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## Cryptic within cryptic: genetics, morphometrics, and bioacoustics delimitate a new species of *Eleutherodactylus* (Anura: Eleutherodactylidae) from Eastern Cuba

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### Abstract

We studied the variation in genetics, bioacoustics, and morphology in *Eleutherodactylus glamyrus*, a regionally endemic frog species restricted to high elevations in the Sierra Maestra Massif, Western Cuba that was originally described as a cryptic species hidden under the name *E. auriculatus*. Genetic analysis of mtDNA sequences of the *16S* and *cob* genes identify two allopatric and strongly supported mitochondrial clades (phylogroups) which also showed no haplotype sharing in the nuclear *Rag-1* gene. Bioacoustic, and morphological comparisons concordantly identify these two phylogroups as independent evolutionary lineages. Therefore, we herein restrict the name *Eleutherodactylus glamyrus* Estrada and Hedges to populations represented in our analyses as the western phylogroup (Cordillera del Turquino to Pico La Bayamesa) and consider specimens from the eastern phylogroup (Sierra del Cobre) to represent a new species described and named as *Eleutherodactylus cattus*. Our results add to the growing list of *Eleutherodactylus* species endemic to Cuba and highlight the importance of combining different sources of evidence for obtaining robust assessments of species limits in amphibians.

**Key words:** Terrarana, species delimitation, integrative taxonomy, Caribbean

### Introduction

Due to extreme morphological similarities some species remain undetected to the eyes of taxonomists and when discovered, often by the application of more refined techniques or increased sampling effort, are termed cryptic species. Among animals, such taxonomic discoveries are more frequent in nocturnal taxa, like arthropods and amphibians, which often rely on chemical or acoustic cues for communication and provide scarce information to our visually-oriented brain (Bickford, *et al.*, 2006). The definition of a species as cryptic is to certain degree subjective, i.e., subtle morphological differences might be obvious to specialists but remain undetected by untrained observers. Yet, there is little doubt that a large number of truly cryptic species exist, and amphibians are an animal group where this phenomenon has become obvious in the past decades (e.g., Fouquet, *et al.*, 2007; Stuart, *et al.*, 2006). The explosion in amphibian species discoveries during this period (Hanken, 1999; Köhler, *et al.*, 2005) certainly can be partially explained by the improved toolkit of molecular and bioacoustic methods that allow an integrative delimitation of species which by morphology alone would have been highly difficult to detect (Dayrat, 2005; Padial, *et al.*, 2010). This unknown fraction of cryptic diversity results in taxonomic uncertainty and remains one of the greatest challenges for basic and applied studies of biodiversity, also misleading threat assessments and conservation efforts.

Although in many situations a steep increase of species numbers reflects a genuine discovery of unequivocal new species, the uncritical use of a single line of evidence (e.g. a single locus of mitochondrial DNA, mtDNA, or a single morphological character) can lead to overestimation of species numbers because small populations isolated only for a very short period of time could already become differentiated with respect to some character and thus diagnosable under the classic cumulative integration framework (Padial, *et al.*, 2010). Single lines of evidence might however delimit units that do not represent lineages, for instances in cases of introgression or incomplete lineage sorting, or phenotypic plasticity. This problem can be particularly acute when the populations under study are restricted to small isolated habitat patches, the typical case in island faunas. Technically speaking, under the Unified or General Lineage Species Concept (de Queiroz, 2007), even a single founder reaching an isolated island might conceptually immediately become a new species, although it might be argued that it should not be taxonomically recognized and described as such (Kuchta & Wake, 2016). In any case, species delimitation can largely benefit from the application of a concordant integration framework that incorporates multiple lines of evidence (Padial, *et al.*, 2010) because the farther along lineages are in the process of divergence, the larger the number of differences they can be expected to have acquired relative to one another, and therefore the easier it should be to find evidence of separation (de Queiroz, 2007; Padial, *et al.*, 2010) and the less controversial a delimitation and description of a new species will be.

With 192 species described so far (AmphibiaWeb 2016), frogs of the genus *Eleutherodactylus* are arguably the dominant amphibian group in the Antilles where they represent 84 % of the diversity of amphibians (Hedges, 1999). The number of species of this genus still continues to increase and only in the last decade seven new species have been described. Five of these (*E. beguei* Díaz and Hedges; *E. feichtingeri* Díaz, Hedges, and Schmid; *E. juanariveroi* Rios-López and Thomas; *E. maestrensis* Díaz, Cádiz, and Navarro; and *E. michaelschmidi* Díaz, Cádiz, and Navarro) can be considered cryptic taxa and were only delimited after the application of refined taxonomic tools.

*Eleutherodactylus glamyrus* Estrada and Hedges is a montane frog endemic from Cuba with a distribution restricted to the areas above 800m a.s.l in the Sierra Maestra Massif. It was originally described as a cryptic species with only acoustic features distinguishing it from its closest relative, *Eleutherodactylus auriculatus* (Cope) (Estrada & Hedges, 1997). The specific status of *E. glamyrus* and its close relationship to the *E. auriculatus* species complex was later confirmed with the analysis of mitochondrial DNA sequences (Rodríguez, *et al.*, 2010b). Bioacoustic analyses have also indicated that despite its restricted distribution, a slight divergence exists between populations in the Turquino and La Bayamesa massifs in terms of call rate, call duration and call rise time (Rodríguez, *et al.*, 2010a). Recently, an additional population of *E. glamyrus* was discovered at two localities 53 km to the east of the previous known distribution, showing longer advertisement call duration than that previously reported for *E. glamyrus* (Rodríguez & Alonso, 2012). Considering the preference for high altitudes of the species and the lower altitude of the intervening region, gene flow is likely to be restricted between these populations (Rodríguez & Alonso, 2012). We herein incorporate multiple sources of evidence (mtDNA, nuclear DNA (nuDNA), morphometric, and bioacoustic characters) to test for evidence of species limits between populations currently assigned to *Eleutherodactylus glamyrus*. Results of these analyses are combined in a concordant integrative framework leading to the description of a new species.

## Materials and methods

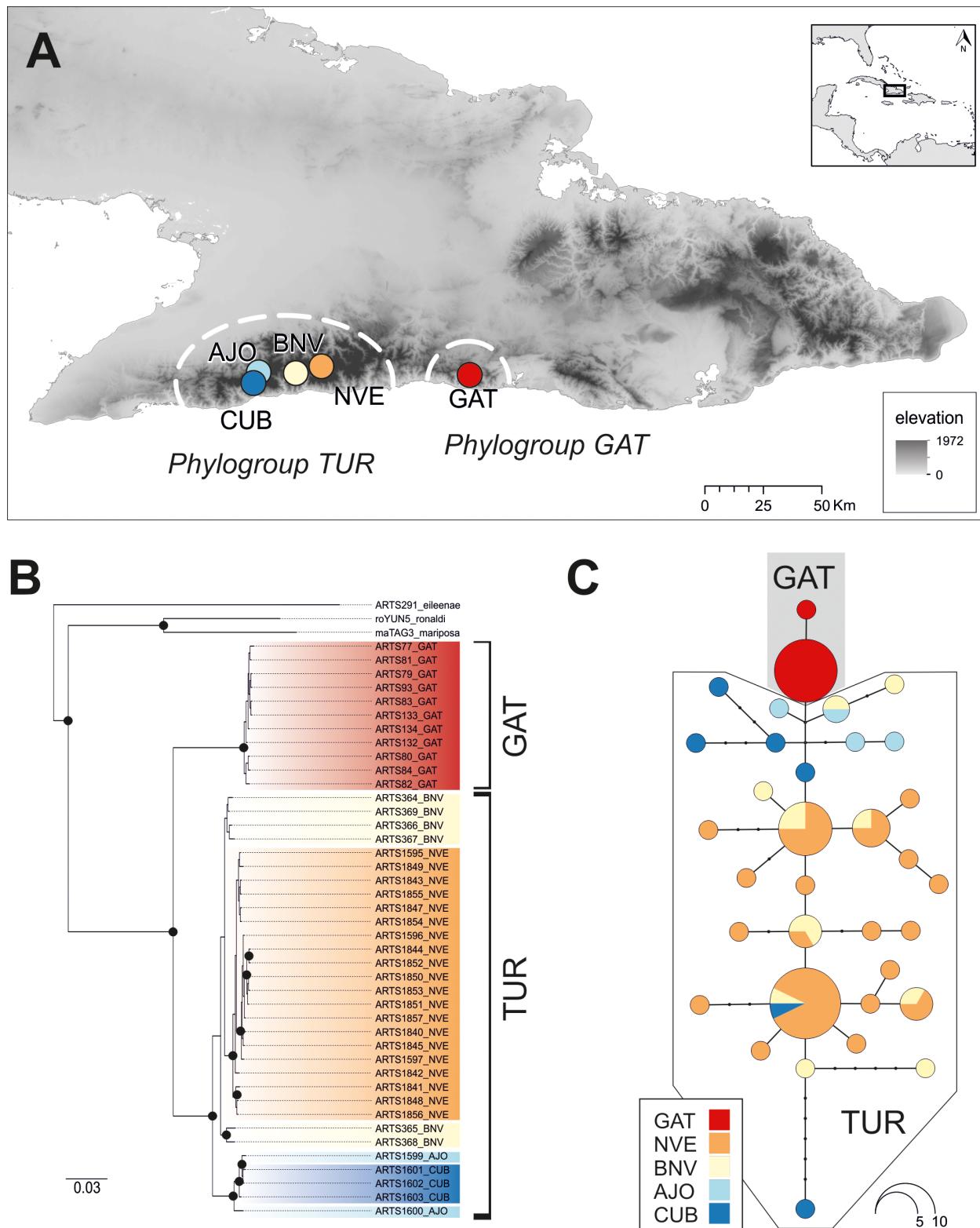
**Samples/materials.** Fieldwork was conducted between 2005 and 2010. Frogs were sampled during expeditions to the following localities: Pico Cuba, Sierra Maestra, Municipio Guamá, Santiago de Cuba (19.98410 N, 76.84540 W, 1872 m a.s.l.) (CUB); Aguada del Joaquín, Sierra Maestra, Municipio Bartolomé Masó, Granma (20.01380 N, 76.83900 W, 1376 m a.s.l.) (AJO); Barrio Nuevo, Sierra Maestra, Municipio Buey Arriba, Granma (20.02677 N, 76.695990 W, 1319 m a.s.l.) (BNV); La Nueve, Sierra Maestra, Municipio Buey Arriba, Granma (20.054348 N, -76.601463 W, 1400 m a.s.l.) (NVE); and trail to Pico El Gato, Sierra del Cobre, Municipio Santiago de Cuba, Santiago de Cuba (20.01364 N, 76.04809 W, 844 m a.s.l.) (GAT) (see Figure 1A). Calling individuals were located between 1900 and 0700 h using headlamps. Calls were recorded with different recorder/microphone combinations (Sennheisser ME-80/ ME-66 unidirectional microphones and tape –Marantz PMD 430 / Sony WM-D6C– or digital –TASCAM DR05 / Marantz PMD 660– recorders). Microphones were held at approximately 50 cm from the focal male and recording proceeded for 2–5 min. Air temperature at the recording site was measured to the nearest 0.2°C

with a Miller & Webber quick-reading thermometer. Following the recordings, males were captured and sacrificed using a chlorobutanol solution and later fixed and preserved in 70% ethanol. Tissue samples were obtained from toe clips or thigh muscles and specimens were later deposited at the Zoological Collection of the Institute of Ecology and Systematics, Havana, Cuba (Appendix I). The recordings from CUB, NVE, and GAT were already used in Rodríguez *et al.* (2010a) and Rodríguez and Alonso (2012). We complemented these with the newly obtained recordings from BNV. Original recordings are stored in the first author personal collection and in the Fonozoo sound archive ([www.fonozoo.org](http://www.fonozoo.org)).

**Molecular genetics.** Genomic DNA was extracted from tissue samples using proteinase K (10 mg/ml) digestion followed by a standard salt extraction protocol (Bruford, *et al.*, 1992). Fragments of two mitochondrial (cytochrome b—*cob*; and 16S rRNA—*16S*) and one nuclear gene (recombination activating protein 1—*Rag-1*), were amplified with polymerase chain reactions (PCR). PCRs were carried in a final volume of 11 µl and using 0.3 µl of each primer (10 µM), 0.25 µl of total dNTP (10 mM), 0.08 µl of 5 u/µl GoTaq and 2.5 µl of 5X GoTaq Reaction Buffer (Promega). The following primer combinations were used to amplify the target fragments: 12SAL /16SBH-new (Palumbi, *et al.*, 2002; So, 2001), Cytb\_a / Cytb\_c (Bossuyt & Milinkovitch, 2000), and Rag1\_EleuF2 / Rag1\_EleuR2 (Rodríguez, *et al.*, 2013). All successfully amplified PCR products were purified using Exonuclease I and Shrimp Alkaline Phosphatase (SAP) or Antarctic Phosphatase (AP) according to the manufacturer's protocols (NEB). Purified PCR templates were sequenced with the same primers using dye-labeled dideoxy terminator cycle sequencing on an ABI 3130 automated DNA sequencer. Chromatograms were checked and sequences were corrected by hand, where necessary, using CodonCode Aligner (v. 3.5.6, Codon Code Corporation). The newly obtained sequences were submitted to GenBank (accession numbers: KY000712–KY000822), a detailed list is provided in Appendix II.

In order to obtain a detailed picture of the genetic variation among the samples, we aligned and concatenated the newly obtained sequences of the two mitochondrial markers with previously published GenBank sequences (see Appendix II), using MEGA 5 (Tamura, *et al.*, 2011). We added to the data set homologous sequences of *Eleutherodactylus eileenae* Dunn, *E. mariposa* Hedges, Estrada and Thomas and *E. ronaldi* Schwartz already available in GenBank (accession numbers: KR908435, FJ527395, FJ527397, GQ426516, and GQ426517) and considered these as outgroup based on published phylogenetic reconstructions of the *E. auriculatus* group (Rodríguez, *et al.*, 2010b). We partitioned the concatenated data into locus and codon position (in the case of the *cob* gene) and used PartitionFinder 1.0.1 (Lanfear, *et al.*, 2012) to infer the best-fitting model of molecular evolution and partition scheme under the corrected Akaike Information Criteria (AICc) (Akaike, 1974; Sugiura, 1978). The optimal partition and evolutionary models for the alignment (third position *cob*: GTR+G, first position *cob* + *16S*: GTR+G, and second position *cob*: GTR) was implemented in a Bayesian phylogenetic reconstruction using MrBayes 3.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). We set up two independent runs of five million generations, each with four Markov Chains (three heated and one cold) sampled every 5,000 generations. Samples corresponding to the initial phase of the Markov chain (25%) were discarded and the applicability of this burn-in value was determined by the inspection of the likelihood scores and effective sample sizes with the software Tracer 1.5 (Rambaut & Drummond, 2007). Post-burn-in trees were combined in a single majority rule consensus tree. Based on the resulting tree, we used a tree-based delimitation procedure (Wiens & Penkrot, 2002) and identified as candidate species all exclusive genetic clusters, strongly supported, and geographically isolated (hereafter phylogroups). We calculated a matrix of pairwise uncorrected p-distances between the phylogroups for each of the two mitochondrial markers using MEGA 5, excluding all ambiguous positions in pairwise comparisons.

We used the information from the nuclear *Rag-1* sequences to evaluate the extent of genetic differentiation in nuclear DNA between the mtDNA-identified candidate species. We aligned the *Rag-1* sequences by hand and translated them into amino acids for authentication with MEGA 5. Nuclear haplotypes were inferred using the coalescent-based Bayesian method of the Phase algorithm (Stephens & Donnelly, 2003; Stephens, *et al.*, 2001) as implemented in DNAsP 5 (Librado & Rozas, 2009) using 10,000 replicates and assuming no recombination among the sequences, as assessed with RDP3 (Martin, *et al.*, 2010). The relationship between phased *Rag-1* sequences was represented in a haplotype network with the aid of Haploviever, which uses an algorithm to produce high-quality haplotype genealogies from tree data (Salzburger, *et al.*, 2011). As guide tree, we used a Maximum Likelihood tree obtained from the phased *Rag-1* sequences with PhyML (Guindon & Gascuel, 2003) under the best fitting molecular substitution model (HKY+I), as determined by the corrected Akaike information criteria (AICc) in the software jModeltest (Posada, 2008).



**FIGURE 1.** Genetic diversity in *Eleutherodactylus glamyrus* sensu lato. A) Topographic map of Eastern Cuba indicating the sampled localities (CUB: Pico Cuba, Sierra Maestra, Municipio Guamá, Santiago de Cuba; AJO: Aguada del Joaquín, Sierra Maestra, Municipio Bartolomé Masó, Granma; BNV: Barrio Nuevo, Municipio Buey Arriba, Granma; NVE: La Nieve, Sierra Maestra, Municipio Buey Arriba, Granma; and GAT: trail to Pico El Gato, Sierra del Cobre, Municipio Santiago de Cuba, Santiago de Cuba). The dashed lines encircle the two phylogroups detected in molecular analyses. B) Bayesian phylogenetic tree of aligned mitochondrial sequences of *16S* and *cob* genes (1158bp) highlighting the two phylogroups identified (TUR and GAT), black dots indicate nodes supported by posterior probabilities > 0.9. C) Haplotype network of aligned 579 bp DNA sequences of the nuclear *Rag-1* gene from 36 individuals with colors indicating sampling localities and mtDNA phylogroups indicated by polygons.

**Bioacoustics.** To maximize the sample size and number of localities compared, our analysis focused on the single-note advertisement call typical of the species (Rodríguez, *et al.*, 2010a), which was recorded in all localities. Tape recordings were digitized at the same sampling rate used in digital recordings (i.e. 44100 samples/s and 16bit depth). Spectrogram and oscillogram illustrations were produced in R (R Core Team, 2016) with the Seewave package (Sueur, *et al.*, 2008). Acoustic analysis were performed with SoundRuler 0.9.4.1 (Gridi-Papp, 2003), as described in Rodríguez *et al.* (2010a). The following temporal and spectral features were measured in 10–11 consecutive calls in the middle of the longest call group: call duration (measured at zero amplitude level on the oscillogram with  $\pm 0.1$  ms of error); rise time (time from call onset to peak amplitude in the oscillogram,  $\pm 0.1$  ms); call rate (reciprocal of the intercall interval,  $\pm 0.1$  ms); dominant frequency (frequency of maximum energy in the spectrogram of a call,  $\pm 43$  Hz); frequency modulation (frequency range between onset and offset of the dominant harmonic in the call spectrogram,  $\pm 43$  Hz); and bandwidth (measured in the power spectrum of a call -6dB from the peak,  $\pm 43$  Hz).

All measurements were averaged per male and only individual means were used in subsequent analysis. Individuals were grouped into candidate species according to the genetic results. Acoustic differentiation between groups was tested with analyses of variance for each acoustic feature. Environmental temperature and caller size are known to affect advertisement call properties in frogs (Wells, 2007) and can confound taxonomic comparisons. To statistically control these effects, we included temperature and size as covariates of each acoustic feature in analyses of covariance (ANCOVA) designed to test for differences among the genetically-identified groups. ANCOVA tests assume the homogeneity of slopes across groups being compared. In our case, this assumption could not be tested due to the small sample size of one of the groups (GAT: N=11, see results), but was assumed as plausible given the close relationship between the groups. ANCOVA models were implemented in R and the fit of the models was assessed via residual plots.

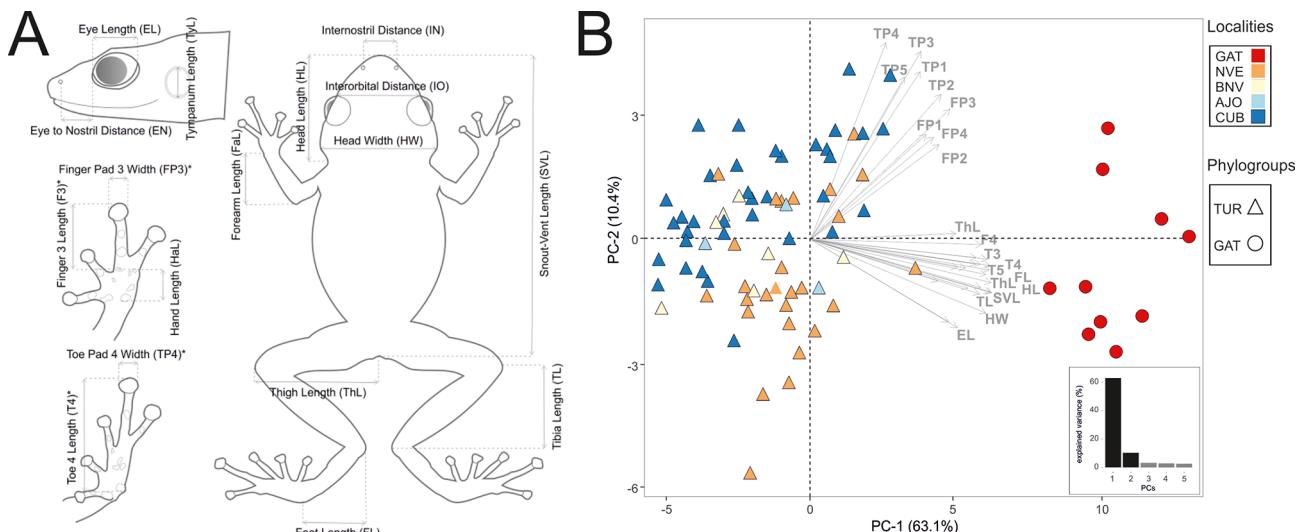
**Morphometrics.** To quantify the body shape and size of the specimens we recorded the following thirty-one linear measurements from 104 specimens (Figure 2 A): snout-vent length (from the tip of the snout to the cloaca, SVL); head length (from posterior margin of the lower jaw to the tip of the snout, HL); head width (at the widest point of the head, HW); internostri distance (IN); interorbital distance (distance between the anterior margins of the eyes, IO); eye to nostril distance (EN); eye length (EL); tympanum length (at its maximum diameter, TyL); forearm length (from elbow to the proximal edge of thenar tubercle, FaL); hand length (from the proximal edge of thenar tubercle to the proximal edge of the first subarticular tubercle on the third finger, HaL); finger lengths (from their insertion into the hand to their tip, F1–4); finger pad widths (at their widest point, FP1–4); thigh length (from the cloaca to the tip of the knee, ThL); tibia length (from the tip of the knee to the tip of the heel, TL); foot length (from the tip of the heel to the proximal edge of the outer metatarsal tubercle, FL); toe lengths (from their insertion into the foot to their tip, T1–5); and toe pads (at their widest point, TP1–5). All raw measurements (Appendix III) were taken by ADC using a Mitutoyo digital caliper to the nearest 0.01 mm, and are here reported rounded to the nearest 0.1 mm from adult males. Sex was determined by the presence of secondary sexual characters (vocal sac and slits). Collection data of measured specimens is provided in Appendix I.

To obtain a morphological perspective on the differentiation between the genetically-identified haplogroups, we selected the data from male individuals (the most abundant sex in collection) from the five localities adding to a total of 82 specimens (after excluding CZACC14.14329 and CZACC14.14304 which lacked some measurements due to the absence of toes/ feet). Data distribution of the variables did not depart significantly from normality (as assessed by Shapiro-Wilks tests, alpha = 0.05) and no logarithmic transformation was applied. To obtain an overall picture of the variation in the data, a principal component analysis (PCA) was conducted on the raw measurements, plotting the scores of the axes with eigenvalues  $> 1$ . For a more robust assessment of the species limit between the phylogroups, and to define which variables contributed more to their differentiation, we statistically tested their morphometric differences using a multivariate analysis of variance (MANOVA). As typical in morphometric studies, measured variables were largely correlated with SVL and we grouped measurements by phylogroups and regressed all measurements against SVL in order to obtain size-independent morphometric variables (Dormann, *et al.*, 2013; Hayek, *et al.*, 2001). A common slope was used for all measurements since no difference between groups was evident in exploratory plots and ultimately because a test of slope homogeneity was not viable due to marked sample size differences (only 11 individuals of the putative new species were available). Uncorrelated regression residuals (Pearson's  $r < 0.7$ ) of each morphometric variable were used as independent variables in a MANOVA.

## Results

**Molecular genetics.** We obtained mitochondrial DNA sequences from 42 individuals including *cob* (591 bp) and *16S* (567 bp) sequences. The *cob* alignment of the ingroup sequences included 68 variable sites containing 71 substitutions of which 62 were parsimony informative, defining 19 haplotypes. The *16S* alignment contained 26 variable sites containing 27 substitutions of which 19 were parsimony informative, defining 13 haplotypes. The majority-rule consensus tree derived from Bayesian inference analysis of the combined mitochondrial DNA sequences indicated the existence of two highly supported and reciprocally monophyletic lineages (Figure 1 B). One of these phylogroups included 31 samples from the localities in the Cordillera del Turquino (CUB, AJO, NVE, and BNV) and is hereafter named phylogroup TUR while the other included all 11 samples from Loma del Gato (locality GAT) in Sierra del Cobre, hereafter phylogroup GAT. Pairwise divergence between these two phylogroups was 8.2% for *cob* and 3.2% for *16S*. For the rest of the analyses, we considered these two allopatric and strongly supported mitochondrial clades as candidate species *sensu* Vieites *et al.* (2009). Within phylogroup TUR, haplotypes also appear to be spatially structured, with sequences from the westernmost populations (AJO and CUB) and also from NVE forming monophyletic groups, while sequences from BNV appeared in two monophyletic groups (Figure 1B). However, the differences between these clades were small and not fully supported in all cases.

Nuclear *Rag-1* gene sequences were obtained for 36 individuals. We trimmed the sequences to exclude missing data ambiguities at the extremes of the alignment, reducing the alignment length to 579 bp. The aligned and phased sequences (72 sequences) contained 42 variable positions of which 15 were parsimony informative, defining 34 different haplotypes. The haplotype network obtained from these sequences indicated, for this nuclear gene, no haplotype sharing between the individuals corresponding to phylogroups TUR and GAT. A single substitution separated the two *Rag-1* GAT haplotypes from those of the other localities (Figure 1 C). The majority of the *Rag-1* alleles (19) were observed in the locality with the largest number of samples (NVE). However, all haplotypes observed at least twice were shared between localities within Cordillera del Turquino (phylogroup TUR) where all localities had at least one shared haplotype.



**FIGURE 2.** Morphometric characters measured and descriptive multivariate ordination of data. A) Measuring landmarks, variable names and abbreviations used. For visual simplicity, only the measurements of a finger/toe length and a finger/toe pad width are displayed. B) Scores of the first two principal components derived from the non-transformed morphometric data (31 variables, 82 specimens) from the five localities (color key as in figure 1) corresponding to phylogroups TUR (triangles) and GAT (circles). Gray arrows represent variable loadings for both axes (overlapping names were excluded, see Appendix V for a full numerical description of components); the inset shows the variance explained by the first five PCs, highlighting in black those displayed in the plot.

**Morphometrics.** Descriptive statistics for the 31 morphometric variables measured in 104 specimens from both candidate species are presented in Table 1 (see Appendix IV for more detailed statistics on the variation in males of *Eleutherodactylus glamyrus* from phylogroup TUR across the nine sampled localities). Results of the principal component analysis revealed a clear differentiation in morphometric space between the males from the

two phylogroups. The first two principal components represented 73.5% of the variation observed in the data and the plot indicated a clear separation along the first axis, with very similar loadings for many variables which were highly correlated with size (Figure 2 B, Appendix V). Regression residuals of the toe lengths and widths were highly correlated (Pearson's  $r > 0.7$ ) and therefore only the residuals of the length and width of the longest toe (T4 and TP4) plus the other 24 uncorrelated residual morphometric variables were included in the multivariate analysis of variance. MANOVA results indicated that the two candidate species differed only in size and not in any other residual variable (i.e. shape). The males from GAT are significantly larger than those from TUR (SVL mean  $\pm$  SD =  $23.9 \pm 0.4$  mm versus  $19.2 \pm 0.8$  mm;  $F = 285.8$ ,  $p < 0.001$ ). Females from the TUR phylogroup have sizes comparable to GAT males (Table 1).

**TABLE 1.** Descriptive statistics of the 31 morphometric variables measured (mm) on 104 specimens of phylogroup TUR (*Eleutherodactylus glamyrus*) and phylogroup GAT (*E. cattus* sp. nov.). For each variable the mean  $\pm$  SD and range (in parenthesis) are presented after grouping by sex and species (N= sample size), no female specimens were available from *E. cattus*. See Appendix I for a complete list of examined specimens.

Measurement	<i>E. glamyrus</i> (♀, N = 6)	<i>E. glamyrus</i> (♂, N = 87)	<i>E. cattus</i> sp. nov. (♂, N = 11*)
SVL	$24.6 \pm 1.8$ (22.4–27.2)	$19.2 \pm 0.8$ (17.4–21.3)	$23.9 \pm 0.4$ (23.1–24.7)
HW	$9.1 \pm 0.8$ (8.2–10.1)	$6.6 \pm 0.4$ (5.7–7.5)	$9.1 \pm 0.1$ (8.9–9.3)
HL	$9.1 \pm 0.9$ (8.1–10.2)	$7.1 \pm 0.3$ (6.3–7.9)	$9 \pm 0.2$ (8.5–9.3)
IN	$2.1 \pm 0.2$ (1.9–2.4)	$1.6 \pm 0.2$ (1.3–2)	$2 \pm 0.2$ (1.8–2.1)
EtN	$3 \pm 0.2$ (2.7–3.2)	$2.2 \pm 0.2$ (1.6–2.7)	$2.7 \pm 0.1$ (2.5–2.9)
IOD	$4.2 \pm 0.3$ (3.8–4.6)	$3.2 \pm 0.2$ (2.6–3.7)	$4.3 \pm 0.2$ (3.9–4.5)
EL	$2.9 \pm 0.3$ (2.6–3.3)	$2.5 \pm 0.2$ (2.1–3)	$3.2 \pm 0.1$ (3–3.4)
TyL	$1.4 \pm 0.1$ (1.2–1.6)	$0.9 \pm 0.1$ (0.6–1.3)	$1.1 \pm 0.1$ (1–1.2)
FAL	$6.1 \pm 0.7$ (5.3–7)	$4.6 \pm 0.3$ (3.6–5.2)	$5.6 \pm 0.2$ (5.2–5.9)
HaL	$1.9 \pm 0.3$ (1.6–2.3)	$1.3 \pm 0.1$ (1.1–1.7)	$1.6 \pm 0.1$ (1.5–1.8)
F1	$2.6 \pm 0.3$ (2.2–2.9)	$2 \pm 0.2$ (1.6–2.4)	$2.6 \pm 0.2$ (2.4–3)
F2	$3.1 \pm 0.3$ (2.7–3.5)	$2.4 \pm 0.2$ (2.1–2.9)	$2.9 \pm 0.1$ (2.7–3)
F3	$4.6 \pm 0.3$ (4.1–5.1)	$3.4 \pm 0.2$ (2.7–4)	$4.4 \pm 0.2$ (4.1–4.8)
F4	$2.9 \pm 0.4$ (2.4–3.3)	$2.2 \pm 0.2$ (1.8–2.8)	$2.8 \pm 0.1$ (2.6–3)
FP1	$0.7 \pm 0.1$ (0.6–0.8)	$0.5 \pm 0.1$ (0.3–0.6)	$0.6 \pm 0.1$ (0.5–0.7)
FP2	$0.9 \pm 0.1$ (0.8–1)	$0.6 \pm 0.1$ (0.4–0.8)	$0.7 \pm 0.1$ (0.6–0.8)
FP3	$1.1 \pm 0.1$ (1–1.3)	$0.7 \pm 0.1$ (0.5–0.9)	$0.9 \pm 0.1$ (0.8–1)
FP4	$1 \pm 0.1$ (0.8–1.2)	$0.7 \pm 0.1$ (0.4–0.8)	$0.9 \pm 0.1$ (0.6–1)
ThL	$11.1 \pm 0.9$ (9.8–12.4)	$8.6 \pm 0.5$ (7.5–9.7)	$10.9 \pm 0.3$ (10.2–11.4)
TL	$11.9 \pm 1$ (10.5–13.2)	$9.3 \pm 0.4$ (8.3–10.2)	$11.9 \pm 0.4$ (11.2–12.4)
FL	$7.4 \pm 0.6$ (6.7–8.1)	$5.8 \pm 0.3$ (5–6.4)	$7.1 \pm 0.3$ (6.8–7.6)
T1	$3.9 \pm 0.3$ (3.4–4.2)	$2.7 \pm 0.2$ (2.1–3.3)	$3.1 \pm 0.3$ (2.6–3.6)
T2	$4.5 \pm 0.6$ (3.6–5.2)	$3.3 \pm 0.2$ (2.8–3.8)	$3.8 \pm 0.3$ (3.2–4.2)
T3	$6.4 \pm 0.6$ (5.5–7.1)	$4.7 \pm 0.3$ (3.9–5.5)	$5.9 \pm 0.3$ (5.3–6.2)
T4	$9.8 \pm 0.9$ (8.6–10.7)	$7.4 \pm 0.5$ (6.1–8.7)	$9.2 \pm 0.4$ (8.7–10)
T5	$7.5 \pm 0.7$ (6.4–8.4)	$5.5 \pm 0.4$ (4.7–6.6)	$7.1 \pm 0.4$ (6.6–7.7)
TP1	$0.9 \pm 0.1$ (0.8–1)	$0.6 \pm 0.1$ (0.4–0.7)	$0.7 \pm 0.1$ (0.6–0.8)
TP2	$0.8 \pm 0.1$ (0.7–1)	$0.5 \pm 0.1$ (0.4–0.7)	$0.7 \pm 0.1$ (0.6–0.7)
TP3	$0.8 \pm 0.1$ (0.7–1)	$0.5 \pm 0.1$ (0.4–0.8)	$0.6 \pm 0.1$ (0.5–0.8)
TP4	$1.1 \pm 0.1$ (1–1.3)	$0.7 \pm 0.1$ (0.4–0.9)	$0.8 \pm 0.1$ (0.6–1)
TP5	$0.9 \pm 0.1$ (0.8–1)	$0.6 \pm 0.1$ (0.4–0.8)	$0.7 \pm 0.1$ (0.5–0.8)

\* Due to the lack of the fourth toe in one specimen of *E. cattus*, the descriptive statistics for TL4 and TP4 in this group had a sample size of 10.

**Bioacoustics.** We analyzed 855 advertisement calls from 74 individuals from four localities. Calls were all composed of a single sound unit with a narrow spectral bandwidth, a slightly increasing frequency modulation, and no secondary harmonics (Figure 3). Individual averages were grouped into candidate species according to their respective localities and genetic groups—63 males of phylogroup TUR (CUB, NVE, BNV) and 11 males from phylogroup GAT. Calls from individuals of phylogroup GAT were distinctly longer than those of phylogroup TUR showing no overlap in the ranges of call duration (TUR: 47.2–112 msec; GAT: 128.4–243.1 msec) (Figures 3 and 4). Average values for each of the measured call features are reported in Table 2.

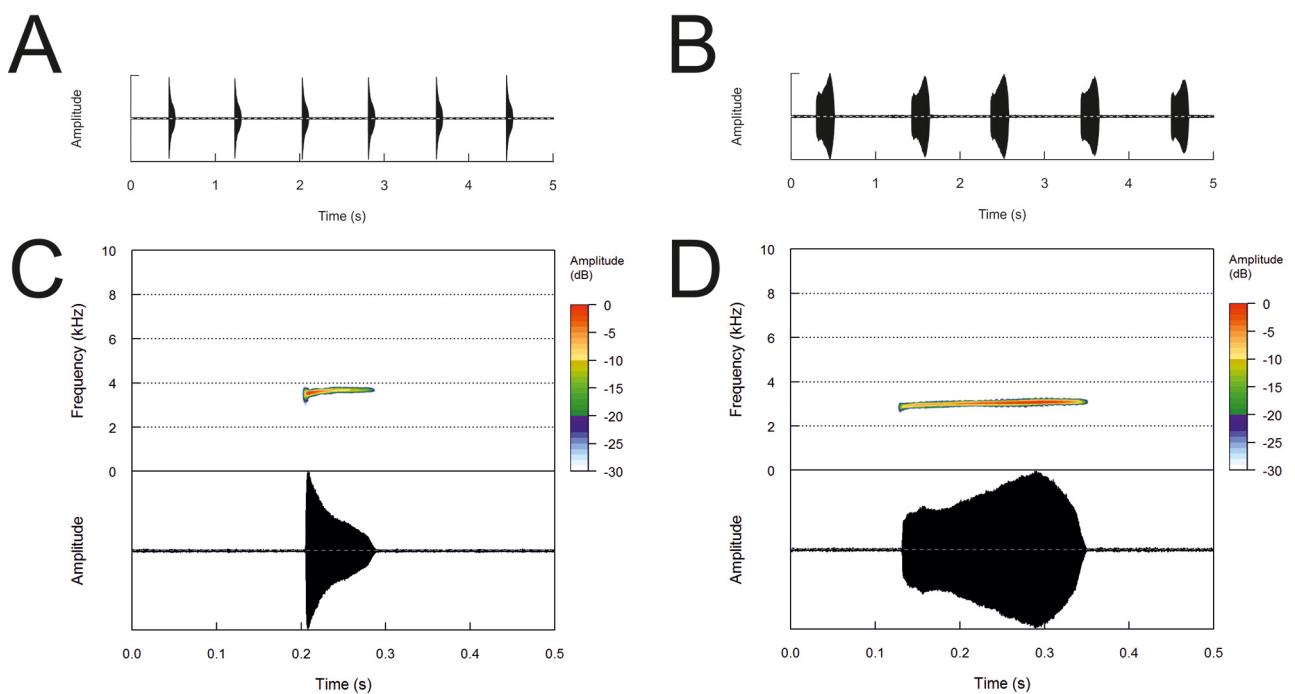
ANCOVA results indicated that, after controlling for the effects of caller size and air temperature, significant differences between the two phylogroups were observed in call duration, call rise time, dominant frequency and frequency modulation (Table 2, Figure 4). Only two call features, call rate and bandwidth, showed no statistical differences between phylogroups (Table 2). Statistically significant effects of SVL were observed in all call features, whereas temperature only showed statistically significant effects on call duration and bandwidth (Table 2). Acoustic features were largely uncorrelated among them (Pearson's  $r < 0.7$ ) except for call duration and call rise time ( $r = 0.93$ ).

**TABLE 2.** Descriptive statistics (mean  $\pm$  SD, minimum–maximum) and ANCOVA results for the seven bioacoustic characters measured in the calls of specimens corresponding to the mitochondrial phylogroups TUR and GAT. Bioacoustic characters abbreviated as: CR, call rate; CD, call duration; RT, call rise time; DF, dominant frequency; FM, frequency modulation; BW, -6dB bandwidth. The last three columns summarize the results of the ANCOVA, including the partial regression slopes test for each covariate: size (SVL), temperature (temp), and the main independent variable (phylogroup); statistically significant results ( $p < 0.05$ ) are highlighted in bold.

	Descriptive statistics		ANCOVA results		
	TUR (N=63)	GAT (N=11)	SVL	temp	phylogroup
CR (calls/min)	70.2 $\pm$ 16.3 (33–101.7)	55.8 $\pm$ 4.3 (46–60.6)	<b>F = 26.4</b> <b>(p &lt; 0.001)</b>	<b>F = 80.1</b> <b>(p &lt; 0.001)</b>	F = 0.01 (p = 0.954)
CD (ms)	80.9 $\pm$ 12.6 (47.2–112)	196 $\pm$ 37.1 (128.4–243.1)	<b>F = 228.3</b> <b>(p &lt; 0.001)</b>	F = 1.2 (p = 0.279)	<b>F = 79.3</b> <b>(p &lt; 0.001)</b>
RT (ms)	5.6 $\pm$ 5.4 (1.9–32.2)	116 $\pm$ 55.6 (16–173.7)	<b>F = 190.0</b> <b>(p &lt; 0.001)</b>	F = 0.8 (p = 0.357)	<b>F = 55.1</b> <b>(p &lt; 0.001)</b>
DF (Hz)	3367.5 $\pm$ 176 (3006–3799.6)	3147.4 $\pm$ 102.4 (3036.2–3337.6)	<b>F = 49.2</b> <b>(p &lt; 0.001)</b>	F = 2.4 (p = 0.127)	<b>F = 8.2</b> <b>(p &lt; 0.01)</b>
FM (Hz)	147.6 $\pm$ 41.8 (73.2–262.3)	247.8 $\pm$ 54.5 (140.9–332.8)	<b>F = 28.4</b> <b>(p &lt; 0.001)</b>	F = 1.0 (p = 0.311)	<b>F = 31.6</b> <b>(p &lt; 0.001)</b>
BW (Hz)	99.3 $\pm$ 8 (86.1–121.6)	88.9 $\pm$ 1.7 (87–93)	<b>F = 40.6</b> <b>(p &lt; 0.001)</b>	<b>F = 9.0</b> <b>(p &lt; 0.01)</b>	F = 2.5 (p = 0.120)

## Taxonomic conclusion

According to the concordant results of the genetic, bioacoustic, and morphological comparisons, the phylogroups TUR and GAT clearly represent independent evolutionary lineages. We consider their differentiation relevant enough to represent distinct species-level units, and taxonomic actions are therefore warranted. Based on geographic, acoustic, and morphologic data, the name *Eleutherodactylus glamyrus* Estrada and Hedges 1997, can be confidently assigned to specimens of the phylogroup TUR. Most of the localities herein listed as phylogroup TUR coincide with several of the paratypes examined in the description of *E. glamyrus* and, although no molecular or bioacoustic data are available from the holotype of *E. glamyrus* (MNHNCU 660), the type locality (Pino del Agua Arriba, Municipio Guisa, Granma Province, Cuba, 1200 m) is only 2–3 km to the northeast from locality NVE of the present study. Additionally, there is an overall match between the morphological and acoustic features employed in the original description of *Eleutherodactylus glamyrus* and those presented here for phylogroup TUR (which includes morphometric measurements from six of the paratypes of *E. glamyrus*). The phylogroup GAT, therefore, represents another species for which no name is currently available. Consequently, we herein provide its formal description.



**FIGURE 3.** Advertisement call differences between the two phylogroups of *Eleutherodactylus glamyruis* sensu lato. Oscillograms of a five seconds fragment from a call series of a male from the phylogroup TUR (A), recorded on June 22, 2005 at Pico Cuba, Santiago de Cuba, air temperature = 16.0 °C and that of a male from phylogroup GAT (B), recorded on 14 May, 2010 at Loma El Gato, Santiago de Cuba, air temperature = 19.2 °C. C) and D) spectrogram (top) and oscillogram (bottom) of a single call from an individual of phylogroup TUR and GAT respectively. Spectrogram parameters: FFT size = 512 points, overlap = 90%, window = Hanning.

#### *Eleutherodactylus cattus* sp. n.

(Fig. 5 A,B)

**Holotype.** CZACC14.14152, adult male collected while vocalizing in the trail to Pico El Gato, Sierra del Cobre, 20.01364 N, 76.04809 W, 844 m a.s.l, by A. Rodríguez and R. Alonso in May 2010.

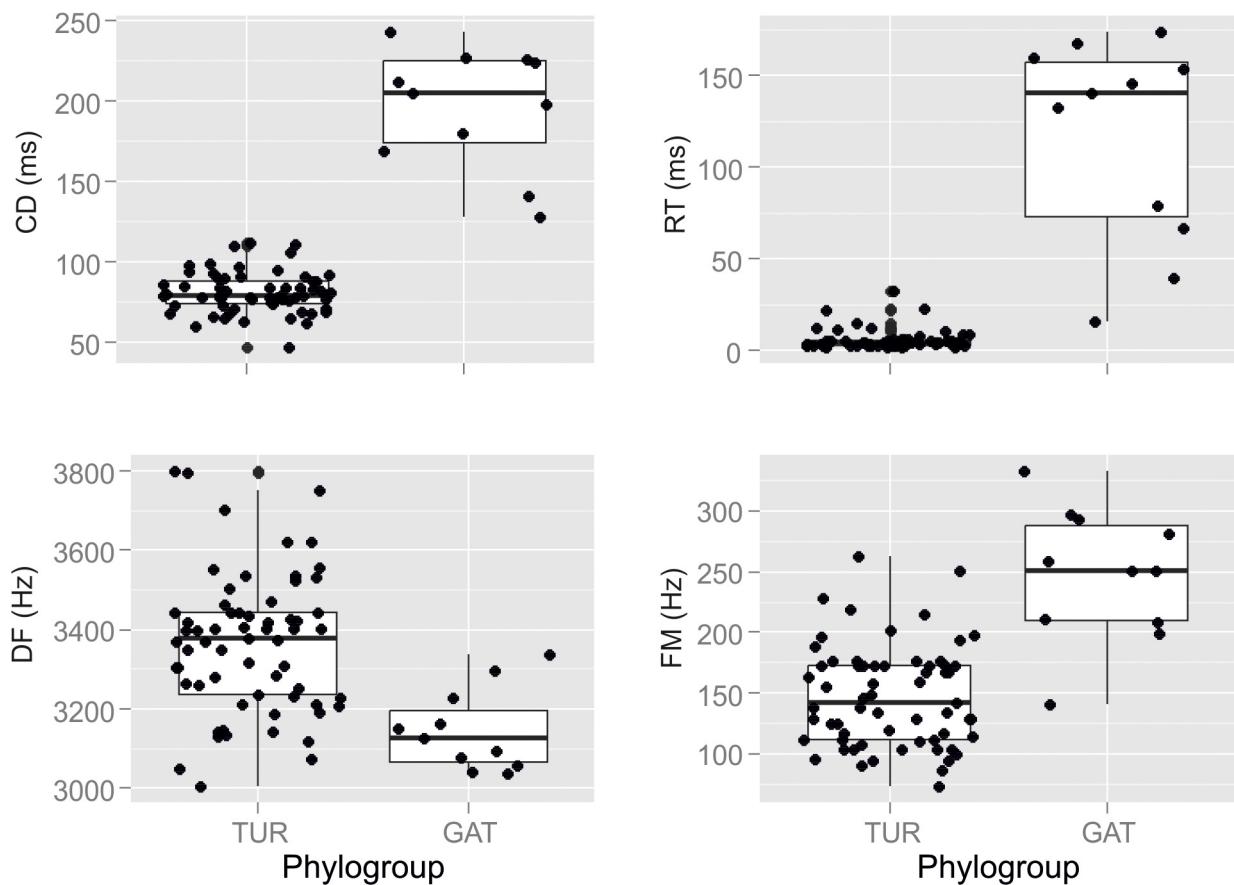
**Paratypes.** CZACC14.14150–51, 14.14153–60, adult males with the same data as the holotype.

**Etymology.** The species name is an invariable noun in apposition to the genus name, derived from Latin *cattus* = cat. It refers to the type locality Loma del Gato (Cat Mountain Ridge) in the Sierra Maestra Mountains, a locality that was neglected for long time in herpetological explorations of Cuba and that surely deserves further attention.

**Diagnosis.** A small species of *Eleutherodactylus* that can be assigned to the subgenus *Eleutherodactylus* based on its genetic, acoustic, and morphological similarities with members of the *E. auriculatus* species group (Hedges, *et al.*, 2008; Rodríguez, *et al.*, 2010b). It is most closely related to *E. glamyruis* with which it shares several morphological, ecological, and behavioral features. However, males of *Eleutherodactylus cattus* can be readily differentiated from *E. glamyruis* by their larger size (SVL = 23.1–24.7 mm in *E. cattus* vs 17.4–21.3 mm in *E. glamyruis*) and the following combination of advertisement call features: longer duration (call duration = 128.4–243.1 ms in *E. cattus* vs 47.2–112 ms in *E. glamyruis*), longer rise time (call rise time = 16–173.7 ms in *E. cattus* vs 1.9–32.2 ms in *E. glamyruis*), lower dominant frequency (dominant frequency = 3036.2–3337.6 Hz in *E. cattus* vs 3006–3799.6 Hz in *E. glamyruis*), and greater frequency modulation (frequency modulation = 140.9–332.8 Hz in *E. cattus* vs 73.2–262.3 Hz in *E. glamyruis*). Additionally, *E. cattus* differs from *E. glamyruis* in the studied mitochondrial DNA sequences by 8.2% (*cob*) and 3.2% (*16S*) and by one substitution in the nuclear *Rag-1* gene (see results).

**Description.** Head as wide as body, width smaller than length; snout subacute in dorsal and lateral views overhanging the jaw; narines laterally oriented and moderately protuberant; rostral canthus rounded and straight, loreal region smooth and abruptly tilted, lips not enlarged. Superior eyelids with conical and small tubercles also

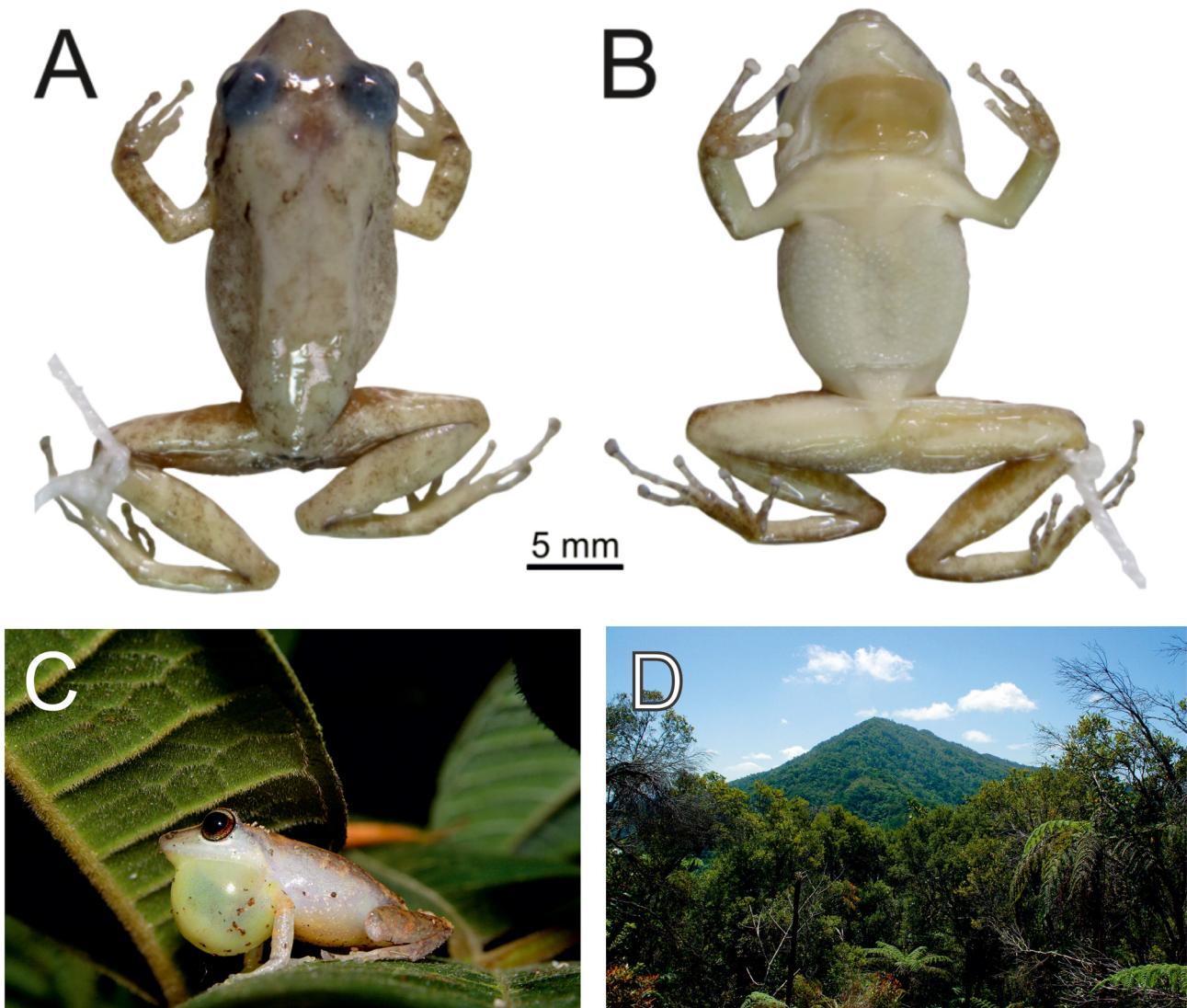
present but less evident in the interorbital area. Tympanum present without supratympanic fold, posttritral tubercles present. Coanae oval, partially concealed by the maxillary arch in ventral view. Vomerine odontophores short, straight, separated and nearly perpendicular to the longitudinal body axis. Tongue rounded, longer than wide with notched posterior edge, fixed by the anterior edge and free in its 3/4 of length. Vocal slits and enlarged vocal sac present in males.



**FIGURE 4.** Call features showing statistically significant differences between the two phylogroups (TUR, N=63) and (GAT, N=11). Box plots and original data points are shown for each acoustic feature abbreviated as: call duration (CD), call rise time (RT), dominant frequency (DF), and frequency modulation (FM).

Dorsal skin slightly tuberculated, without dorsolateral folds, and less tuberculated in the lower half of flanks. Ventral skin slightly areolated but without folds. Cloacae not expanded. Glandular regions not evident. Hands with ulnar tubercle subconical, palmar tubercle simple and of similar size as the thenar; thenar tubercle oval and pronounced. Supernumerary tubercles absent; fingers with rounded and subconical subarticular tubercles and without lateral expansions. Finger tips rounded and expanded in all cases with the ventral surface forming a circular digital pad bordered by a circumferential groove in 2/3 of its distal edge. Width of the largest toe pad (finger III) roughly similar to tympanum diameter. Finger order III > II > IV > I. One moderate tubercle present on heel, no tubercles on the outer edge of the tarsal, metatarsal tubercles subconical and smaller than subarticular tubercles; internal oval, same size as external; plantar supernumerary tubercles absent; subarticular tubercles rounded and subconical. Toes not webbed, toe tips rounded and expanded in all cases with the ventral surface forming a circular digital pad bordered by a circumferential groove in 2/3 of its distal edge. Heels overlap when thighs are placed perpendicularly to the longitudinal body axis. Toe size order IV > III > V > II > I.

**Color in alcohol.** Color pattern was similar among the individuals of the type series. Dorsum varies from brownish to beige with darker mottled and two mostly well-defined dark brownish or black anterior dorsolateral marks; the flanks show a similar pattern, lighter from dorsum to belly, a relatively wide dark brown strip from the insertion of the forelimb to the eye; belly pale and throat (vocal sac) from yellowish to light brown, underside of the limbs slightly yellowish (see Figure 5 A–B for dorsal and ventral views of the holotype).



**FIGURE 5.** A–B) Holotype of *Eleutherodactylus cattus* (CZACC14.14152) in dorsal and ventral views. C) Male (CZACC14.14153, paratype) calling in the trail to Pico El Gato, Sierra del Cobre, 844m a.s.l.. D) Habitat in the type locality, dominated by montane rainforest, Pico del Gato in the background.

**Color in life.** Dorsum color varies from brownish to greenish tan, with an interocular bar of darker brown color followed by an X shaped mark both darker than the background color. A distinctive pattern of chevron-like bands is evident in the sacral region extending to the hind legs. Eyelids with a greenish wash, pupils creamy to cookey colored with horizontal slit, iris black. The flanks are lighter colored than the dorsum, tympanum creamy white. Loreal region color varies from tan to greenish with an obvious black stripe that extends from snout to supratympanic fold and becomes progressively diffuse towards the insertion of the forelimb. Venter whitish to translucent, vocal sac yellow. A photograph of a living paratype is presented in figure 5 C.

**Measurements of the holotype (mm):** (see Methods for abbreviations) SVL 23.9; HW 9.0; HL 9.1; IN 2.1; EN 2.9; IO 4.2; EL 3.0; TyL 1.2; FaL 5.7; HaL 1.8; F1 2.7; F2 3.0; F3 4.6; F4 2.9; FP1 0.6; FP2 0.7; FP3 0.9; FP4 1.0; ThL 10.7; TL 11.6; FL 7.1; T1 3.6; T2 4.2; T3 6.1; T4 9.4; T5 7.3; TP1 0.7; TP2 0.6; TP3 0.6; TP4 0.7; TP5 0.6.

**Remarks.** Although the proposed diagnostic features allow a clear discrimination between adult males of *Eleutherodactylus cattus* and *E. glamyrus*, a straightforward classification of non-vocal juveniles and females is so far impossible without genetic analyses. It is likely that adult females of *E. cattus* oversize those of *E. glamyrus* but no female specimens are available for *E. cattus* and a proper test of this hypothesis will require the collection of a sufficient number of female specimens of both taxa. It can also be hypothesized that additional diagnostic features

for both taxa will be recovered after a detailed examination of osteological and soft tissue examinations are conducted.

**Distribution.** This species is only known from the type locality but assuming it has specialized to high elevations like its sister taxon, *Eleutherodactylus glamyrus*, it could well be found in neighboring areas above 800 m a.s.l..

**Natural history.** Field data indicate that *Eleutherodactylus cattus* is a nocturnal species that inhabits the montane rainforests and elfin woodlands above 800 m a.s.l., in areas of Loma del Gato-Monte Líbano Ecological Reserve, in the Sierra del Cobre massif, Santiago de Cuba province, in eastern Cuba. In this region, the mean monthly air temperature is 18.4 °C with minimal and maximum mean values around to 15.7 °C and 22.4 °C, respectively. Mean monthly relative humidity is high year round, and ranges between 87–92%, the mean annual precipitation is 1.220 mm, with May and October being the雨iest months (Potrony, *et al.*, 1994; Reyes, 1999). Males of *Eleutherodactylus cattus* were observed calling in the vegetation at  $1.16 \pm 0.47$ m (mean  $\pm$  SD; range: 0.50–2.20 m) above the ground in the understory (Figure 5 C). This forest stratum is dense and rich in shrubs and herbaceous plants, with ferns, liverworts, mosses and terrestrial orchids (Figure 5 D). Calling males were heard in three nearby localities in Loma El Gato, between 844–1070 m a.s.l (ascent trail on the northern slope of Pico El Gato, 844 m a.s.l; surroundings of Loma de la Cruz, 1070 m a.s.l.; and Loma de La Juana, 900 m a.s.l.). *E. cattus* showed an apparent acoustic activity peak at dusk (between 19:00–21:00 hrs) and shortly before dawn (5:00–6:30hrs). Calling males appear to show preference for exposed surfaces of leaves and ferns, but they can call at different orientations, facing down or horizontally.

At least another eight species of *Eleutherodactylus* are known to occur in sympatry with *E. cattus* in the surroundings of the type locality: *Eleutherodactylus atkinsi* Dunn, *E. auriculatus*, *E. cuneatus* (Cope), *E. dimidiatus* (Cope), *E. gundlachi* Schmidt, *E. ionthus* Schwartz, *E. limbatus* (Cope), *E. ricordii* (Duméril and Bibron), plus the hylid *Osteopilus septentrionalis* (Duméril and Bibron). Two species of the *Eleutherodactylus auriculatus* species group, *E. ionthus* and *E. auriculatus* (Hedges, *et al.*, 2008; Padial, *et al.*, 2014) were heard and observed vocalizing simultaneously with *E. cattus* at the type locality. The first species, *E. ionthus*, vocalizes from high perches (above three meters) on the arboreal stratum often on epiphytic plants (Bromeliaceae). This species produces a very different advertisement call in terms of temporal and spectral structure (Hedges *et al.*, 1992; Díaz and Cádiz, 2008). The second species, *E. auriculatus*, uses similar calling sites (substrates and heights) in the understory as *E. cattus*, but the acoustic features of its advertisement call are distinctly different (e.g. faster call rate, shorter call duration and higher dominant frequency), as already noted by Estrada and Hedges (1997).

## Discussion

Over the last two decades (1996–2016) a total of 20 new species of frogs of the genus *Eleutherodactylus* have been described (AmphibiaWeb, 2016). Although the genus is distributed throughout the West Indies, peninsular Florida and southern Texas (USA), Mexico, Belize, and Guatemala (Hedges, *et al.*, 2008), 15 of these newly-described species (75 %) have been discovered in Cuba. These figures account for the impressive faunal diversity of the largest Caribbean island but also for the importance of increasing field explorations and the application of new methodologies like DNA sequencing and bioacoustics in anuran systematics. Our results add to the growing list of endemic amphibian species and suggest that more taxa could be uncovered in remote montane areas of Cuba that remain unexplored by herpetologists.

In many cases cryptic species remain erroneously classified, hidden in collections under another species name (Bickford, *et al.*, 2006). In the case of *E. cattus* this period was relatively short; AR and RA collected the type series in 2010, and published their results as a new locality record for *E. glamyrus* shortly after (Rodríguez & Alonso, 2012). Six years after collection, we herein formally describe this population as a new species. Our results are based on data stemming from independent lines of evidence –mtDNA, nuDNA, morphology, bioacoustics, and geography—which concordantly suggest a cessation of gene flow, hence supporting the recognition of a species limit between *Eleutherodactylus glamyrus* and *E. cattus*. It must be acknowledged that the term cryptic species is broad and not all the cases labeled as cryptic discoveries are equivalent. Theoretically, the term could encompass a diversity of cases reflecting the extent of uncertainty faced by taxonomists, varying from extremely cryptic forms (only distinguishable by genetic methods) to those that are different in phenotype (such as morphology, behavior,

or ecology) but out of the resolution range of the methods applied. Examples of extreme cryptic taxa are rare and to some extent the number of cryptic taxa is a reflection of taxonomic effort applied in each group.

Species delimitation is a very active field of research and a plethora of methods have emerged for testing species hypotheses, based of different species criteria, which often result in highly different estimates of species numbers when applied to a given taxonomic group (Miralles & Vences, 2013). Although modern sequencing technologies now allow for an unprecedented high throughput capable of sequencing thousands of loci overnight, the costs of such applications remains relatively high and they are not broadly applied in taxonomy. Most of the time, taxonomists must rely in incomplete or suboptimal information on which to draw the limits between species and take taxonomic action (Katz, *et al.*, 2015). However, species limits for a given taxonomic group can be more robustly defined by combining different sources of evidence, and we argue that every effort should be made to include genetic information from both mitochondrial and nuclear loci together with morphological, ecological and/or behavioral data.

As pointed by Rodríguez & Alonso (2012) a large extension of areas of lower altitude (< 800 m a.s.l) exists in the region of the Sierra Maestra mountains between the easternmost known population of *E. glamyrus* (La Bayamesa) and the distribution of *E. cattus* in Sierra del Cobre constituting a likely barrier for gene flow between these two forms putatively adapted to high altitudes. Further insights on the evolution and biogeography of these sister taxa will require additional sampling in the Sierra Maestra mountains and especially in the intervening region. Interestingly, none of these species appears to occur in the nearby Sierra de La Gran Piedra, as suggested by the absence of collection records (Díaz & Cádiz, 2008; Estrada & Ruibal, 1999; Fong, 2000; Hedges, 1999) and our own (AR & RA) field data. The Sierra de la Gran Piedra reaches an altitude of 1225 m a.s.l and maintains ecologic and climatic conditions very similar to those observed the distribution ranges of both taxa on the Sierra Maestra mountains. The absence of these two species in the Gran Piedra mountains, but not of other members of the subgenus (*E. auriculatus* and *E. ronaldi*) and several other *Eleutherodactylus* species typical of the Sierra Maestra mountains, is therefore intriguing and requires further investigation.

An accurate assessment of the conservation status of *Eleutherodactylus cattus* sp nov. is not yet possible given the paucity of information. But given the imperative need to prioritize species in light of the ongoing biodiversity crisis, and following a conservative approach, the species should be categorized as vulnerable (VU) under criterion D2 of the IUCN standards. Criterion D2 applies to taxa with extremely reduced geographic distribution (typically less than 20 km<sup>2</sup>) or that exist at typically five or fewer locations, and where there is a plausible natural or anthropogenic threat (IUCN, 2013). The Loma del Gato region has been severely impacted by agricultural practices in the past decades and it is likely that these have reduced and continue reducing the quality and extension of the habitat of *E. cattus*, which added to the extremely small extent of occurrence sufficiently fulfills all three requirements for VU status. It is however premature to affirm that a population decline has occurred and additional data on the population trend, natural history, and current threats are urgently needed to formulate better informed conservation plans for this species and ensure its long-term survival.

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**APPENDIX I.** Specimens examined in morphological comparisons and genetic analyses. The following collection acronyms are used: AR (field numbers of A. Rodríguez), CZACC (zoological collection of the Institute of Ecology and Systematics, Havana, Cuba), and USNM (United States National Museum –now National Museum of Natural History, Smithsonian Institution, Washington DC, USA). Specimens also included in genetic (g) or acoustic (a) analyses are indicated within brackets.

*Eleutherodactylus glamyrus*: **Granma province**: USNM509043 (paratype), Minas de Frío, Sierra Maestra, Municipio Bartolomé Masó; USNM509044 (paratype), CZACC14.14271–73 [14271–73 (a), 14272–73 (g)], Aguada del Joaquín, Sierra Maestra, Municipio Bartolomé Masó; CZACC14.14368–73 [14368–73 (a, g)], Barrio Nuevo, Sierra Maestra, Municipio Buey Arriba; CZACC14.13061–63 [13061–63 (a, g)], CZACC14.14297–314 [14297–314 (a), 14298–300 (g), 14303–305 (g), 14307–309 (g)], CZACC14.14317–18 [a], CZACC14.14320–21[a], AR459 [a], AR473 [a], La Nueve, Sierra Maestra, Municipio Buey Arriba; CZACC14.14322–29 [a] Pico La Bayamesa, Municipio Buey Arriba; USNM 509052 (paratype) Pico Botella, Municipio Buey Arriba, Granma. **Santiago de Cuba province**: USNM509045–48 (paratypes), CZACC14.13059–60 [a, g], 14.14229–62 [14229 (a, g), 14230–62 (a)], Pico Cuba, Sierra Maestra, Municipio Guamá; CZACC14.14274, 14.14276, USNM509049–51 (paratypes), Pico Turquino, Sierra Maestra, Municipio Guamá; CZACC14.14228, Pico Suecia, Sierra Maestra, Municipio Guamá.

*Eleutherodactylus cattus*: **Santiago de Cuba province**: CZACC14.14150–60 [a, g], trail to Pico El Gato, Sierra del Cobre, Municipio Santiago de Cuba.

**APPENDIX II.** Locality data and GenBank sequence accession numbers for specimens used in the molecular genetic analyses, sequences obtained in this study are highlighted in bold. Coordinates expressed in decimal degrees, CZACC = Zoological collection from Instituto de Ecología y Sistemática, Havana; ARTS = field numbers of the first author.

Field nr.	Voucher	Locality code	Latitude	Longitude	16S	cob	Rag-1
ARTS1601	CZACC:14.14229	CUB	19.98644	-76.84181	FJ527386	<b>KY000767</b>	<b>KY000804</b>
ARTS1602	CZACC:14.13059	CUB	19.98644	-76.84181	FJ527387	<b>KY000768</b>	<b>KY000805</b>
ARTS1603	CZACC:14.13060	CUB	19.98644	-76.84181	FJ527388	<b>KY000769</b>	<b>KY000806</b>
ARTS1599	CZACC:14.14272	AJO	20.01453	-76.83975	FJ527389	<b>KY000765</b>	<b>KY000802</b>
ARTS1600	CZACC:14.14273	AJO	20.01453	-76.83975	FJ527390	<b>KY000766</b>	<b>KY000803</b>
ARTS364	CZACC:14.14368	BNV	20.02677	-76.69599	<b>KY000723</b>	<b>KY000757</b>	<b>KY000793</b>
ARTS365	CZACC:14.14369	BNV	20.02677	-76.69599	<b>KY000724</b>	<b>KY000758</b>	<b>KY000794</b>
ARTS366	CZACC:14.14370	BNV	20.02677	-76.69599	<b>KY000725</b>	<b>KY000759</b>	<b>KY000795</b>
ARTS367	CZACC:14.14371	BNV	20.02677	-76.69599	<b>KY000726</b>	<b>KY000760</b>	<b>KY000796</b>
ARTS368	CZACC:14.14372	BNV	20.02677	-76.69599	<b>KY000727</b>	<b>KY000761</b>	<b>KY000797</b>
ARTS369	CZACC:14.14373	BNV	20.02677	-76.69599	<b>KY000728</b>	<b>KY000762</b>	<b>KY000798</b>
ARTS1595	CZACC:14.13061	NVE	20.05501	-76.603643	FJ527391	<b>KY000763</b>	<b>KY000799</b>
ARTS1596	CZACC:14.13062	NVE	20.05501	-76.603643	FJ527392	GQ426500	<b>KY000800</b>
ARTS1597	CZACC:14.13063	NVE	20.05501	-76.603643	FJ527393	<b>KY000764</b>	<b>KY000801</b>
ARTS1840	CZACC:14.14299	NVE	20.05501	-76.603643	<b>KY000729</b>	<b>KY000770</b>	<b>KY000807</b>
ARTS1841	CZACC:14.14312	NVE	20.05501	-76.603643	<b>KY000730</b>	<b>KY000771</b>	<b>KY000808</b>
ARTS1842	CZACC:14.14309	NVE	20.05501	-76.603643	<b>KY000731</b>	<b>KY000772</b>	<b>KY000809</b>
ARTS1843	CZACC:14.14301	NVE	20.05501	-76.603643	<b>KY000732</b>	<b>KY000773</b>	<b>KY000810</b>
ARTS1844	CZACC:14.14303	NVE	20.05501	-76.603643	<b>KY000733</b>	<b>KY000774</b>	<b>KY000811</b>
ARTS1845	CZACC:14.14304	NVE	20.05501	-76.603643	<b>KY000734</b>	<b>KY000775</b>	<b>KY000812</b>
ARTS1847	CZACC:14.14313	NVE	20.05501	-76.603643	<b>KY000735</b>	<b>KY000776</b>	<b>KY000813</b>
ARTS1848	CZACC:14.14300	NVE	20.05501	-76.603643	<b>KY000736</b>	<b>KY000777</b>	<b>KY000814</b>
ARTS1849	CZACC:14.14298	NVE	20.05501	-76.603643	<b>KY000737</b>	<b>KY000778</b>	<b>KY000815</b>
ARTS1850	CZACC:14.14314	NVE	20.05501	-76.603643	<b>KY000738</b>	<b>KY000779</b>	<b>KY000816</b>
ARTS1851	CZACC:14.14317	NVE	20.05501	-76.603643	<b>KY000739</b>	<b>KY000780</b>	<b>KY000817</b>
ARTS1852	CZACC:14.14306	NVE	20.05501	-76.603643	<b>KY000740</b>	<b>KY000781</b>	<b>KY000818</b>
ARTS1853	CZACC:14.14308	NVE	20.05501	-76.603643	<b>KY000741</b>	<b>KY000782</b>	<b>KY000819</b>
ARTS1854	CZACC:14.14305	NVE	20.05501	-76.603643	<b>KY000742</b>	<b>KY000783</b>	
ARTS1855	CZACC:14.14307	NVE	20.05501	-76.603643	<b>KY000743</b>	<b>KY000784</b>	<b>KY000820</b>
ARTS1856	CZACC:14.14318	NVE	20.05501	-76.603643	<b>KY000744</b>	<b>KY000785</b>	<b>KY000821</b>
ARTS1857	CZACC:14.14320	NVE	20.05501	-76.603643	<b>KY000745</b>	<b>KY000786</b>	<b>KY000822</b>
ARTS132	CZACC:14.14159	GAT	20.01364	-76.04809	<b>KY000720</b>	<b>KY000754</b>	
ARTS133	CZACC:14.14158	GAT	20.01364	-76.04809	<b>KY000721</b>	<b>KY000755</b>	<b>KY000792</b>
ARTS134	CZACC:14.14160	GAT	20.01364	-76.04809	<b>KY000722</b>	<b>KY000756</b>	
ARTS77	CZACC:14.14150	GAT	20.01364	-76.04809	<b>KY000712</b>	<b>KY000746</b>	<b>KY000787</b>
ARTS79	CZACC:14.14153	GAT	20.01364	-76.04809	<b>KY000713</b>	<b>KY000747</b>	
ARTS80	CZACC:14.14155	GAT	20.01364	-76.04809	<b>KY000714</b>	<b>KY000748</b>	<b>KY000788</b>
ARTS81	CZACC:14.14157	GAT	20.01364	-76.04809	<b>KY000715</b>	<b>KY000749</b>	<b>KY000789</b>
ARTS82	CZACC:14.14151	GAT	20.01364	-76.04809	<b>KY000716</b>	<b>KY000750</b>	
ARTS83	CZACC:14.14156	GAT	20.01364	-76.04809	<b>KY000717</b>	<b>KY000751</b>	<b>KY000790</b>
ARTS84	CZACC:14.14152	GAT	20.01364	-76.04809	<b>KY000718</b>	<b>KY000752</b>	
ARTS93	CZACC:14.14154	GAT	20.01364	-76.04809	<b>KY000719</b>	<b>KY000753</b>	<b>KY000791</b>

770 **APPENDIX III.** Morphometric measurements (in mm) of the 104 specimens studied (see main text for a description of the morphometric variables).

Species	Catalog Nr.	Sex	SVL	HW	HL	IN	EN	IO	EL	TYL	FaL	HaL	F1	F2	F3	F4	FP1	FP2	FP3	FP4	ThL	TL	FL	T1	T2	T3	T4	T5	TPI	TP2	TP3	TP4	TP5
<i>E. cattus</i> sp. nov.	CZACC14.14151	M	23.7	9.1	8.9	1.8	2.7	4.4	3.2	1.2	5.7	1.5	2.6	2.8	4.2	2.8	0.7	0.7	1.0	0.9	10.9	11.8	6.8	2.7	3.6	5.8	8.8	7.0	0.8	0.7	0.8		
<i>E. cattus</i> sp. nov.	CZACC14.14152	M	23.9	9.0	9.1	2.1	2.9	4.2	3.0	1.2	5.7	1.8	2.7	3.0	4.6	2.9	0.6	0.7	0.9	1.0	10.7	11.6	7.1	3.6	4.2	6.1	9.4	7.3	0.7	0.6	0.7	0.6	
<i>E. cattus</i> sp. nov.	CZACC14.14153	M	24.7	9.2	9.3	2.2	2.9	4.2	3.3	1.2	5.9	1.6	2.4	2.7	4.5	2.7	0.5	0.6	0.8	0.7	11.3	12.2	7.2	3.1	3.9	6.2	10.0	7.7	0.6	0.7	0.6		
<i>E. cattus</i> sp. nov.	CZACC14.14154	M	24.2	9.1	9.3	2.1	2.8	4.5	3.0	1.1	5.9	1.6	2.6	2.9	4.3	2.9	0.7	0.8	0.9	1.0	11.4	12.4	7.6	3.1	4.1	6.1	9.0	7.4	0.7	0.7	0.7		
<i>E. cattus</i> sp. nov.	CZACC14.14155	M	23.7	9.3	9.3	2.0	2.8	4.2	3.3	1.1	5.9	1.6	2.7	2.8	4.4	2.8	0.7	0.7	1.0	0.9	10.7	11.7	7.2	3.3	3.7	5.8	9.0	6.8	0.6	0.6	0.7		
<i>E. cattus</i> sp. nov.	CZACC14.14156	M	23.5	8.9	8.9	1.8	2.7	4.3	3.2	1.0	5.4	1.6	2.5	3.0	4.4	2.8	0.6	0.8	0.9	0.6	10.9	11.7	6.9	3.5	3.7	6.0	9.0	7.3	0.7	0.7	0.7		
<i>E. cattus</i> sp. nov.	CZACC14.14157	M	23.1	9.0	8.5	1.8	2.6	4.2	3.1	1.1	5.4	1.5	2.6	2.8	4.2	2.7	0.5	0.7	0.8	0.8	10.6	11.2	6.8	2.8	3.9	5.7	NA	6.6	0.7	0.6	0.6		
<i>E. cattus</i> sp. nov.	CZACC14.14158	M	24.2	9.3	9.0	2.0	2.8	4.2	3.4	1.2	5.8	1.6	3.0	3.0	4.8	3.0	0.6	0.7	1.0	1.0	11.2	12.4	7.6	3.3	4.0	6.1	9.9	7.7	0.7	0.7	0.8		
<i>E. cattus</i> sp. nov.	CZACC14.14159	M	23.7	9.1	9.1	2.1	2.8	4.3	3.3	1.1	5.7	1.6	2.8	3.0	4.4	3.0	0.6	0.8	1.0	0.9	10.9	12.1	7.2	2.8	3.2	5.4	8.9	6.7	0.6	0.5	0.6		
<i>E. cattus</i> sp. nov.	CZACC14.14160	M	23.8	9.0	9.0	1.8	2.5	3.9	3.2	1.1	5.3	1.6	2.5	2.9	4.3	2.6	0.6	0.9	1.0	0.9	10.2	11.8	7.3	3.0	3.8	6.1	9.3	6.8	0.7	0.8	1.0		
<i>E. cattus</i> sp. nov.	CZACC14.14150	M	23.9	9.1	8.9	2.0	2.7	4.5	3.3	1.1	5.2	1.8	2.5	2.8	4.1	2.6	0.5	0.7	0.8	0.8	11.1	11.8	6.8	2.6	3.3	5.5	8.7	6.8	0.7	0.7	0.6		
<i>E. glamyrtus</i>	AR459	M	19.3	7.1	7.1	1.8	2.2	3.6	2.9	0.9	4.7	1.3	2.1	2.5	3.3	2.1	0.3	0.4	0.5	0.5	8.5	9.6	6.1	2.9	3.6	5.1	7.6	5.8	0.4	0.4	0.5		
<i>E. glamyrtus</i>	AR473	M	20.0	6.9	7.3	1.7	2.2	3.6	2.9	0.9	4.5	1.3	1.8	2.3	3.3	1.9	0.4	0.6	0.7	0.6	8.2	9.4	5.8	2.9	3.4	4.2	6.9	4.9	0.5	0.4	0.6		
<i>E. glamyrtus</i>	CZACC14.13059	M	19.3	6.6	7.1	1.4	2.0	3.4	2.3	0.8	4.5	1.4	2.0	2.3	3.5	2.1	0.5	0.6	0.7	0.6	9.1	10.2	5.8	2.5	3.1	4.7	7.4	5.6	0.6	0.5	0.6		
<i>E. glamyrtus</i>	CZACC14.13060	M	19.3	6.7	7.1	1.5	2.3	3.4	2.3	0.9	4.9	1.3	2.0	2.4	3.2	2.1	0.5	0.6	0.8	0.8	9.2	10.2	5.8	2.7	3.3	4.3	6.8	5.0	0.6	0.6	0.7		
<i>E. glamyrtus</i>	CZACC14.13061	M	19.2	6.1	6.8	1.5	2.1	3.0	2.5	0.7	4.6	1.2	1.9	2.2	3.0	1.8	0.5	0.6	0.7	0.6	8.9	9.4	5.7	2.6	2.8	4.3	7.0	5.4	0.7	0.6	0.7		
<i>E. glamyrtus</i>	CZACC14.13062	M	19.8	6.1	6.9	1.7	2.0	3.2	2.6	0.7	4.7	1.4	1.8	2.1	3.1	1.9	0.4	0.5	0.6	0.7	9.0	9.3	5.8	2.6	3.0	4.5	7.1	5.2	0.5	0.5	0.5		
<i>E. glamyrtus</i>	CZACC14.13063	M	20.3	7.2	7.2	1.5	2.2	3.3	2.7	1.1	4.8	1.4	2.0	2.2	3.5	2.2	0.6	0.6	0.7	0.7	8.7	9.9	6.1	2.7	3.1	4.0	6.8	5.4	0.5	0.4	0.5		
<i>E. glamyrtus</i>	CZACC14.14228	M	20.3	7.6	7.9	1.7	2.4	3.5	2.5	1.1	4.9	1.3	2.1	2.2	3.3	2.2	0.6	0.7	0.9	0.9	8.1	9.3	6.3	2.8	3.6	5.3	6.8	5.0	0.7	0.6	0.7		
<i>E. glamyrtus</i>	CZACC14.14229	M	18.3	6.3	6.9	1.4	2.1	2.9	2.3	0.8	4.4	1.2	1.9	2.3	3.3	2.0	0.5	0.6	0.7	0.6	8.1	9.3	5.5	2.5	2.9	4.3	6.8	5.3	0.5	0.5	0.6		
<i>E. glamyrtus</i>	CZACC14.14230	M	19.0	6.6	7.2	1.5	2.0	3.3	2.4	0.9	4.4	1.4	2.1	2.7	3.9	2.5	0.5	0.7	0.8	0.8	8.1	9.0	5.9	2.8	3.5	4.9	7.8	5.8	0.7	0.6	0.6		
<i>E. glamyrtus</i>	CZACC14.14231	M	19.2	6.4	7.0	1.3	2.4	2.9	2.6	1.0	4.7	1.4	1.8	2.2	3.6	2.1	0.5	0.6	0.8	0.7	9.0	9.6	6.0	2.5	3.1	4.5	7.4	5.5	0.6	0.6	0.7		
<i>E. glamyrtus</i>	CZACC14.14232	M	19.2	6.6	7.4	1.5	2.4	3.4	2.3	0.8	4.9	1.5	2.3	2.6	3.8	2.3	0.6	0.6	0.8	0.8	9.1	9.7	6.2	2.6	3.4	5.0	8.0	5.9	0.7	0.6	0.7		
<i>E. glamyrtus</i>	CZACC14.14233	M	19.1	6.5	6.9	1.5	2.1	3.3	2.3	0.9	4.7	1.4	2.1	2.6	3.9	2.4	0.5	0.6	0.8	0.7	9.1	9.4	5.8	2.9	3.5	5.0	8.0	5.8	0.7	0.6	0.7		
<i>E. glamyrtus</i>	CZACC14.14234	M	18.7	6.5	7.3	1.6	2.1	3.5	2.6	1.0	4.4	1.4	2.1	2.5	3.5	2.3	0.5	0.7	0.9	0.8	8.9	9.2	5.9	2.6	3.3	4.9	7.8	6.0	0.7	0.6	0.8		
<i>E. glamyrtus</i>	CZACC14.14235	M	19.6	6.7	7.3	1.5	2.1	3.4	2.5	1.0	5.1	1.3	2.1	2.6	3.8	2.5	0.6	0.7	0.8	0.8	9.6	10.2	6.2	2.6	3.5	5.1	8.3	6.2	0.7	0.6	0.7		
<i>E. glamyrtus</i>	CZACC14.14236	M	17.5	6.2	6.7	1.6	2.2	2.9	2.2	0.9	4.0	1.3	1.8	2.1	3.3	2.1	0.5	0.6	0.8	0.7	8.3	8.9	5.7	2.6	3.2	4.2	7.0	5.4	0.5	0.6	0.8		
<i>E. glamyrtus</i>	CZACC14.14237	M	19.0	6.5	7.1	1.5	1.9	3.4	2.3	1.0	4.6	1.2	1.9	2.2	3.2	2.1	0.5	0.6	0.8	0.8	7.8	8.7	5.6	2.6	3.0	4.2	6.7	5.1	0.6	0.6	0.8		
<i>E. glamyrtus</i>	CZACC14.14238	M	17.4	6.5	6.9	1.7	2.0	3.4	2.2	0.9	4.5	1.2	1.7	2.1	3.3	2.1	0.4	0.5	0.7	0.7	9.0	9.5	5.1	2.5	3.1	4.3	6.9	5.0	0.5	0.5	0.6		
<i>E. glamyrtus</i>	CZACC14.14239	M	17.8	6.1	6.4	1.4	2.0	3.2	2.2	0.8	4.3	1.3	1.9	2.3	3.4	2.1	0.4	0.6	0.7	0.7	8.1	8.8	5.6	2.4	3.0	3.9	6.6	5.0	0.5	0.5	0.7		
<i>E. glamyrtus</i>	CZACC14.14240	M	18.9	5.9	6.8	1.5	1.9	3.0	2.5	0.8	4.5	1.3	2.1	2.4	3.6	2.3	0.5	0.6	0.8	0.7	8.1	9.0	5.8	2.1	3.0	4.5	6.9	5.2	0.6	0.6	0.7		
<i>E. glamyrtus</i>	CZACC14.14241	M	18.3	6.1	6.6	1.5	2.0	2.8	2.6	0.8	4.2	1.2	1.8	2.2	3.4	2.0	0.5	0.6	0.6	0.6	8.1	8.6	5.5	2.4	2.9	4.0	6.5	4.7	0.5	0.4	0.6		
<i>E. glamyrtus</i>	CZACC14.14242	M	19.8	6.5	7.8	1.6	1.9	3.3	2.7	0.9	4.7	1.5	2.2	2.3	3.6	2.2	0.6	0.6	0.7	0.7	8.0	9.6	5.8	2.5	3.3	4.6	7.3	5.5	0.6	0.5	0.7		
<i>E. glamyrtus</i>	CZACC14.14243	M	18.0	6.4	6.5	1.3	1.9	2.9	2.5	0.8	4.5	1.4	1.9	2.3	3.2	2.1	0.5	0.6	0.6	0.6	8.8	8.9	5.8	2.5	3.1	4.5	7.2	5.6	0.6	0.5	0.5		
<i>E. glamyrtus</i>	CZACC14.14244	M	19.6	6.6	6.9	1.7	2.0	3.3	2.6	0.8	4.5	1.2	2.2	2.3	3.4	2.1	0.5	0.6	0.7	0.7	8.4	9.3	5.8	2.8	3.3	4.6	7.0	5.4	0.5	0.4	0.5		

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## APPENDIX III. (Continued)

Species	Catalog Nr.	Sex	SVL	HW	HL	IN	EN	IO	EL	TyL	FaL	HaL	F1	F2	F3	F4	F5	FP1	FP2	FP3	FP4	ThL	TL	FL	T1	T2	T3	T4	T5	TP1	TP2	TP3	TP4	TP5
<i>E. glamyris</i>	CZACCI14.14245	M	20.0	6.6	7.3	1.9	2.2	3.5	2.7	1.0	5.1	1.4	2.2	2.6	3.8	2.3	0.6	0.7	0.8	0.8	9.4	9.4	6.0	2.9	3.6	4.8	7.3	5.3	0.6	0.6	0.7	0.6		
<i>E. glamyris</i>	CZACCI14.14246	M	17.8	6.0	6.8	1.4	1.9	3.0	2.3	0.9	4.4	1.3	2.2	2.5	3.4	2.1	0.5	0.5	0.7	0.7	8.0	8.7	5.5	2.4	2.9	4.2	6.1	4.9	0.6	0.5	0.5	0.5		
<i>E. glamyris</i>	CZACCI14.14247	M	18.0	6.2	6.7	1.6	1.7	2.8	2.3	0.8	4.2	1.2	1.7	2.1	3.2	2.0	0.5	0.6	0.8	0.8	7.5	8.3	5.4	2.5	3.1	4.1	6.6	5.0	0.6	0.5	0.5	0.5		
<i>E. glamyris</i>	CZACCI14.14248	M	18.7	6.3	7.0	1.7	2.1	3.3	2.6	0.8	4.3	1.3	1.9	2.3	3.4	2.2	0.6	0.7	0.6	0.6	8.2	8.9	5.8	2.6	3.3	4.8	7.8	5.7	0.6	0.6	0.7	0.6		
<i>E. glamyris</i>	CZACCI14.14249	M	20.2	7.0	7.4	1.8	2.2	3.6	2.6	1.0	4.8	1.5	2.1	2.5	3.2	2.3	0.6	0.8	0.8	0.8	9.2	9.7	6.1	2.9	3.6	5.1	7.8	5.6	0.7	0.8	0.9	0.8		
<i>E. glamyris</i>	CZACCI14.14250	M	18.3	6.5	6.9	1.6	1.9	3.0	2.8	0.7	3.7	1.2	2.2	2.4	3.6	2.3	0.5	0.6	0.7	0.7	7.9	8.9	5.5	2.4	3.2	4.5	7.1	5.3	0.5	0.5	0.5	0.5		
<i>E. glamyris</i>	CZACCI14.14251	M	18.8	6.7	7.2	1.6	2.2	3.3	2.6	1.1	4.4	1.3	2.2	2.4	3.3	2.3	0.6	0.7	0.9	0.8	8.5	9.2	5.6	2.9	3.6	4.9	7.6	5.7	0.7	0.7	0.7	0.7		
<i>E. glamyris</i>	CZACCI14.14252	M	17.5	5.7	6.4	1.5	1.9	2.7	2.3	0.8	4.2	1.3	2.1	2.2	3.3	2.1	0.5	0.6	0.8	0.7	8.0	8.4	5.0	2.5	3.0	4.4	6.9	5.3	0.6	0.6	0.7	0.7		
<i>E. glamyris</i>	CZACCI14.14253	M	18.2	6.2	6.7	1.5	2.1	2.9	2.4	0.9	4.3	1.4	2.1	2.3	2.7	2.2	0.5	0.7	0.7	0.7	8.2	9.0	5.6	2.9	3.7	4.8	7.3	5.5	0.7	0.6	0.5	0.5		
<i>E. glamyris</i>	CZACCI14.14254	M	18.1	5.9	6.8	1.7	1.9	3.3	2.6	1.0	4.3	1.2	1.9	2.4	3.2	2.2	0.5	0.6	0.6	0.6	7.5	8.5	5.4	2.7	3.3	4.6	7.2	5.3	0.5	0.5	0.5	0.4		
<i>E. glamyris</i>	CZACCI14.14255	M	18.7	5.9	6.8	1.6	2.0	3.1	2.8	1.0	4.0	1.3	2.2	2.4	3.4	2.2	0.6	0.6	0.8	0.7	7.5	8.6	5.6	2.6	3.1	4.5	7.1	5.2	0.6	0.5	0.5	0.5		
<i>E. glamyris</i>	CZACCI14.14256	M	18.3	6.1	6.8	1.5	2.1	3.0	2.4	0.9	4.2	1.2	1.8	2.3	3.3	2.1	0.4	0.6	0.6	0.7	8.3	8.7	5.4	2.4	3.2	4.5	6.8	4.9	0.5	0.5	0.5	0.5		
<i>E. glamyris</i>	CZACCI14.14257	M	19.1	6.2	6.9	1.6	2.1	3.2	2.4	1.0	4.7	1.4	1.9	2.4	3.4	2.1	0.6	0.8	0.7	0.9	9.3	9.6	6.0	2.9	3.3	4.9	7.6	5.9	0.7	0.6	0.8	0.6		
<i>E. glamyris</i>	CZACCI14.14258	M	20.0	6.2	7.6	1.7	2.2	3.2	2.8	0.9	4.6	1.3	2.1	2.5	3.6	2.2	0.6	0.7	0.7	0.7	8.8	9.6	6.0	2.9	3.5	4.5	7.7	5.6	0.6	0.6	0.6	0.6		
<i>E. glamyris</i>	CZACCI14.14259	M	18.4	5.7	6.9	1.6	1.9	2.6	2.4	0.8	4.2	1.2	1.9	2.3	3.3	2.0	0.5	0.5	0.6	0.6	8.1	8.9	5.4	2.4	2.9	4.2	6.7	5.3	0.5	0.5	0.5	0.5		
<i>E. glamyris</i>	CZACCI14.14260	M	19.3	6.4	6.9	1.6	1.9	3.2	2.3	0.8	4.1	1.4	1.7	2.3	3.1	2.0	0.5	0.6	0.7	0.8	8.4	8.8	5.6	2.8	3.4	4.4	6.9	5.4	0.6	0.6	0.6	0.5		
<i>E. glamyris</i>	CZACCI14.14261	M	19.8	6.4	7.2	1.8	2.1	3.3	2.7	1.0	4.8	1.4	2.1	2.6	3.6	2.3	0.6	0.7	0.9	0.8	8.6	9.0	5.7	3.2	3.3	4.8	7.8	5.7	0.6	0.5	0.5	0.6		
<i>E. glamyris</i>	CZACCI14.14262	M	18.4	5.9	6.6	1.3	1.8	3.0	2.4	0.8	4.3	1.3	2.0	2.1	3.3	2.0	0.5	0.6	0.7	0.7	8.1	8.8	5.3	2.8	3.1	4.2	6.4	4.7	0.6	0.5	0.5	0.5		
<i>E. glamyris</i>	CZACCI14.14271	M	19.3	6.9	7.5	1.8	2.2	3.3	2.7	0.9	5.2	1.5	2.1	2.4	3.7	2.3	0.5	0.5	0.7	0.7	9.2	9.5	5.9	2.7	3.4	4.9	7.7	5.8	0.6	0.5	0.5	0.7		
<i>E. glamyris</i>	CZACCI14.14272	M	19.3	6.5	7.1	1.7	2.2	3.1	2.5	0.8	4.7	1.2	2.1	2.5	3.5	2.3	0.5	0.6	0.7	0.8	8.0	9.2	6.0	2.6	3.3	4.8	7.8	5.7	0.6	0.5	0.5	0.6		
<i>E. glamyris</i>	CZACCI14.14273	M	18.9	6.4	7.2	1.6	1.9	2.9	2.6	0.8	4.4	1.3	2.0	2.4	3.6	1.9	0.5	0.5	0.7	0.7	8.0	8.9	5.5	2.4	3.1	4.0	6.9	4.9	0.5	0.5	0.5	0.6		
<i>E. glamyris</i>	CZACCI14.14274	M	17.9	6.3	6.9	1.7	2.1	3.5	2.3	0.8	4.3	1.4	2.2	2.6	3.3	2.1	0.6	0.7	0.9	0.7	8.4	9.5	5.8	3.1	3.5	4.7	7.3	5.5	0.7	0.6	0.9	0.7		
<i>E. glamyris</i>	CZACCI14.14275	F	24.9	9.6	9.5	2.1	2.8	4.5	3.3	1.3	5.6	1.7	2.9	3.3	4.8	2.7	0.7	0.9	1.0	1.0	10.8	11.5	7.3	3.8	4.3	6.5	9.8	7.6	0.9	0.8	1.0	0.9		
<i>E. glamyris</i>	CZACCI14.14276	M	19.1	6.7	7.3	1.7	2.4	3.3	2.7	0.9	5.0	1.4	2.3	2.4	3.5	2.3	0.6	0.6	0.7	0.7	9.7	9.6	5.7	2.8	3.2	4.6	7.0	5.5	0.5	0.5	0.5	0.6		
<i>E. glamyris</i>	CZACCI14.14297	M	19.8	7.1	7.5	1.6	2.4	3.1	2.7	0.9	4.7	1.4	2.3	2.6	3.6	2.4	0.5	0.6	0.7	0.6	8.3	9.7	6.1	2.9	3.7	4.8	7.7	5.9	0.5	0.5	0.5	0.5		
<i>E. glamyris</i>	CZACCI14.14298	M	18.2	6.5	6.8	1.7	2.2	3.1	2.7	0.9	4.6	1.3	1.8	2.3	3.1	1.9	0.5	0.6	0.6	0.6	8.4	9.1	5.9	2.8	3.5	4.7	7.2	5.5	0.5	0.5	0.6	0.4		
<i>E. glamyris</i>	CZACCI14.14299	M	20.2	6.9	7.5	1.3	2.3	3.1	2.4	0.9	5.0	1.5	2.0	2.5	3.5	2.1	0.5	0.6	0.7	0.7	8.4	10.0	6.4	2.4	3.6	5.0	7.9	6.1	0.5	0.4	0.7	0.6		
<i>E. glamyris</i>	CZACCI14.14300	M	20.3	7.0	7.3	1.6	2.2	3.1	2.5	0.9	4.9	1.3	2.1	2.5	3.5	2.2	0.5	0.6	0.7	0.8	8.9	10.0	5.9	2.5	3.1	5.1	7.6	5.5	0.6	0.5	0.5	0.5		
<i>E. glamyris</i>	CZACCI14.14301	M	19.0	7.2	7.4	1.6	2.2	3.2	2.6	1.0	4.6	1.4	2.1	2.4	3.8	2.1	0.5	0.6	0.7	0.7	9.2	9.7	6.2	2.5	3.2	4.8	7.4	5.5	0.4	0.4	0.4	0.4		
<i>E. glamyris</i>	CZACCI14.14302	M	19.1	6.7	7.3	1.4	2.4	3.3	3.0	0.9	4.7	1.4	2.4	2.4	3.3	2.4	0.4	0.6	0.6	0.6	9.2	9.7	5.8	2.7	3.3	4.7	7.2	5.5	0.6	0.5	0.6	0.4		
<i>E. glamyris</i>	CZACCI14.14303	M	20.0	6.9	7.0	1.6	2.5	3.1	2.7	0.9	5.0	1.3	2.0	2.6	3.7	2.3	0.5	0.6	0.9	0.8	8.5	10.0	6.0	2.7	3.4	4.4	7.5	5.6	0.5	0.6	0.9	0.6		
<i>E. glamyris</i>	CZACCI14.14305	M	19.8	7.5	7.5	1.7	2.5	3.4	2.6	1.0	4.9	1.4	1.9	2.4	3.4	2.2	0.4	0.6	0.6	0.6	8.9	9.9	6.0	2.4	3.3	4.9	8.0	6.1	0.5	0.6	0.4	0.5		
<i>E. glamyris</i>	CZACCI14.14306	M	20.4	6.9	7.6	1.5	2.3	3.3	2.5	0.9	4.8	1.4	2.3	2.8	4.0	2.3	0.5	0.6	0.9	0.8	8.9	9.6	5.9	2.6	3.5	4.9	8.0	6.1	0.7	0.6	0.8	0.6		
<i>E. glamyris</i>	CZACCI14.14307	M	19.9	6.8	7.7	1.7	2.5	3.3	2.2	0.9	4.8	1.4	2.1	2.4	3.5	2.2	0.6	0.5	0.8	0.6	9.2	9.6	6.2	2.9	3.5	5.0	8.0	6.2	0.7	0.6	0.7	0.6		
<i>E. glamyris</i>	CZACCI14.14308	M	18.5	6.4	7.2	1.4	2.4	3.2	2.6	0.8	4.4	1.3	2.1	2.1	3.4	2.0	0.5	0.6	0.6	0.8	8.7	9.3	5.7	2.6	3.1	4.2	7.2	5.2	0.5	0.5	0.7	0.6		

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## APPENDIX III. (Continued)

Species	Catalog Nr.	Sex	SVL	HW	HL	IN	EN	IO	EL	Tyl	Fal	Hal	F1	F2	F3	F4	FP1	FP2	FP3	FP4	Thl	TL	FL	T1	T2	T3	T4	T5	TP1	TP2	TP3	TP4	TP5
<i>E. glamydus</i>	CZACCC14.14309	M	19.6	7.0	7.6	1.6	3.1	2.7	1.0	4.6	1.4	2.1	2.4	3.6	2.3	0.4	0.5	0.7	0.6	8.9	9.4	6.1	3.1	3.5	5.0	7.8	5.9	0.6	0.5	0.5	0.6	0.5	
<i>E. glamydus</i>	CZACCC14.14310	M	19.1	6.6	7.3	1.4	2.5	3.1	2.5	0.8	5.0	1.4	1.9	2.4	3.4	1.9	0.5	0.6	0.6	0.7	8.5	9.3	5.7	2.4	3.4	4.7	7.3	5.6	0.5	0.5	0.5	0.6	0.5
<i>E. glamydus</i>	CZACCC14.14311	M	21.3	7.0	7.3	1.7	2.2	3.6	2.8	0.9	4.7	1.5	2.1	2.4	3.6	2.1	0.6	0.6	0.7	0.7	9.0	9.7	5.9	2.9	3.4	5.0	8.0	6.1	0.6	0.5	0.5	0.6	0.5
<i>E. glamydus</i>	CZACCC14.14312	M	20.7	6.4	7.4	1.4	2.4	3.2	2.4	0.8	4.9	1.2	2.2	2.4	3.5	2.3	0.6	0.6	0.7	0.7	9.1	9.6	6.1	2.5	3.1	4.7	7.2	5.6	0.6	0.6	0.6	0.7	0.6
<i>E. glamydus</i>	CZACCC14.14313	M	20.1	7.0	7.6	1.7	2.3	3.4	2.6	0.9	5.0	1.5	2.2	2.9	3.9	2.9	0.5	0.7	0.8	0.7	9.4	10.1	6.4	3.2	3.6	5.5	8.7	6.6	0.6	0.6	0.6	0.7	0.5
<i>E. glamydus</i>	CZACCC14.14314	M	19.8	6.7	7.2	1.4	2.1	3.2	2.5	0.9	4.6	1.4	2.0	2.2	3.5	1.9	0.5	0.6	0.7	0.7	8.7	9.1	5.9	2.5	3.4	5.1	7.6	5.8	0.7	0.6	0.6	0.7	0.5
<i>E. glamydus</i>	CZACCC14.14317	M	20.9	7.2	7.5	1.8	2.3	3.7	2.5	0.7	5.2	1.4	2.0	2.6	3.4	2.2	0.5	0.6	0.5	0.5	9.4	9.7	5.9	2.6	3.5	4.4	7.9	5.7	0.5	0.5	0.5	0.5	0.5
<i>E. glamydus</i>	CZACCC14.14318	M	20.2	6.6	7.1	1.5	2.3	3.3	2.5	0.9	4.6	1.7	2.2	2.3	3.6	2.4	0.6	0.7	0.8	0.8	9.5	9.4	6.0	2.5	3.1	5.0	8.1	6.1	0.7	0.6	0.7	0.8	0.6
<i>E. glamydus</i>	CZACCC14.14320	M	19.5	7.0	7.4	1.8	2.0	3.3	2.3	0.8	4.5	1.4	2.1	2.3	3.4	2.2	0.5	0.6	0.7	0.7	9.0	9.4	5.8	2.7	3.1	4.4	7.8	5.9	0.5	0.5	0.5	0.6	0.5
<i>E. glamydus</i>	CZACCC14.14321	M	19.4	7.2	7.3	1.6	2.1	3.4	2.9	1.0	4.6	1.2	2.0	2.1	3.2	2.0	0.5	0.5	0.8	0.7	8.8	9.5	5.7	2.4	3.3	4.8	7.1	5.4	0.6	0.6	0.6	0.7	0.7
<i>E. glamydus</i>	CZACCC14.14322	M	19.2	6.4	7.0	1.4	2.0	3.2	2.4	0.8	4.5	1.2	2.1	2.3	3.3	2.1	0.6	0.6	0.7	0.6	8.6	8.9	6.1	2.6	3.3	4.8	7.4	5.7	0.7	0.6	0.6	0.7	0.6
<i>E. glamydus</i>	CZACCC14.14323	M	19.3	6.9	7.2	1.5	2.3	3.4	2.7	0.9	4.7	1.4	2.2	2.5	3.5	2.3	0.4	0.5	0.5	0.5	7.8	9.4	5.7	2.6	3.3	5.0	7.9	6.2	0.4	0.5	0.4	0.5	0.5
<i>E. glamydus</i>	CZACCC14.14324	M	19.9	6.6	7.5	1.4	2.3	3.3	2.8	1.0	4.2	1.4	2.1	2.4	3.5	2.2	0.6	0.7	0.9	0.8	8.1	8.9	5.7	2.8	3.3	4.7	7.4	5.7	0.6	0.6	0.6	0.8	0.8
<i>E. glamydus</i>	CZACCC14.14325	M	18.5	6.7	7.3	1.3	2.0	3.2	2.5	0.9	4.7	1.2	1.8	2.6	3.6	2.3	0.5	0.7	0.9	0.8	8.2	9.2	5.8	2.8	3.3	4.8	7.5	5.4	0.5	0.5	0.5	0.7	0.7
<i>E. glamydus</i>	CZACCC14.14326	M	20.2	7.1	7.3	1.8	2.2	3.6	2.9	0.9	4.9	1.4	2.0	2.3	3.4	2.0	0.6	0.6	0.8	0.8	8.6	9.2	5.6	2.6	3.4	5.0	8.0	6.0	0.7	0.5	0.6	0.8	0.8
<i>E. glamydus</i>	CZACCC14.14327	M	19.6	6.5	7.2	1.5	2.2	3.0	2.5	0.9	4.7	1.3	2.2	2.5	3.8	2.3	0.5	0.6	0.7	0.6	7.8	9.7	5.9	2.6	3.3	4.9	7.9	5.8	0.6	0.5	0.6	0.7	0.7
<i>E. glamydus</i>	CZACCC14.14328	M	20.1	7.0	7.5	1.6	2.3	3.4	2.6	1.1	4.9	1.2	2.2	2.4	3.4	2.2	0.5	0.6	0.6	0.6	8.3	9.4	5.9	2.8	3.3	4.8	7.8	5.6	0.7	0.5	0.5	0.7	0.6
<i>E. glamydus</i>	CZACCC14.14368	M	18.9	6.7	6.9	1.6	2.3	3.2	2.4	0.8	4.3	1.3	2.0	2.2	3.2	2.1	0.4	0.6	0.7	0.7	8.4	9.2	5.5	2.6	3.1	4.3	6.8	5.2	0.6	0.5	0.5	0.8	0.6
<i>E. glamydus</i>	CZACCC14.14369	M	19.7	6.7	6.8	1.5	2.1	3.1	2.7	0.8	4.6	1.4	2.1	2.3	3.3	2.2	0.5	0.5	0.7	0.7	8.6	9.2	5.6	2.9	3.4	4.9	7.6	5.8	0.6	0.5	0.5	0.7	0.6
<i>E. glamydus</i>	CZACCC14.14370	M	18.8	6.6	6.9	1.6	2.2	3.0	2.4	1.0	4.5	1.2	1.9	2.1	3.2	2.2	0.5	0.5	0.7	0.7	7.6	8.9	5.4	2.8	3.1	4.3	6.9	5.2	0.5	0.5	0.6	0.7	0.6
<i>E. glamydus</i>	CZACCC14.14371	M	19.8	7.0	7.2	1.6	2.2	3.6	2.8	0.9	4.7	1.3	2.1	2.3	3.3	2.2	0.4	0.5	0.7	0.7	8.2	9.4	5.5	2.7	3.3	4.3	7.0	5.2	0.5	0.5	0.5	0.7	0.6
<i>E. glamydus</i>	CZACCC14.14372	M	18.6	6.7	6.7	1.5	2.1	3.2	2.4	0.8	4.3	1.2	1.6	2.2	3.2	2.1	0.3	0.5	0.6	0.6	8.3	9.0	5.6	2.7	3.0	4.1	6.7	5.0	0.5	0.4	0.4	0.7	0.6
<i>E. glamydus</i>	CZACCC14.14373	M	20.0	7.4	7.5	1.4	2.3	3.5	2.7	1.0	4.8	1.4	2.4	2.6	3.5	2.2	0.5	0.6	0.7	0.7	9.1	9.7	5.9	2.9	3.6	5.1	7.7	6.0	0.6	0.5	0.7	0.6	0.7
<i>E. glamydus</i>	USNM509043	M	18.1	6.8	6.9	1.8	2.0	3.2	2.5	1.2	4.4	1.3	1.8	2.1	3.2	2.1	0.5	0.6	0.5	0.5	8.2	9.0	5.5	2.2	3.1	4.6	7.5	5.4	0.4	0.4	0.4	0.4	0.4
<i>E. glamydus</i>	USNM509044	M	20.7	7.5	7.5	2.1	2.7	3.6	2.8	0.9	4.5	1.4	2.1	2.5	3.6	2.4	0.6	0.6	0.7	0.8	9.1	9.6	6.0	2.5	3.4	4.9	7.8	6.0	0.6	0.6	0.6	0.7	0.6
<i>E. glamydus</i>	USNM509045	F	27.2	10.1	10.2	3.0	3.4	4.6	3.2	1.5	7.0	2.1	2.7	3.5	5.1	3.1	0.8	1.0	1.3	1.2	12.4	13.3	8.1	4.2	5.1	6.9	10.6	8.1	1.0	1.0	1.3	1.0	1.0
<i>E. glamydus</i>	USNM509046	F	25.1	8.7	8.7	2.1	3.1	4.0	2.8	1.4	5.8	2.0	2.9	3.4	4.6	3.3	0.7	0.8	1.1	0.9	11.7	12.2	7.4	4.1	4.9	6.4	10.0	7.5	1.0	0.9	0.8	1.0	0.9
<i>E. glamydus</i>	USNM509047	F	22.7	8.3	8.1	1.9	2.9	4.0	2.6	1.3	5.3	1.8	2.3	2.7	4.1	2.4	0.7	0.9	1.1	0.8	9.8	10.5	6.7	3.4	3.6	5.5	8.6	6.4	0.8	0.7	0.7	1.0	0.8
<i>E. glamydus</i>	USNM509048	F	22.4	8.2	8.2	2.0	2.7	3.8	2.6	1.2	5.9	1.6	2.2	2.8	4.4	2.7	0.7	0.9	1.0	1.0	10.7	11.5	6.9	3.6	4.2	6.0	8.9	7.0	0.8	0.9	1.1	0.9	0.9
<i>E. glamydus</i>	USNM509049	M	20.7	7.5	7.3	1.9	2.5	3.6	2.4	1.3	5.0	1.4	2.0	2.5	3.9	2.3	0.6	0.7	0.8	0.8	9.5	9.7	6.1	3.3	3.8	5.3	7.9	5.9	0.7	0.7	0.7	0.9	0.7
<i>E. glamydus</i>	USNM509050	M	18.9	7.3	7.3	1.9	2.0	3.7	2.3	1.2	4.5	1.3	1.7	2.2	2.9	1.8	0.5	0.6	0.8	0.8	8.9	9.2	5.5	2.7	3.4	4.7	7.0	5.4	0.6	0.7	0.7	0.8	0.7
<i>E. glamydus</i>	USNM509051	M	19.4	7.2	7.1	1.9	2.2	3.5	2.5	1.2	4.6	1.5	2.3	2.5	3.7	2.2	0.5	0.7	0.8	0.8	8.5	9.2	5.7	3.0	3.7	4.9	7.5	5.7	0.7	0.6	0.9	0.8	0.8
<i>E. glamydus</i>	USNM509052	M	19.5	7.3	7.2	2.0	2.5	3.5	2.1	1.2	4.9	1.4	2.1	2.6	3.8	2.3	0.4	0.6	0.7	0.6	9.5	9.7	6.1	3.3	3.5	5.1	7.8	5.7	0.6	0.6	0.7	0.6	0.6
<i>E. glamydus</i>	USNM564987	F	25.1	9.8	10.0	2.3	3.1	4.3	2.8	1.6	6.8	2.3	2.8	3.1	4.5	3.3	0.7	0.9	1.1	1.1	11.4	12.6	8.0	4.2	5.2	7.1	10.7	8.4	0.9	0.8	0.8	1.0	0.9

**APPENDIX IV.** Descriptive statistics (mean  $\pm$  SD) of morphometric variables (in mm) measured in 87 males of *Eleutherodactylus glamyrus* from nine localities. Locality names abbreviated as: AJO: Aguada del Joaquín, Sierra Maestra, Municipio Bartolomé Masó, Granma; BNV: Barrio Nuevo, Municipio Buey Arriba, Granma; BYM: Pico La Bayamesa, Sierra Maestra, Municipio Buey Arriba, Granma; NVE: La Nieve, Sierra Maestra, Municipio Buey Arriba, Granma; MIN: Minas de Frío, Sierra Maestra, Municipio Bartolomé Masó, BOT: Pico Botella, Municipio Buey Arriba, Granma; CUB: Pico Cuba, Sierra Maestra, Municipio Guamá, Santiago de Cuba; SUE: Pico Suecia, Sierra Maestra, Municipio Guamá; TUR: Pico Turquino, Sierra Maestra, Municipio Guamá, Santiago de Cuba. See Appendix I for collection data of these specimens.

	AJO (N=4)	BNV (N=6)	BYM (N=7)	NVE (N=26)	MIN (N= 1)	BOT (N=1)	CUB (N=36)	SUE (N=1)	TUR (N=5)
SVL	19.6 $\pm$ 0.8	19.3 $\pm$ 0.6	19.5 $\pm$ 0.6	19.8 $\pm$ 0.7	18.1	19.5	18.8 $\pm$ 0.8	20.3	19.2 $\pm$ 1
HW	6.8 $\pm$ 0.5	6.9 $\pm$ 0.3	6.7 $\pm$ 0.3	6.8 $\pm$ 0.3	6.8	7.3	6.3 $\pm$ 0.3	7.6	7 $\pm$ 0.5
HL	7.3 $\pm$ 0.2	7 $\pm$ 0.3	7.3 $\pm$ 0.2	7.3 $\pm$ 0.2	6.9	7.2	7 $\pm$ 0.3	7.9	7.2 $\pm$ 0.2
IN	1.8 $\pm$ 0.2	1.5 $\pm$ 0.1	1.5 $\pm$ 0.2	1.6 $\pm$ 0.1	1.8	2	1.6 $\pm$ 0.1	1.7	1.8 $\pm$ 0.1
EN	2.3 $\pm$ 0.3	2.2 $\pm$ 0.1	2.2 $\pm$ 0.1	2.2 $\pm$ 0.2	2	2.5	2 $\pm$ 0.2	2.4	2.2 $\pm$ 0.2
IO	3.2 $\pm$ 0.3	3.3 $\pm$ 0.2	3.3 $\pm$ 0.2	3.3 $\pm$ 0.2	3.2	3.5	3.2 $\pm$ 0.3	3.5	3.5 $\pm$ 0.1
EL	2.7 $\pm$ 0.1	2.6 $\pm$ 0.2	2.6 $\pm$ 0.2	2.6 $\pm$ 0.2	2.5	2.1	2.5 $\pm$ 0.2	2.5	2.4 $\pm$ 0.2
TyL	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1	1.2	1.2	0.9 $\pm$ 0.1	1.1	1.1 $\pm$ 0.2
FaL	4.7 $\pm$ 0.4	4.5 $\pm$ 0.2	4.7 $\pm$ 0.2	4.7 $\pm$ 0.2	4.4	4.9	4.5 $\pm$ 0.3	4.9	4.7 $\pm$ 0.3
HaL	1.4 $\pm$ 0.1	1.3 $\pm$ 0.1	1.3 $\pm$ 0.1	1.4 $\pm$ 0.1	1.3	1.4	1.3 $\pm$ 0.1	1.3	1.4 $\pm$ 0.1
F1	2.1 $\pm$ 0	2 $\pm$ 0.3	2.1 $\pm$ 0.1	2.1 $\pm$ 0.2	1.8	2.1	2 $\pm$ 0.2	2.1	2.1 $\pm$ 0.3
F2	2.5 $\pm$ 0.1	2.3 $\pm$ 0.2	2.4 $\pm$ 0.1	2.4 $\pm$ 0.2	2.1	2.6	2.4 $\pm$ 0.2	2.2	2.4 $\pm$ 0.2
F3	3.6 $\pm$ 0.1	3.3 $\pm$ 0.1	3.5 $\pm$ 0.2	3.5 $\pm$ 0.2	3.2	3.8	3.4 $\pm$ 0.2	3.3	3.5 $\pm$ 0.4
F4	2.2 $\pm$ 0.2	2.2 $\pm$ 0.1	2.2 $\pm$ 0.1	2.2 $\pm$ 0.2	2.1	2.3	2.2 $\pm$ 0.1	2.2	2.1 $\pm$ 0.2
FP1	0.5 $\pm$ 0	0.4 $\pm$ 0.1	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	0.5	0.4	0.5 $\pm$ 0.1	0.6	0.6 $\pm$ 0.1
FP2	0.6 $\pm$ 0.1	0.5 $\pm$ 0.1	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1	0.6	0.6	0.6 $\pm$ 0.1	0.7	0.7 $\pm$ 0.1
FP3	0.7 $\pm$ 0	0.7 $\pm$ 0	0.7 $\pm$ 0.1	0.7 $\pm$ 0.1	0.5	0.7	0.7 $\pm$ 0.1	0.9	0.8 $\pm$ 0.1
FP4	0.8 $\pm$ 0.1	0.7 $\pm$ 0	0.7 $\pm$ 0.1	0.7 $\pm$ 0.1	0.5	0.6	0.7 $\pm$ 0.1	0.9	0.8 $\pm$ 0.1
ThL	8.6 $\pm$ 0.7	8.4 $\pm$ 0.5	8.2 $\pm$ 0.3	8.9 $\pm$ 0.4	8.2	9.5	8.4 $\pm$ 0.6	8.1	9 $\pm$ 0.6
TL	9.3 $\pm$ 0.3	9.2 $\pm$ 0.3	9.2 $\pm$ 0.3	9.6 $\pm$ 0.3	9	9.7	9.1 $\pm$ 0.4	9.3	9.4 $\pm$ 0.2
FL	5.9 $\pm$ 0.2	5.6 $\pm$ 0.2	5.8 $\pm$ 0.2	6 $\pm$ 0.2	5.5	6.1	5.7 $\pm$ 0.3	6.3	5.8 $\pm$ 0.2
T1	2.6 $\pm$ 0.1	2.8 $\pm$ 0.1	2.7 $\pm$ 0.1	2.7 $\pm$ 0.2	2.2	3.3	2.6 $\pm$ 0.2	2.8	3 $\pm$ 0.2
T2	3.3 $\pm$ 0.1	3.3 $\pm$ 0.2	3.3 $\pm$ 0	3.3 $\pm$ 0.2	3.1	3.5	3.2 $\pm$ 0.2	3.6	3.5 $\pm$ 0.2
T3	4.7 $\pm$ 0.4	4.5 $\pm$ 0.4	4.9 $\pm$ 0.1	4.7 $\pm$ 0.4	4.6	5.1	4.5 $\pm$ 0.3	5.3	4.8 $\pm$ 0.3
T4	7.6 $\pm$ 0.4	7.1 $\pm$ 0.4	7.7 $\pm$ 0.3	7.6 $\pm$ 0.5	7.5	7.8	7.2 $\pm$ 0.5	6.8	7.3 $\pm$ 0.4
T5	5.6 $\pm$ 0.5	5.4 $\pm$ 0.4	5.8 $\pm$ 0.3	5.7 $\pm$ 0.4	5.4	5.7	5.4 $\pm$ 0.4	6	5.6 $\pm$ 0.2
TP1	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1	0.6 $\pm$ 0.1	0.4	0.6	0.6 $\pm$ 0.1	0.7	0.6 $\pm$ 0.1
TP2	0.6 $\pm$ 0.1	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	0.4	0.6	0.6 $\pm$ 0.1	0.7	0.7 $\pm$ 0.1
TP3	0.5 $\pm$ 0	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	0.4	0.6	0.6 $\pm$ 0.1	0.6	0.6 $\pm$ 0.1
TP4	0.7 $\pm$ 0.1	0.7 $\pm$ 0	0.7 $\pm$ 0.1	0.6 $\pm$ 0.1	0.4	0.7	0.7 $\pm$ 0.1	0.8	0.8 $\pm$ 0.1
TP5	0.6 $\pm$ 0.1	0.6 $\pm$ 0	0.7 $\pm$ 0.1	0.5 $\pm$ 0.1	0.4	0.6	0.6 $\pm$ 0.1	0.7	0.7 $\pm$ 0.1

**APPENDIX V.** Loadings of the first five components of a principal component analysis conducted on 31 morphometric variables from 82 male specimens of *E. glamyrus* sensu lato (including specimens assigned to phylogroups TUR and GAT).

	PC-1	PC-2	PC-3	PC-4	PC-5
SVL	0.93	-0.18	-0.14	0.01	-0.07
HW	0.91	-0.26	-0.19	0.03	0.04
HL	0.94	-0.18	-0.11	0.05	-0.02
IN	0.70	-0.28	-0.02	0.12	0.24
EN	0.81	-0.18	-0.24	-0.09	-0.04
IO	0.88	-0.17	-0.17	-0.01	0.08
EL	0.76	-0.29	-0.16	0.12	0.12
TyL	0.74	0.02	-0.07	0.12	0.33
FaL	0.87	-0.21	-0.15	-0.05	-0.07
HaL	0.79	-0.12	0.10	-0.05	-0.20
F1	0.85	-0.09	0.09	0.11	-0.15
F2	0.85	-0.10	0.21	0.04	-0.06
F3	0.87	-0.09	-0.02	0.14	-0.10
F4	0.88	-0.04	0.14	0.10	-0.01
FP1	0.58	0.44	0.23	0.27	-0.25
FP2	0.65	0.38	0.22	0.21	-0.10
FP3	0.68	0.52	0.00	0.32	0.13
FP4	0.61	0.41	-0.03	0.49	0.08
ThL	0.90	-0.12	-0.10	-0.07	-0.19
TL	0.94	-0.20	-0.18	0.00	-0.08
FL	0.93	-0.15	-0.03	-0.01	-0.10
T1	0.66	-0.13	0.41	-0.05	0.46
T2	0.76	-0.10	0.32	-0.23	0.31
T3	0.91	-0.08	0.16	-0.20	-0.02
T4	0.92	-0.10	0.10	-0.19	-0.03
T5	0.92	-0.13	0.09	-0.20	-0.09
TP1	0.54	0.61	0.19	-0.23	-0.17
TP2	0.66	0.50	0.01	-0.26	-0.02
TP3	0.56	0.69	-0.04	-0.13	-0.10
TP4	0.38	0.73	-0.21	-0.18	0.23
TP5	0.45	0.61	-0.41	-0.14	0.18