

Discovering an Antillean *Anolis* (Squamata: Polychrotidae) with contrasting sexual dichromatism in otherwise sexually monomorphic “chamaeleolis” group

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Abstract. The anole (genus *Anolis* Daudin, 1802) dewlap is a rapidly evolving trait. Sexually dichromatic anole species usually occur in the mainland, while the island species display only little dichromatism in particular. The so-called “chamaeleolis” group of anoles endemic to Cuba Island, traditionally classified as the ‘twig giant’ ecomorph, consists of large, slow and very cryptic species with very similar sexes. Our study describes a new population of “chamaeleolis” anoles which, unlike other related species, display a surprising sexual dichromatism in dewlaps. Males have conspicuously red dewlaps, while the dewlaps of females are whitish. We compared the specimens from the newly discovered populations with related *Anolis barbatus* Garrido, 1982, *A. chamaeleonides* Duméril et Bibron, 1837, *A. guamuhaya* Garrido, Pérez-Beato et Moreno, 1991 and *A. porcus* Cope, 1864 through the means of spectrophotometry, visual modelling, morphology and mtDNA analysis. The results show that the red coloration substantially increases both chromatic and achromatic contrasts, while the dichromatism in the remaining species is only in the achromatic channel, if any. Both genetic and morphometric comparisons suggest distinctness of the dichromatic populations which may represent a separate species. The reason for the unusual dewlap coloration remains unclear, though an ecological explanation is discussed.

Key words. Sexual size dimorphism, body shape, mtDNA, reflectance, *Anolis*, *Chamaeleolis*, Cuba, Neotropical Region.

INTRODUCTION

Coloration of males and females differs considerably in many animal species. This sexual dichromatism is a specific form of sexual dimorphism. Sexual dichromatism has attracted attention of researchers and theoreticians for centuries (e.g., Darwin 1871), but the phylogenetic studies analysing macroevolutionary patterns of sexual dichromatism within major clades of terrestrial vertebrates appeared only recently (frogs: Bell & Zamudio 2012; lizards: Stuart–Fox & Ord 2004; snakes: Shine & Madsen 1994; turtles: Liu et al. 2013; birds: Badayev & Hill 2003, Stoddard & Prum 2011).

The evolution of sexual dichromatism is usually explained by the two following opposing selective forces: (1) sexual selection favouring conspicuous coloration of the sex with higher variance in its reproductive output, typically males; (2) predation pressure favouring cryptic coloration in both sexes, but more strongly in females which are vulnerable to predation during gravidity and/or maternal care (Darwin 1871, for a review see also Stutchbury & Morton 2001). Risks for females associated with mating and male harassment were currently proposed as a driving force leading to the reduction of conspicuous ornamentation in females (Hosken et al. 2016).

Anoles are one of the best model systems for the evolutionary, ecological and phylogenetic studies of morphological traits including sexual dimorphism and dichromatism (for a review see Losos 2009). The most important part of anole body for the study of dichromatism is the dewlap. Dewlap is a flap of skin under the throat of the lizard that is extended and retracted rapidly during signalling and has an important species recognition functions (Ord & Martins 2006, Ng & Glor 2011, Ng et al. 2013). In many anole species females have smaller and/or less conspicuously coloured dewlaps than males (Harrison & Poe 2012). Nevertheless, fundamental sexual differences in dewlap coloration and pattern are rare and such cases are mostly reported from mainland anoles (Köhler 2003, Ugueto et al. 2007, 2009, Köhler et al. 2014).

Intersexual differences in dewlaps could be understood as a result of different pressures on males and females. While the reproductive success of males is determined mostly by success in mate acquisition which is positively associated with signalling, female success depends mainly on egg production and survival. This results in different design of dewlaps between sexes (Vanhooydonck et al. 2009). Females generally use their dewlaps much less than males and rarely in reproductive context (Nunez et al. 1997, Losos 2009, Johnson & Wade 2010). Females also exhibit no relationship between display behaviour and dewlap morphology.

Thus, the use of the dewlap is not associated with the size of the cartilaginous structures that support its movement (Johnson et al. 2011). Harrison & Poe (2012) focused on females dewlap size variation in huge comparative study and found evidence that females have large dewlaps in species with little sexual size dimorphism, while having small or no dewlaps in species with wider sexual size dimorphism. From all Antillean ecomorphs, the largest dewlaps are found in crown-giants and twig anoles. One hypothesis for this phenomenon is that large dewlaps are essential in dense habitats as they would be seen better, and second hypothesis is based on low densities of these two ecomorphs – larger dewlaps facilitate long distance communication (Harrison & Poe 2012).

In this study we focus on the “chamaeleolis” group endemic to Cuba Island (Schettino 2003, Losos 2009). They used to have their own ecomorph class named “twig giants” (Haas et al. 1993) referring to their unique body shape and lifestyle but they were moved to wide-size ecomorph class “twig anoles” (Beuttell & Losos 1999, Mahler et al. 2016). Nevertheless these striking lizards show list of unusual characteristics among other anoles- large body size, short limbs, huge bony casques on heads, lack of tail autotomy (Garrido & Schwartz 1968), independent eye movement, cryptic coloration and extraordinary scalation, molariform teeth in adults (Estes & Williams 1984, Schwartz & Henderson 1991) specialized for crushing snails (Schettino 1999, 2003, Herrel & Holáňová 2008) and cryptic and slow motion lifestyle (Leal & Losos 2000). Consequently, these anoles have been traditionally recognized as a distinct genus *Chamaeleolis* Cocteau, 1838. Because this group forms a derived internal clade of the genus *Anolis* Daudin, 1802 sensu lato, it is usually treated as a junior synonym of *Anolis*. The studies discovering the cladogenesis of the anoles have placed the genus *Chamaeleolis* within the main body of the tree of Antillean anoles. These form a sister group of a clade consisting of the Puerto Rican species *Anolis cuvieri* Merrem, 1820 and Hispaniolan *A. barahonae* Williams, 1962 and *A. christophei* Williams, 1960 (e.g., Haas et al. 1993, Poe 2004, Nicholson et al. 2005, 2012). In this study we discovered a population exhibiting fundamental sexual dichromatism, males possessing red dewlap strongly contrasting with white dewlaps of conspecific females.

The aim of this paper was (1) to describe dewlap coloration and reflectance spectra in the discovered dichromatic population and other four species/populations of anoles belonging to the “chamaeleolis” group; (2) to assess sexual dichromatism and sexual size dimorphism in the studied “chamaeleolis” species; (3) to analyse morphometric and genetic variation in this group and related anoles in order to discuss evolution of the dimorphic traits.

MATERIAL AND METHODS

Species determination

The examined material (see below) was assigned to species according to morphological criteria (Holáňová et al. 2012). We provisionally determined the specimens of dichromatic population from vicinity of San German (Holguín province, Cuba) and Gran Piedra (Santiago de Cuba province, Cuba; Figs. 1–3) as *Anolis porcus*. Considering multiple distinct morphometric and genetic characters of these animals (see under the results), we further refer to this population as *Anolis* sp. In original description of *A. porcus* Cope, 1864 there is no information about dewlap coloration nor about type locality. We avoid taxonomic discussions concerning species identity of these specimens until a thorough revision of *A. porcus* sensu lato including properly localized materials will be performed. Clarification of the geographic origin of the holotype is needed prior to any nomenclatural suggestion.

Spectrophotometry

We measured 3 males and 2 females of *Anolis* sp. from San German population together with *Anolis barbatus* Garrido, 1982 (Soroa), *A. chamaeleonides* Duméril et Bibron, 1837 (Viñales), *A. guamuhaia* Garrido, Pérez-Beato et Moreno, 1991 (Topes de Collantes) and *A. porcus* (Baracoa), each represented by a single male and a single female. All specimens were obtained from collections of private European and Russian breeders. All were captive bred after parental animals with known original localities.

The dewlap colour reflectance was determined between 300 and 700 nm with an OceanOptics USB4000 spectrophotometer, using a PX-2 Pulsed Xenon lamp source. We used the Ocean Optics WS-1 white standard for calibration, which was performed after every three measurements. The probe was held in a constant perpendicular 5mm distance from the gently stretched dewlap skin and the measurements were performed in a darkened room. Each colour patch was measured 3 times and then calculated its mean reflectance value. For visual modelling, we used the photoreceptor sensitivity data for *Anolis lineatopus* Gray, 1840 (Loew et al. 2002, Marshall & Stevens 2014). We calculated both chromatic and achromatic



Figs. 1–3. 1 – Original locality of *Anolis* sp. at Gran Piedra, Santiago de Cuba province, Cuba (photo by VHZ). 2 and 3 – Wild adult male of *Anolis* sp. at locality Gran Piedra, Santiago de Cuba province, Cuba (both photos by VHZ).

Table 1. Genetic samples and their GenBank accession numbers

samples	origin	GenBank Accession Numbers
<i>Anolis barbatus</i> AB 1	Soroa	MF157534
<i>Anolis barbatus</i> AB 2	Soroa	MF157535
<i>Anolis porcus</i> AP 1	El Yunque	MF157536
<i>Anolis porcus</i> AP 2	Duaba	MF157537
<i>Anolis porcus</i> AP 3	Duaba	MF157538
<i>Anolis guamuhaya</i> AG 1	Topes de Collantes	MF157539
<i>Anolis</i> sp. APS 6	Gran Piedra	MF157540
<i>Anolis</i> sp. APS 4	Gran Piedra	MF157541
<i>Anolis</i> sp. APS 5	Gran Piedra	MF157542
<i>Anolis sierramaestrae</i> AS	La Mula	MF157543
<i>Anolis chamaeleonides</i> AC 3	Viñales	MF157544
<i>Anolis chamaeleonides</i> AC 2	Viñales	MF157545
<i>Anolis chamaeleonides</i> AC 1	Viñales	MF157546
<i>Anolis</i> sp. APS 1	San German	MF157547
<i>Anolis</i> sp. APS 2	San German	MF157548
<i>Anolis</i> sp. APS 3	San German	MF157549
<i>Anolis guamuhaya</i> AG 2	Topes de Collantes	MF157550

contrasts between (1) “males and females” dewlaps (the pale colour), and (2) the pale and red colour within the dewlap of the males of *A. sp.* from San German. The chromatic contrast expressed in “just noticeable differences” (JND) was calculated according to Vorobyev & Osorio (1998); values below 1 indicate that two colours are unrecognizable within the particular visual system, values between 1–3 are considered to be distinguishable under ideal lighting conditions and with the increasing value the colours gradually become more distinct. Data for relative photoreceptor densities and the Weber fraction value 0.05 were taken from Marshall & Stevens (2014). The calculation of the achromatic contrast was performed after the model of Siddiqi et al. (2004) and Loyeau et al. (2007). All calculations were performed in Avicol v.6 (Gomez 2006).

Morphometric traits

We examined 128 adult anoles of the “chamaeleolis” group of which 83 were live animals or preserved specimens provided by European private breeders (18 *A. barbatus* from Soroa, Cuba; 23 *A. chamaeleonides* from Viñales, Cuba; 19 *A. guamuhaya* from Topes de Collantes, Cuba; 17 *A. porcus* Baracoa, Cuba and 6 *Anolis* sp. from San German, Cuba) and 44 were museum specimens from the herpetological collection of the National Museum in Prague (NMP), Czech Republic (list of museum specimens in Appendix 1).

The following measurements were made with digital callipers to the nearest 0.1 mm: snout-to-vent length (SVL: measured from the tip of the snout to the vent); body length (LIE: longitudo interextremitatis – the distance between front and hind legs); jaw out-lever distance (JOL: the distance between the jaw articulation and the tip of the jaw); head length (HL: measured from the edge of the head casque to tip of the snout); head width (HW: measured at the intersection with the angle of the jaws); head height (HH: measured just posterior to the orbits); snout-orbit distance (SO: the distance between the tip of the snout to the nearest point of the orbit); snout-nostril distance (SN: the distance between the tip of the snout to the edge of the left nostril); snout-mouth end (SME: the distance from the tip of the snout to the corner of the mouth); lower jaw length (LJL: the distance from the back of the retroarticular process to the tip of the lower jaw); snout length (SL: the length of the snout measured from the back of the jugal bone to the tip of the upper jaw); closing in-lever (CL: the distance between the jaw articulation and the back of the jugal bone; this distance was calculated by subtracting the snout length from the distance measured from the jaw articulation to the tip of the jaw = QT); opening in-lever (OL: the distance from the jaw articulation to the back of the retroarticular proces; this distance was calculated by subtracting QT from lower jaw length); internasal distance (IN: the distance between the nostrils); orbit-casque distance (OC: the distance between the posterior-most point of the orbit and the highest point of the casque); interorbital distance (IO: the shortest distance between the orbits); ear opening (EO: the maximum vertical length of an ear opening); tibia (TB: the length of the left tibia); femur (FEM: the length of the left femur); hind metatarsus (HM: the length of the left hind metatarsus); hind finger (HF: the length of the longest –the fourth- hind finger excluding the claw); humerus (HU: the length of the left humerus); radius (RA: the length of the left radius); front metatarsus (FM: the length of the left front metatarsus); barb scales (BS: the maximum length of the barb-like scales on a dewlap).

Statistical analysis of morphometric data

Size component of morphometric variation may mask differences in body shape (e.g., Frýdlová et al. 2011). Thus, we performed size adjustment of morphometric traits prior further analyses comparing the sexes and/or populations. For this purpose, we adopted a method published by Somers (1986, 1989) as implemented in the Size analysis v02 (Thompson & Withers 2005a, b, c). This software computes from original untransformed measurements not only generalised (multivariate) isometric size, but also partial isometric size-adjusted measurements. The latter ones were further treated by univariate and/or multivariate statistical procedures. We used STATISTICA, version 6.0, StatSoft Inc., 2001, for these calculations.

Genetic characteristics

We sampled 17 individuals of genus *Anolis* (see Table 1 for GenBank accession numbers of samples). Total genomic DNA was isolated from tissue samples with DNAeasy Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's guidelines. DNA amplification was performed with two previously published primers: L4437 (Macey et al. 1997) and H5730 (Glor et al. 2004). These primers were used to sequence the entire 1035 bp fragment, which includes complete sequence for the gene encoding ND2.

Polymerase chain reactions (PCR) were carried out in 50 µl including 2.5 µl of each 10 µM primer, 5 µl of 10× PCR buffer (Fermentas), 5 µl of 10 mM dNTP, 2.5 µl of 50 mM MgCl₂, 0.5 µl of 5 U/ml Fermentas Taq DNA polymerase, 100 ng of DNA and 27 µl of ddH₂O. The PCR amplification protocol consisted of 30 cycles of denaturation at 95 °C for 35 s, annealing at 48–51 °C for 35 s, and extension at 72 °C for 150 s; a further 7 min elongation step at 72 °C followed the last cycle. All PCR products were purified with the Qiaquick® purification kit (Qiagen, Hilden, Germany) and directly sequenced using the same primers as for the amplification.

Bayesian analysis (BA) was conducted with MrBayes 3.2.2 (Ronquist & Huelsenbeck 2003). The optimal model of DNA sequence evolution was selected using the AIC criterion in Modeltest 3.7 (Posada & Crandall 1998). Two independent runs of Bayesian analyses were conducted with a random starting tree and run for 6×10⁶ generations, with trees sampled every 100 generations. The burn-in command was used to discard the first 6,000 trees (600,000 generations). Posterior-probability values were used to indicate support for nodes of the Bayesian topology.

The outgroup is composed of ND2 sequences of *Anolis argenteolus* Cope, 1861 (GenBank Accession Number AY296154.1), *A. lucius* Duméril et Bibron, 1837 (GenBank Accession Number AF055962.2), *A. etheridgei* Williams, 1962 (GenBank Accession Number AF055934.2), *A. insolitus* Williams et Rand, 1969 (GenBank Accession Number AF055933.2), *A. cuvieri* (GenBank Accession Number AF055973.2), *A. christophei* (GenBank Accession Number AF055957.2), *A. ricordii* Duméril et Bibron, 1837 (GenBank Accession Number AY367138.1), *A. barahonae* (GenBank Accession Number AF055972.2) and *A. baleatus* Cope, 1864 (GenBank Accession Number AY296155.1).

RESULTS

Sexual dichromatism

Pictorial comparison of heads and dewlaps of males and females in each of five examined species/populations of anoles belonging to “chamaeleolis” group is provided in Fig. 4. Our spectrophotometric measurements confirmed the white/gray/brown character of the dewlaps in *Anolis barbatus* (Soroa), *A. chamaeleonides* (Viñales), *A. guamuhaya* (Topes de Collantes) and *A. porcus* (Baracoa), without any significant peak in the UV (see Table 2). Certain UV reflectance (320–400 nm) was detected in both males and females in these four “non-red” species, but was absent in red-throated *Anolis* sp. from San German (Fig. 5). Chromatic contrast suggested little intersexual differences in the pale colour: 2.09–4.28 JND in “non-red” species; 4.02–6.70 JND in *Anolis* sp. from San German (JND values <1, 1–3 and >3 indicate indistinguishable, barely distinguishable and clearly distinguishable stimuli respectively; see Methods). The only sexual difference in “non-red” species, if present, was manifested mainly through the overall brightness of the pale colour, which was brighter in male dewlaps. In *A. porcus*, the reflectance curve differed at longer wavelengths between males and females, suggesting a possible chromatic difference. It is however hard to evaluate this difference with only a single pair of individuals. The achromatic contrasts between male and female dewlaps spanned from nearly non-dimorphic (3.5 JND in *A. chamaeleonides*) to strongly distinct (22 JND in *A. barbatus*) and the same range was present among the individuals of *Anolis* sp. from San German. The red spot in the males from this popu-

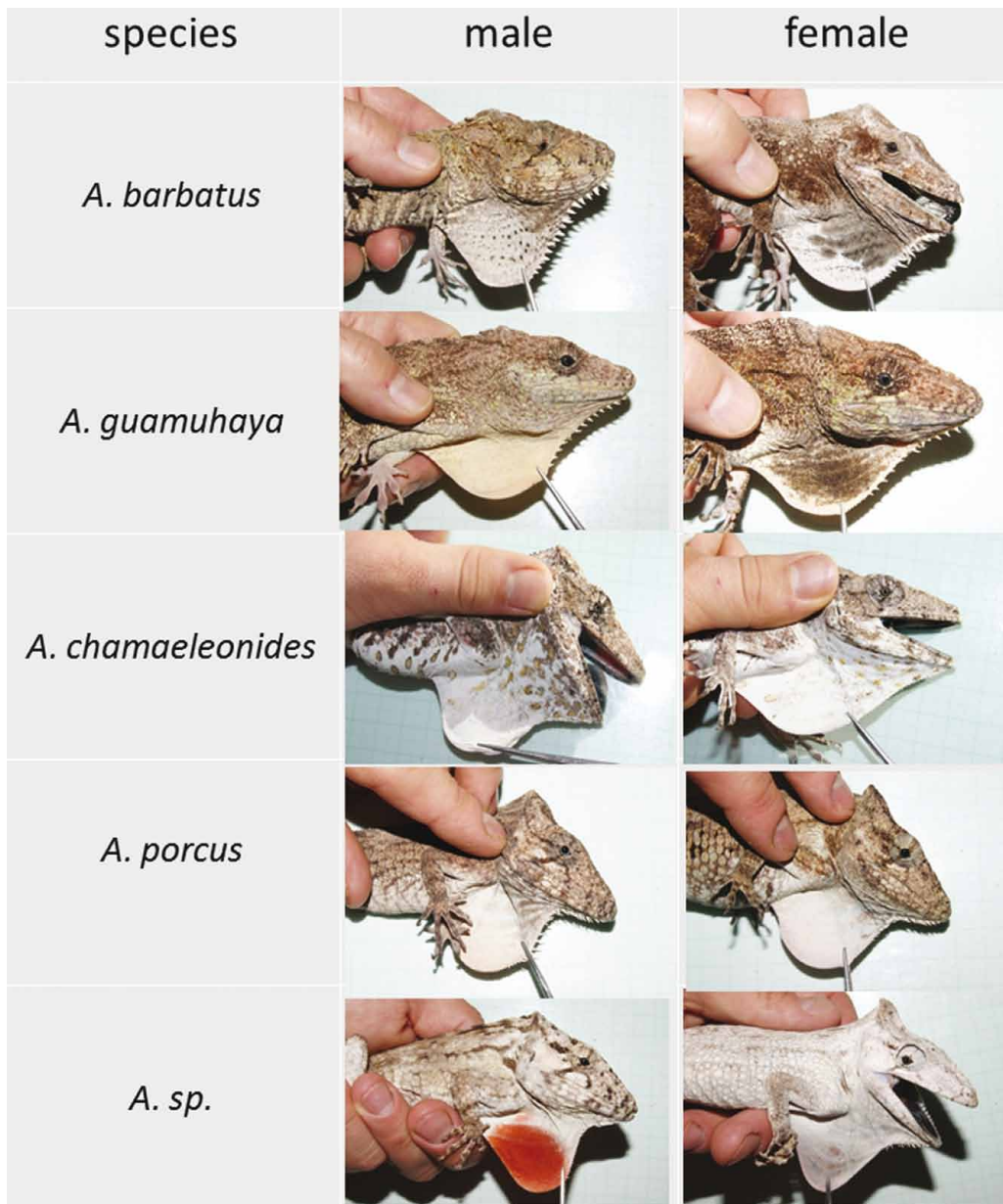


Fig. 4. Lateral view of heads and dewlaps in males and females of five examined *Anolis* species/populations of the “chamaeleolis” group.

lation strongly contrasted with its white background in both chromatic (mean=12.35±0.05 JND) and achromatic (mean=21.40±0.07 JND) visual channels (Table 1).

Table 2. Chromatic and achromatic contrasts between (1) males' and females' dewlaps (the pale color), and (2) the pale and red color within the dewlap of the males of *Anolis* sp. from San German

	chromatic contrast (JND)	achromatic contrast (JND)
<i>Anolis barbatus</i> white M-F	4.193	22.013
<i>Anolis chamaeleonides</i> white M-F	2.094	3.490
<i>Anolis guamuhaya</i> white M-F	4.286	6.759
<i>Anolis porcus</i> white M-F	2.107	20.087
<i>Anolis</i> sp. white M1-F1	6.698	19.956
<i>Anolis</i> sp. white M1-F2	4.496	9.174
<i>Anolis</i> sp. white M2-F1	6.397	9.907
<i>Anolis</i> sp. white M2-F2	4.085	3.467
<i>Anolis</i> sp. white M3-F1	6.071	15.350
<i>Anolis</i> sp. white M3-F2	4.017	4.569
<i>Anolis</i> sp. red-white M1	12.149	20.742
<i>Anolis</i> sp. red-white M2	12.849	21.921
<i>Anolis</i> sp. red-white M3	12.065	21.539

Sexual dimorphism in body size and shape

Multivariate analysis of variance (MANOVA) showed strong effect of species ($F_{100,327}=6.70$, $P<0.0001$) on 25 size-adjusted morphometric variables, but revealed neither the effect of sex ($F_{25,82}=1.14$, $P=0.3221$) nor sex*species interaction ($F_{100,327}=1.26$, $P=0.0704$). This result was confirmed by Analysis of variance examining the effects of species/populations, sex and its interaction on isometric multivariate body size (PC1 produced by the Size analysis v02 software, Thompson & Withers 2005a). Body size differed among species/populations ($F_{4,106}=8.20$, $P<0.0001$), but not between the sexes (sex: $F_{1,106}=1.01$, $P=0.3174$; sex*species interaction: $F_{4,106}=1.39$, $P=0.2415$). Almost the same results were obtained for PC2 and PC3 reflecting body shape (Species: both $P<0.0001$, for sex and interaction all $P>0.20$). This allowed us to pool sexes for further analyses.

Morphometric differentiation among populations/species

We performed discriminant function analysis (DFA) to visualize differences among examined species/populations (except *A. sierramaestrae* Holáňová, Frynta et Rehák, 2012 for which only the holotype specimen was available) in size-free morphometric traits. We applied stepwise forward selection method which resulted in inclusion of 21 of variables (four variables were excluded) and high classification success (Wilks' Lambda=0.0161, $F_{84,361}=7.94$, $P<0.0001$). 108 of 116 specimens (93%) were assigned to proper species/population. All *A. chamaeleonides* (25) and *A. porcus* (17) were reclassified correctly. Two of 40 specimens of *A. barbatus* were misclassified as *A. guamuhaya* while five of 28 *A. guamuhaya* as *A. barbatus*. One of six *Anolis* sp. was misclassified as *A. porcus*. The type specimen of *A. sierramaestrae* was closest to *A. porcus* according to the classification equations derived from DFA.

Cluster analysis (CA) performed by Ward method from the matrix of squared Mahalanobis distances visualized similarities between *A. barbatus* and *A. guamuhaya* as well as between *A. porcus* and *Anolis* sp.; *A. chamaeleonides* was morphometrically least similar to remaining examined species (Fig. 6, Appendix 2).

Canonical analysis revealed four significant ($P<0.0005$) multivariate axes (Appendix 3). The position of examined specimens in the morphospace of the first two canonical axes is plotted on Fig. 7.

Genetic differentiation among populations/species

The phylogenetic analysis was based on sequences of mitochondrial ND2 gene (alignment consisting of 1035 bp). The sequence divergences between haplotypes of “red dewlap” (here referred to *Anolis* sp.) and “whitish-yellowish dewlap” (*A. porcus*) populations belonging to *A. porcus* sensu lato (p -distances ranging from 5.25% to 6.11%, mean=5.62%), is comparable to those among haplotypes of currently recognized species of the “chamaeleolis” group of anoles (i.e.,

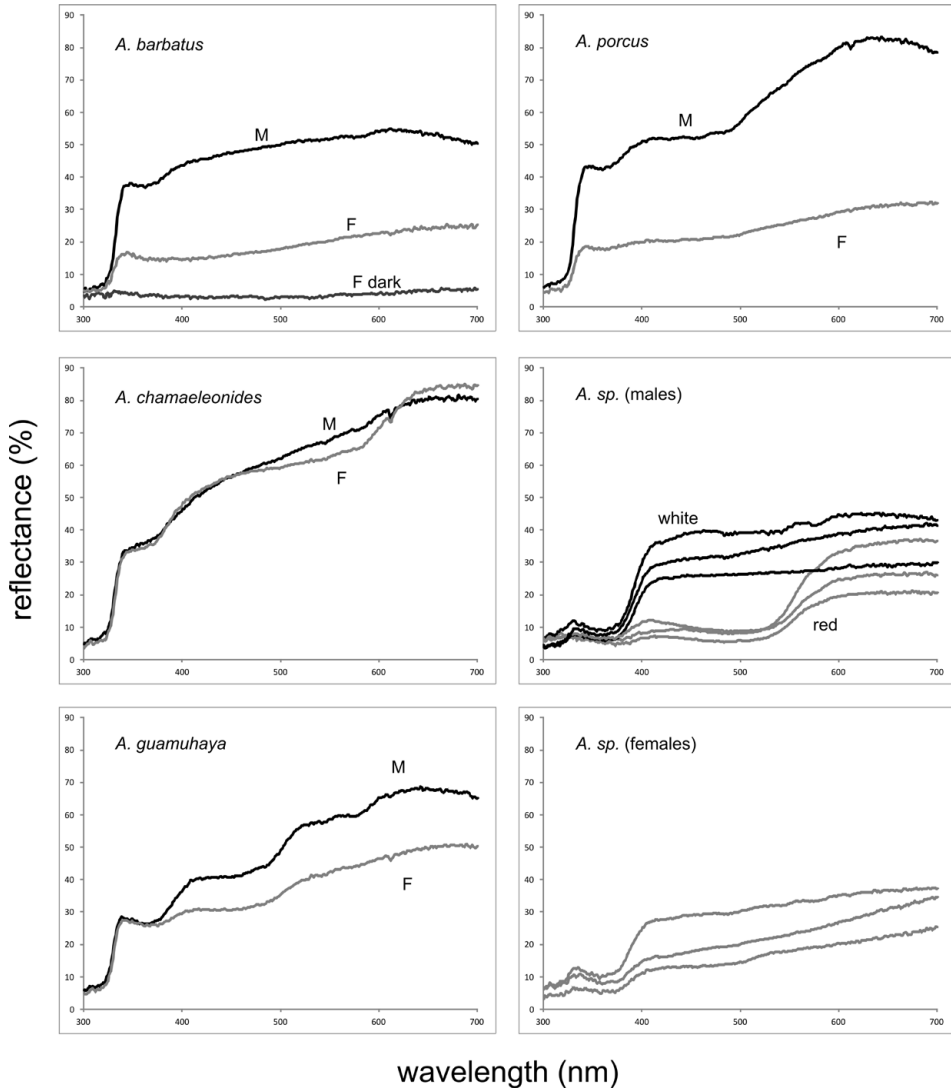


Fig. 5. The dewlap color reflectance between 300 and 700 nm in males and females of five species/populations of anoles of “chamaeleolis” group.

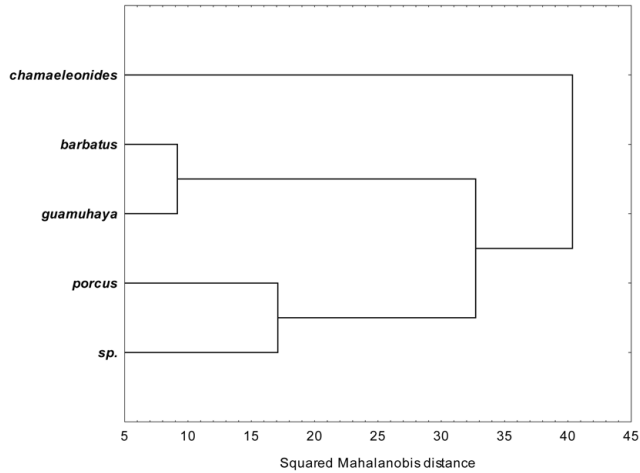


Fig. 6. Phenetic tree of five *Anolis* species based on morphometric data. It is constructed from matrix of Mahalanobis distances clustered by Ward's method.

A. barbatus, *A. chamaeleonides*, *A. guamuhaya*, *A. porcus* and *A. sierramaestrae*). The uncorrected P-distances of these between species comparisons vary within the range of 4.44–10.20%, mean=6.58%, while within species distances are much smaller; maximum values were 0.59, 0.59,

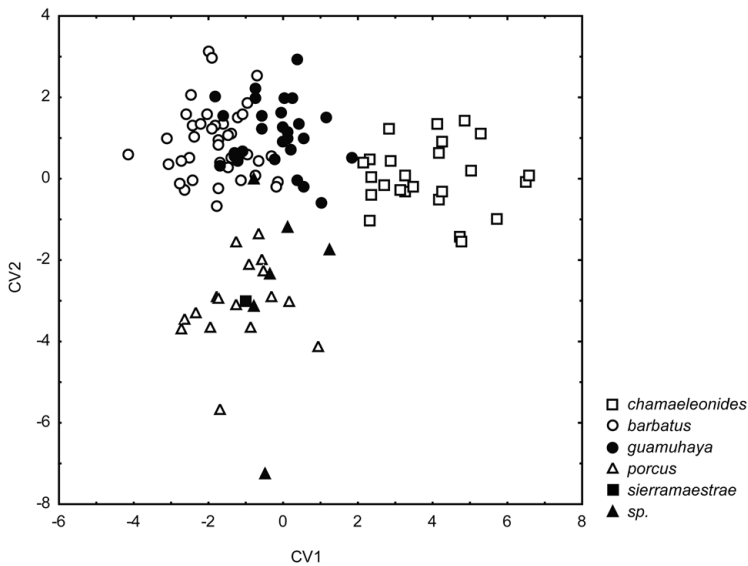


Fig. 7. Position of *Anolis* species/populations of the “chamaeleolis” group in the morphospace of the first two canonical axes (CVA1 and CVA2).

1.19, 0.39, and 2.21 for *A. barbatus*, *A. chamaeleonides*, *A. guamuhaya*, *A. porcus* and *Anolis* sp., respectively. The Bayesian analysis revealed that examined haplotypes belonging to *A. barbatus*, *A. chamaeleonides* and *A. guamuhaya* (all posterior probabilities = 1) form monophyletic groups, but this is not true for *A. porcus sensu lato* including populations of both colour forms. In contrast to this the haplotypes from *A. sp.* (= “red dewlap”) and “whitish-yellowish dewlap” (= *A. porcus*) populations formed mutually exclusive monophyletic groups (posterior probabilities were 0.99 and 1.00 respectively). As expected, monophyletic status of entire “chamaeleolis” group of anoles was strongly supported (Fig. 8).

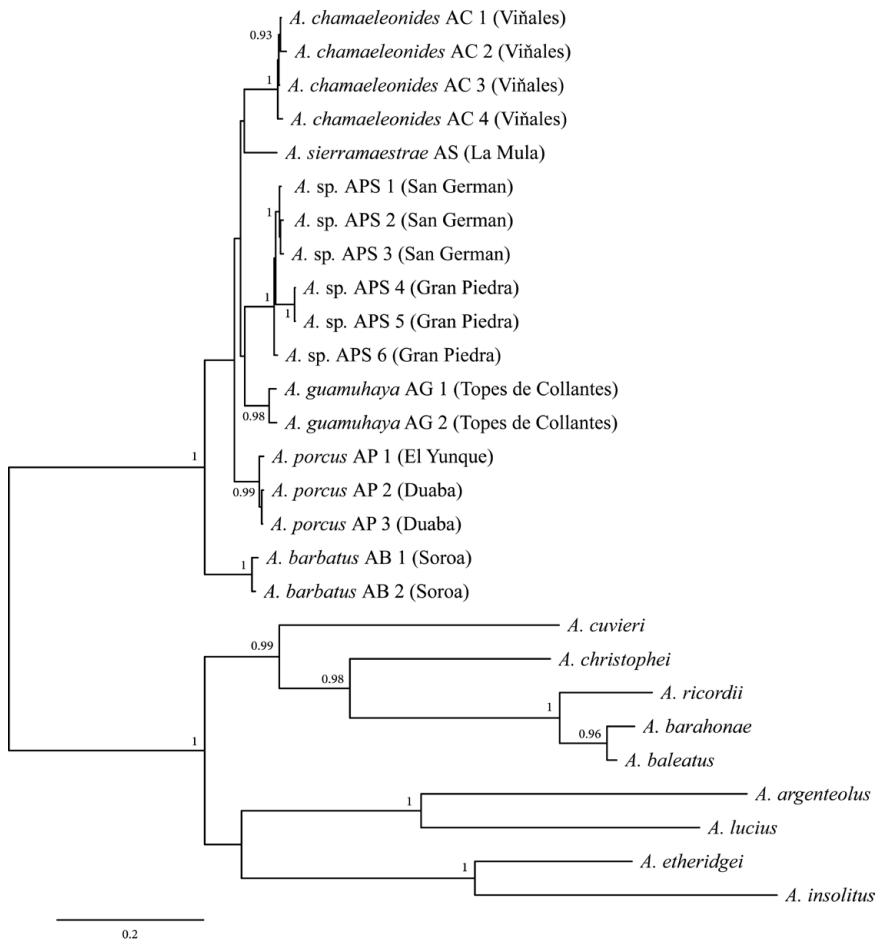


Fig. 8. Bayesian tree of mitochondrial ND2 gene including six species/populations of Cuban anoles belonging to the “chamaeleolis” group as well as nine related anoles introduced as outgroups.

DISCUSSION

Dewlap dichromatism

Our study showed that in comparison to other species/populations of the “chamaeleolis” group, dewlaps of *Anolis* sp. are clearly sexually dichromatic. Taking into account the tetrachromatic vision of the anoles, spectrophotometry and visual modelling revealed no such intersexual difference which would be perceived only by the anoles, but not by humans. While the “dichromatism” in the “non-red” anoles, if present, can be manifested mainly through the lightness of the dewlap, there is both chromatic and achromatic contrast in the red-throated *Anolis* sp., making their dewlaps more conspicuous. Males of the “chamaeleolis” anoles perform the courtship behaviour style as other anoles do, including dewlap displays and head bobs (pers. obs.). The red coloration may therefore represent an honest signal, possibly mediated by carotenoid pigments, which are often involved as indicators of males’ quality (Steffen & McGraw 2009, Steffen et al. 2010). The other frequent honest signal in lizards is the ultraviolet colouration (Font et al. 2009, Fleishman et al. 2011), but although our measurements detected some UV reflectance in the “non-red” species (and none in *Anolis* sp.), this did not seem to be the case. Firstly, the UV reflectance was the lowest within the whole spectrum and secondly, when the sexes differed, they did by the overall shape of the reflectance curve, not just by the UV reflectance itself. However, larger samples are needed to determine reliable statistics of possible trends in this or other traits.

Dewlap function and evolution

There are multiple hypotheses about the function and evolution of the dewlap (Losos 2009, Steffen & Guyer 2014, Hagman & Ord 2016). It may play a role in species recognition, territorial signalling (intrasexual selection), honest signalling of male quality, or in predator deterrence.

Huge dewlap evolution in lizards with clearly allopatric distribution, like in the “chamaeleolis” anoles, does not suggest that it should have function in species recognition as it has in sympatrically living anoles of the same ecomorph. Lizards of the “chamaeleolis” group are classified as the “twig anoles” ecomorph which displays active foraging mode. Species belonging to this ecomorph have slow locomotion but can search for usually cryptic prey for greater distances (Irschick & Losos 1996). These authors suppose that it is possible for this reason that twig anoles have wide size habitats and so it is difficult to defend such a large space. That could be the reason why intrasexual selection usually does not impact twig anoles and dimorphism is not noticeable. But this assumption could be applicable in case of low density of the species. In the species *Anolis* sp., there can be higher local population density which leads to stronger male competition and therefore to stronger intrasexual component of sexual selection. Unfortunately population density has not been measured for this population. The dichromatism subsequently makes the courtship and aggressive communication more unambiguous, unlike in monochromatic species (Regalado 2015).

The habitat type has also considerable influence on the use of the dewlap. Species inhabiting habitats with higher visibility display more frequently. Anole species extend their dewlaps in sunny habitats more often than in shady ones (Ord & Martins 2006), but light conditions appear to be unrelated to the evolution of dewlap colour and signal detectability (Fleishman et al. 2009). Moreover, all “chamaeleolis” anoles live in more or less the same type of habitat (personal observation of the first author), it is therefore unlikely that the dewlap colour is directly associated with their environment.

Different pressures on sexes can also lead to different use and size of dewlaps in some anole species. Johnson & Wade (2010) showed in their comparative study that males have larger dewlaps (and cartilage and muscular components controlling dewlap movement) and use them

more frequently than females. Dewlap size is a significant predictor of the winner in male fights in species with low but not high SSD. Neither the dewlap display rate is associated with SSD (Lailvaux & Irschick 2007). But as we detected no significant SSD in *Anolis* sp., this explanation is unlikely as well.

According to the pursuit-deterrence hypothesis, when the prey is detected by the predator, it gives him an “I am aware of you” signal (shows the dewlap), which might deter the predator from pursuing its prey (Caro 1995). This hypothesis predicts that mainland species living under higher predation risk would display more often. Mainland *A. carolinensis* Voigt, 1832 exhibit elevated rate of dewlap use compared to its island relatives (Vanhooydonck et al. 2009, Johnson & Wade 2010). However, this explanation would be doubtful in the case of “chamaeleolis” anoles, as they are not presumably preyed much, being considerably cryptic. Moreover, if the conspicuous dewlap was to deter potential predators, it should be present in both sexes and not just the males.

In conclusion, because of lack of data on the population density of *Anolis* sp. and its related species, it is difficult to determine the evolutionary causes of the dewlap dichromatism.

Sexual size monomorphism/dimorphism

The differences between the sexes in morphometric traits have been thoroughly and repeatedly analysed in anoles (see Losos 2009). Sexual differences in body size are positively correlated with those in body shape (Losos et al. 2003). Sexual size dimorphism is traditionally explained by three causes: (1) sexual selection or competition for reproductive success, (2) intersexual resource differences and (3) different reproductive roles (Losos et al. 2003). Nevertheless, the relative clutch size in anoles is considerably reduced compared to the other clades of squamates (Kratochvíl & Kubička 2007). This limits a peak load of maternal investment and thus, the effect of sex differences in reproductive roles have in this particular group of lizards. Contribution of the strength of sexual selection was repeatedly confirmed even by intraspecific comparisons, e.g., sexual size dimorphism increases with population density (Stamps et al. 1997). Empirical studies revealed that habitat use of a particular species belongs to key predictors the sexual size dimorphism in anoles (Butler et al. 2000, Losos et al. 2003). Size of defended habitat, foraging style, food source dispersion – these habitat characteristics influence sexual dimorphism.

In examined material of the “chamaeleolis” anoles, we failed to detect sexual size dimorphism neither in the multivariate body size nor in the body shape. Although, the available sample of the dichromatic *Anolis* sp. was too small to allow a separate analysis, the sexes were of comparable size and shape even in this population/species. The entire absence of the sexual size and shape dimorphism in this group is not surprising. Lizards of group are arranged to the “twig anoles” ecomorph which displays active foraging mode. Species belonging to this ecomorph have slow locomotion but can search for usually cryptic prey for greater distances (Irschick & Losos 1996). These authors suppose that it is possible for this reason that twig anoles have wide size habitats and so it is difficult to defend such a large space. That could be the reason why intrasexual selection usually does not impact twig anoles and dimorphism is typically not noticeable.

Morphometric and genetic divergence of “chamaeleolis” species

Anoles of the “chamaeleolis” group are slowly moving lizards with limited home ranges. Thus, they are most likely poor dispersers. This suggestion is supported by distribution patterns of these species in Cuba (Garrido et al. 1991, Díaz et al. 1998, Schettino 1999, Holáňová et al. 2012). Each species is typically restricted to a local mountainous area and its surroundings. The isolation of local populations may explain a considerable degree of morphological divergence we observed. Considering the reports of morphologically suspect individuals from other localities (e.g., Rancho Velaz, Villa Clara province, Sierra de Banao, Sancti Spiritus province) (Garrido 1982, Garrido

et al. 1991), discoveries of additional species of the chamaeleolis group can be expected in the near future.

Our multivariate analysis of morphometric traits showed that the most distinct species is *A. chamaeleonides*, while *A. barbatus* resembles *A. guamuhaia* and non-dichromatic *A. porcus* is similar to dichromatic *Anolis* sp. In this context it is interesting that a morphotype resembling “chamaeleolis group” evolved entirely independently in Hispaniola in a group of related anole species (Mahler et al. 2016).

Genetically, all studied species/populations seem to differ in mitochondrial DNA. But except of finding that *A. barbatus* is the most distinct species of all we cannot determine their phylogenetic relationship. It seems that in view of the fact that they have low dispersion ability there will be separated species/population on every mountainous locality. It only confirms that there is extensive genetical diversity in Cuba, which is known also in other species (Starostová et al. 2010). The sequence divergence of a mitochondrial gene we found among species/populations of “chamaeleolis” anoles is considerable and this supports the view that each of examined form, including dichromatic *A. sp.*, represents distinct species. Nevertheless, recent studies suggest that reproductive isolation and thus speciation process are sometimes not fully completed even between lizards with roughly two times higher genetic distances calculated from the sequence divergence of mitochondrial genes (Jančúchová-Lásková et al. 2015a, b).

CONCLUSIONS

In conclusion, local populations of the “chamaeleolis” groups are distinct genetically as well as morphologically. They are non-dimorphic in size but one population is sexually dichromatic (*Anolis* sp.). Such conspicuous dichromatism is unusual among the island anoles.

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APPENDIX 1

List of museum specimens from the herpetological collection of the National Museum in Prague (NMP), Czech Republic used for morphometric examination.

Anolis barbatus, 29 specimens: NMP6j 25/1992, NMP6V 34520/1-11, NMP6V 71872/1-3, NMP6V 71873, NMP6V 73148/1-4, NMP6d 279/03, NMP6V 34504;
Anolis guamuhaya, 10 specimens: NMP6V 71871, NMP6V 71870/1-8, NMP6V 34517;
Anolis chamaeleonides, 3 specimens: NMP6d 81/06, NMP6V 34505, NMP6V 34518;
Anolis porcus, 1 specimen: NMP6V 34519;
Anolis sierramaestrae, 1 specimen: NMP6V 74453.

APPENDIX 2

Matrix of squared Mahalanobis distances between examined species/populations of “chamaeleolis” group revealed by DFA.

	1	2	3	4	5
1 <i>A. chamaeleonides</i> : <i>A. chamaeleonides</i>	0.000	34.394	23.326	38.285	34.384
2 <i>A. barbatus</i> : <i>A. barbatus</i>	34.394	0.000	9.163	19.005	24.287
3 <i>A. guamuhaya</i> : <i>A. guamuhaya</i>	23.326	9.163	0.000	20.939	27.466
4 <i>A. porcus</i> : <i>A. porcus</i>	38.285	19.005	20.939	0.000	17.099
5 <i>A. sierramaestrae</i> : <i>Anolis</i> sp.	34.384	24.287	27.466	17.099	0.000

APPENDIX 3

Loadings of roots revealed by canonical analysis subroutine of DFA.

root	1	2	3	4
SVL	-0.136	-0.623	-0.164	-0.185
OL	0.370	-0.132	0.039	0.232
FM	-0.301	-0.085	0.192	0.468
HM	0.026	-0.181	0.053	0.481
IN	0.204	-0.204	0.034	-0.347
HL	0.228	0.071	0.228	-0.183
OC	0.062	0.086	-0.400	-0.129
SN	0.119	0.261	-0.089	0.141
RA	-0.002	0.173	-0.035	-0.197
SL	-0.107	0.002	0.273	-0.267
HF	-0.086	0.107	0.087	0.072
IO	0.207	-0.014	0.187	0.109
HH	0.147	0.220	0.121	0.139
LIE	0.042	0.313	-0.078	-0.065
HW	-0.090	0.122	0.343	-0.385
SO	-0.075	-0.100	-0.139	0.024
JOL	0.077	-0.008	0.080	-0.213
TB	-0.037	-0.060	-0.245	-0.171
LJL	0.102	0.239	-0.065	0.080
HU	-0.064	-0.036	-0.013	-0.020
SME	0.053	0.245	0.096	0.052