Herpetologica, **64**(1): 91–108.

Köhler, G. & M. Vesely (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**: 207–228.

Köhler, J. J. (2007): Evolution der Hemipenismorphologie mittelamerikanischer Saumfingerechsen (Polychrotidae). – Diplomarbeit, Johannes Gutenberg Universität Mainz and Forschungsinstitut Senckenberg, Frankfurt am Main, 100 pp. [unpublished]

Kraus, O. (1968): Isolationsmechanismen und Genitalstrukturen bei wirbellosen Tieren. – Zoologischer Anzeiger, **181**: 22–38.

Losos, J. B. (2009): Lizards in an evolutionary tree. Ecology and adaptive radiation of anoles. – Berkley and Los Angeles, California (University of California Press), 507 pp.

Malzer, O. (2001): Geological history of Central America and the Golfo Dulce region. – in Weber, A. (ed.): An introductory field guide to the flowering plants of the Golfo Dulce Rainforest Costa Rica. – Linz, Austria (Biologiezentrum des Oberösterreichischen Landesmuseums), 462 pp.

Mayr, E. (1963): Animal species and evolution. – Cambridge, Massachusetts (Harvard University Press), 191 pp.

Passek, K. M. (2002): Extra-pair paternity within the female-defense polygyny of the lizard, *Anolis carolinensis*: evidence of alternative mating strategies. – PhD Thesis. Faculty of Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Pope, C. H. (1941): Copulatory adjustment in snakes. – Zoological Series of the Field Museum of Natural History, **24**: 249–252.

Posada, D. & K. A. Crandall (1998): MODELTEST: testing the model of DNA substitution. – Bioinformatics, **14**: 817–818.

Ronquist, F. & J. P. Huelsenbeck (2003): MrBayes 3: Bayesian phylogenetic inference under mixed models. – Bioinformatics, **19**: 1572–1574.

Schneider, C. J., J. B. Losos & K. de Queiroz (2001): Evolutionary Relationships of the *Anolis bimaculatus* Group from the Northern Lesser Antilles. – Journal of Herpetology, **35**: 1–12.

Stamps, J. A. (1977): The relationship between resource competition, risk, and aggression in a tropical territorial lizard. – Ecology, **58**: 349–358.

Stenson, A. G., A. Malhotra & R. S. Thorpe (2002): Population differentiation and nuclear gene flow in the Dominican anole (*Anolis oculatus)*. – Molecular Ecology, **11**: 1679–1688.

Stenson, A. G., R. S. Thorpe & A. Malhotra (2004): Evolutionary differentiation of *bimaculatus* group anoles based on analyses of mtDNA and microsatellite data. – Molecular Phylogenetics an Evolution, **32**: 1–10.

Swofford, D. L. (2002): PAUP*: Phylogenetic analysis using parsimony (*and other methods), Version 4.0b10. – Sunderland, Massachusetts (Sinauer Associates).

Tamura, K., M. Nei & S. Kumar (2004): Prospects for inferring very large phylogenies by using the neighbor-joining method. – Proceedings of the National Academy of Sciences of the U.S.A., **101**: 11030–11035.

Tamura, K., J. Dudley, M. Nei & S. Kumar (2007): MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. – Molecular Biology and Evolution, **24**: 1596–1599.

Thorpe, R. S. & A. G. Stenson (2003): Phylogeny, paraphyly and ecological adaptation of the colour and pattern in the *Anolis roquet* complex on Martinique. – Molecular Ecology, **12**: 117–132.

Tokarz, R. R. (1988): Copulatory behaviour of the lizard *Anolis sagrei*: alternation of hemipenis use. –Animal Behaviour, **36**: 1518–1524.

Tokarz, R. R., & Slowinski, J. B. (1990): Alternation of hemipenis use as a behavioural means of increasing sperm transfer in the lizard *Anolis sagrei*. – Animal Behaviour, **40**: 374–379.

Trivers, R. L. (1976): Sexual selection and resource-acruing abilities in *Anolis garmani*. – Evolution, **30**: 253–269.

Ziegler, T. & W. Böhme (1997): Genitalstrukturen und Paarungsbiologie bei squamaten Reptilien, speziell den Platynota, mit Bemerkungen zur Systematik. – Mertensiella, **8**: 1–210.