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# Two new fringe-limbed frogs of the genus *Ecnomiohyla* (Anura: Hylidae) from Panama

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## Abstract

Forest canopy-dwelling frogs are usually among the rarest anuran species observed in the neotropical forest, mainly because they fall outside of the scope of the standard search methods used by herpetologists. During field explorations undertaken in western and eastern Panama in recent years, we discovered two species belonging to the genus *Ecnomiohyla*, which showed significant differences in genetic distances (16S mtDNA gene) and morphological characteristics different from any known *Ecnomiohyla* species. The first specimen originates from the Serranía de Jingurudó, Darién province, southeastern Panamá, and is described herein as *E. bailarina* **sp. nov.**, and the second specimen was found at Santa Fe National Park, Veraguas province, central-western Panama, and is described as *E. veraguensis* **sp. nov.** We provide a detailed description of both new species, including comparisons of morphological and molecular characters of almost all members of the genus in lower Central America, as well as an identification key for the entire genus.

**Key words:** Fringe-limbed frogs, *Ecnomiohyla,* rare species, DNA barcoding, lower Central America, Panama

## Introduction

After the description of a new species, subsequent sampling usually provides additional comparative specimens, which thus increases our knowledge about the biology and distribution of that species with time (Vrcibradic *et al.* 2008; Hertz *et al.* 2012a). Nonetheless, there are some apparently rare species, whose existence we know for decades based only on the type specimen(s) or material from the type locality (Pimenta *et al.* 2005; Frost 2013, Wickramasinghe *et al.* 2013). Burrowing caecilians, salamanders (*Oedipina* spp.) and forest canopy-dwelling frogs (e.g. *Pseudophilautus stellatus*) are examples of such infrequently encountered species, which are then perceived as very rare (García-París & Wake 2000; Hanken *et al.* 2005; Wilkinson *et al.* 2007; Kamei *et al*. 2009; Wickramasinghe *et al.* 2013), because the habitat of these amphibians usually falls outside of the scope of the standard search methods used by herpetologists. Thus, the perception of rarity might be only an artifact of limited or inappropriate search techniques. In this context, any information about such seldom-seen (or "rare") taxa can be relevant.

Among such rare species are most members of the fringe-limbed frogs of the genus *Ecnomiohyla* Faivovich, Haddad, Garcia, Frost, Campbell, & Wheeler 2005, which spend all their life phases in the canopy and only rarely climb down and become visible to us. Fringe-limbed frogs are large, morphologically unusual hylid frogs with a cryptic moss-like color pattern and dermal fringes on portions of the body, rendering them well camouflaged. They breed in phytotelmata (e.g. Savage 2002; Mendelson *et al.* 2008; Savage & Kubicki 2010), and most of them occur in wet lowland, premontane tropical, and cloud forests between 20–2000 m elevation (Wilson *et al*. 1985;

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Duellman 2001; Frost 2013). The genus *Ecnomiohyla* is distributed from southern Mexico through Central America and into north-western South America, containing 12 species to date (Frost 2013), which are: *Ecnomiohyla echinata* (Duellman, 1961), *E. fimbrimembra* (Taylor, 1948), *E. miliaria* (Cope, 1886), *E. minera* (Wilson, McCranie, & Williams, 1985), *E. miotympanum* (Cope, 1863), *E. phantasmagoria* (Dunn, 1943), *E. rabborum* Mendelson, Savage, Griffith, Ross, Kubicki, & Gagliardo, 2008, *E. salvaje* (Wilson, McCranie, & Williams, 1985), *E. sukia* Savage & Kubicki, 2010, *E. thysanota* (Duellman, 1966), *E. tuberculosa* (Boulenger, 1882), and *E. valancifer* (Firschein & Smith, 1956). The holotypes of *Ecnomiohyla* species were often the only specimens known for an extended period of time (Taylor 1948; Duellman 1966). However, intensified sampling during the past three decades has contributed increasingly to our knowledge about the ecology of some species, while recent genetic studies helped to understand better the species relationships inside the genus *Ecnomiohyla* and its position in the amphibian tree of life (Wilson *et al*. 1985; Faivovich *et al*. 2005; Mendelson *et al*. 2008; Crawford *et al.* 2013).

The genus *Ecnomiohyla* can be differentiated from all other genera of Hylinae by the combination of the following characters: having immense hands and feet, scalloped dermal fringes on the outer margin of the forearm and foot, large digital disks, and enlarged prepollices (Firschein & Smith 1956; Savage & Heyer 1969; Duellman 1970; Mendelson *et al*. 2008). The prepollices are more developed in males and usually modified with a projecting terminal spine (protruding as in *E. miliaria*), or a spade-like plate (as in *E. valancifer*). In many species, male prepollices bear also keratinized black spines (Duellman 2001), whereas in females the prepollex is slender, straight and without spines.

Some uncertainty remains in unifying all currently recognized species within *Ecnomiohyla* based on the morphological characters mentioned above (Faivovich *et al.* 2005). *Ecnomiohyla miotympanum* and *E. tuberculosa* have been catalogued as problematic species due to substantial differences in adult and larval morphology and shared behavioral ecological traits in comparison to the other members of the genus (Faivovich *et al.* 2005; Mendelson *et al*. 2008). This problem is not solved yet, partly because of the lack of fresh material for genetic approaches in many species that prevents the construction of a well-resolved phylogeny of the genus. Recent phylogenetic studies lack most species of *Ecnomiohyla* (there are no sequences available for *E. tuberculosa* yet) thus some of its species (*E. miotympanum* and *E. tuberculosa* specially) are assigned to the genus only tentatively (Faivovich *et al*. 2005; Wiens *et al.* 2010; Pyron & Wiens 2011). The issues regarding exclusion of *E. miotympanum* and *E. tuberculosa* from the genus *Ecnomiohyla* (see Savage & Kubicki 2010) are not relevant in the context of this paper, but we include *E. miotympanum* to our phylogenetic analysis to discuss its relationship with other *Ecnomiohyla* from lower Central America.

Herein, we describe two new species of *Ecnomiohyla* from Panama, based on comparative morphology of the twelve known species of *Ecnomiohyla* and a genetic analysis of the species from lower Central America (except *E. thysanota*). The new species from eastern Panama can be distinguished from its congeners by the presence of cranial and dorsal osteoderms, and two clusters of nuptial spines, one at the distal end of prepollex and one at the end of the first phalanx of the thumb in males. The new species from western Panama has scattered minute keratin tipped tubercles on the dorsal skin, and 6–8 widely spaced keratinized black spines along the outer side of the thumb.

## Material and methods

Fieldwork was carried out in eastern Panama in 2011 and 2012 (Fig. 1) and in central-western Panama during two field trips in 2009. Specimens were euthanized with a euthanasia solution (T61), fixed with a mixture of 5 ml formalin (5%) in 1L ethanol (94%), and then stored in ethanol (70%). Morphological nomenclature and diagnoses usually follow the methodology of Duellman (2001), except for standards of dorsal and lateral profiles of the snout that follow Savage (2002). Coding for webbing formulae follows Savage & Kubicki (2010): considerable (C) = not extending to base of disk on one margin of any digit; substantial (S) = extending to base of disk on one margin of one digit; extensive (EX) = extending to base of disk on one margin of two to four digits; full (F) = extending to base of disk on margins of all digits.

All measurements are given in millimeters, were rounded to the nearest 0.1 mm and follow Duellman & Lehr (2009). The following measurements were taken (with abbreviations indicated): length from snout to vent (SVL); head length (HL), measured diagonally from angle of jaw to tip of snout; head width (HW) between angles of jaws;

interorbital distance (IOD); eye diameter (ED); eye length (EL) from anterior to posterior edge; eye to nostril distance (END) from anterior edge of eye to posterior corner of nostril; internarial distance (IND) between centers of nostrils; forearm length (FAL) from proximal edge of palmar tubercle to outer edge of flexed elbow; hand length (HAL) from proximal edge of palmar tubercle to tip of third finger; tibia length (TL), distance from knee to distal end of the tibia; foot length (FL) from proximal edge of outer metatarsal tubercle to tip of fourth toe; width of third finger (3FW) at penultimate phalanx just anterior to disk; width of disk of third finger (3FD) at greatest width; width of third toe (3TW) at penultimate phalanx just anterior to disk; width of disk of third toe (3TD) at greatest width; width of fourth toe (4TW) at penultimate phalanx just anterior to disk; width of disk of fourth toe (4TD) at greatest width; body width (BW) at greatest width of body; tympanum diameter (TD), horizontal distance, based on an estimated circular tympanum. SVL, HL, HW, TL, and FL were measured with vernier calipers; all other variables were measured with an ocular micrometer in a Zeiss stereomicroscope.

Capitalized colors and color codes (the latter in parentheses) used in the color descriptions are those of Smithe (1975–1981), except those in the color description of the holotype of *Ecnomiohyla bailarina*, which are those of Köhler (2012). Specimens were deposited in the herpetological collection of the Senckenberg Forschungsinstitut and Naturmuseum Frankfurt (SMF) in Germany. Comparisons among similar species are based on data provided in the respective original descriptions. Geographic coordinates and altitude were taken with a Garmin GPSmap 60CSx given in decimal degrees and rounded to the fourth decimal place. Elevations are rounded up to the next tenth. All georeferences were recorded in WGS 1984 datum. The map was downloaded from the server of the Smithsonian Tropical Research Institute (http://mapserver.stri.si.edu/), and created using ArcGIS 10 (ESRI 2009). Detailed information about the specimens examined is given in Table 1.

## Molecular Genetics

We took tissue samples from the two new species plus a newly collected specimen of *Ecnomiohyla fimbrimembra* (SMF89857, Hertz et al. 2012b) and a newly collected specimen of *E. sukia* (SMF94578, Köhler *et al.* 2013). Tissue for DNA was extracted by excision on finger-tips of preserved specimens, except for *Ecnomiohyla bailarina*, where the tissue was extracted from a fresh liver sample. A fragment of the mitochondrial 16S mtDNA gene was extracted following the protocol of Ivanova *et al*. (2006), and amplified using a Mastercycler pro S (Eppendorf, Hamburg, Germany) performing an initial denaturation for 60 sec at 94° C followed by 35 steps with denaturation for 15 sec at 94° C, hybridization for 45 sec at 45° C, elongation for 1.5 min at 72° C, final extension at 72° C for 7 min; reaction mix contained 1 µL DNA template, 2.5 µL Reaction Buffer x10 (PeqGold), 4 µL 2.5 mM dNTPs, 0.4 µL (containing 2.5 units) Taq Polymerase (PeqLab), 14.1 µL H2O, 1 µL 25 mM MgCl2, and 1 µL (containing 10 pmol) (forward: L2510, 5'-CGCCTGTTTATCAAAAACAT-3'; reverse: H3056, 5'-CCGGTCTGAACTCAGATCACGT-3'; eurofins MWG Operon).

To compare the 16S mtDNA data of our specimens with published sequences, we conducted a *BLAST* search in GenBank and took the sequences with the highest scores for comparison. Additionally, we used *Bromeliohyla bromeliacia* (Schmidt, 1933), *Duellmanohyla rufioculis* (Taylor, 1952), and *D. soralia* (Wilson & McCranie, 1985) as outgroups (the phylogenetically most closely related species according to Faivovich *et al.* 2005). All sequences were aligned and manually refined using Genious (Drummond *et al*. 2010). In MEGA5 (Tamura *et al.* 2011), we computed uncorrected pairwise genetic distances prior to the maximum likelihood and Bayesian analyses. We used JModeltest 0.1.1 (Posada 2008) with likelihood settings to find the best-fitting substitution model according the Akaike Information Criterion (AICc). The Bayesian phylogenetic analysis (MrBayes 3.1.2, Huelsenbeck & Ronquist 2001) was run under the model TPM3uf+G, for 2,000,000 generations with four Metropolis-coupled Markov Chain Monte Carlo (MCMC) sampled every 100 generations. The first 5% were discarded as burn-in (burn-in= 1000). The ML analysis was assessed via 1000 bootstrap replicates, using PAUP v4.0b10 (Swofford 1998). The Automatic Barcode Gap Discovery (ABGD) algorithm (Puillandre *et al.* 2011), has been recently recommended as a reliable barcode cluster identification algorithm (Paz & Crawford 2012). Therefore, we also evaluated our sequences applying this method, using the Web interface at http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html. The following settings were chosen: steps=20, distance= Kimura 2-parameter 2.0, and the setting for the minimum relative gap width (X) was moved to different values between 0 and 1.5.

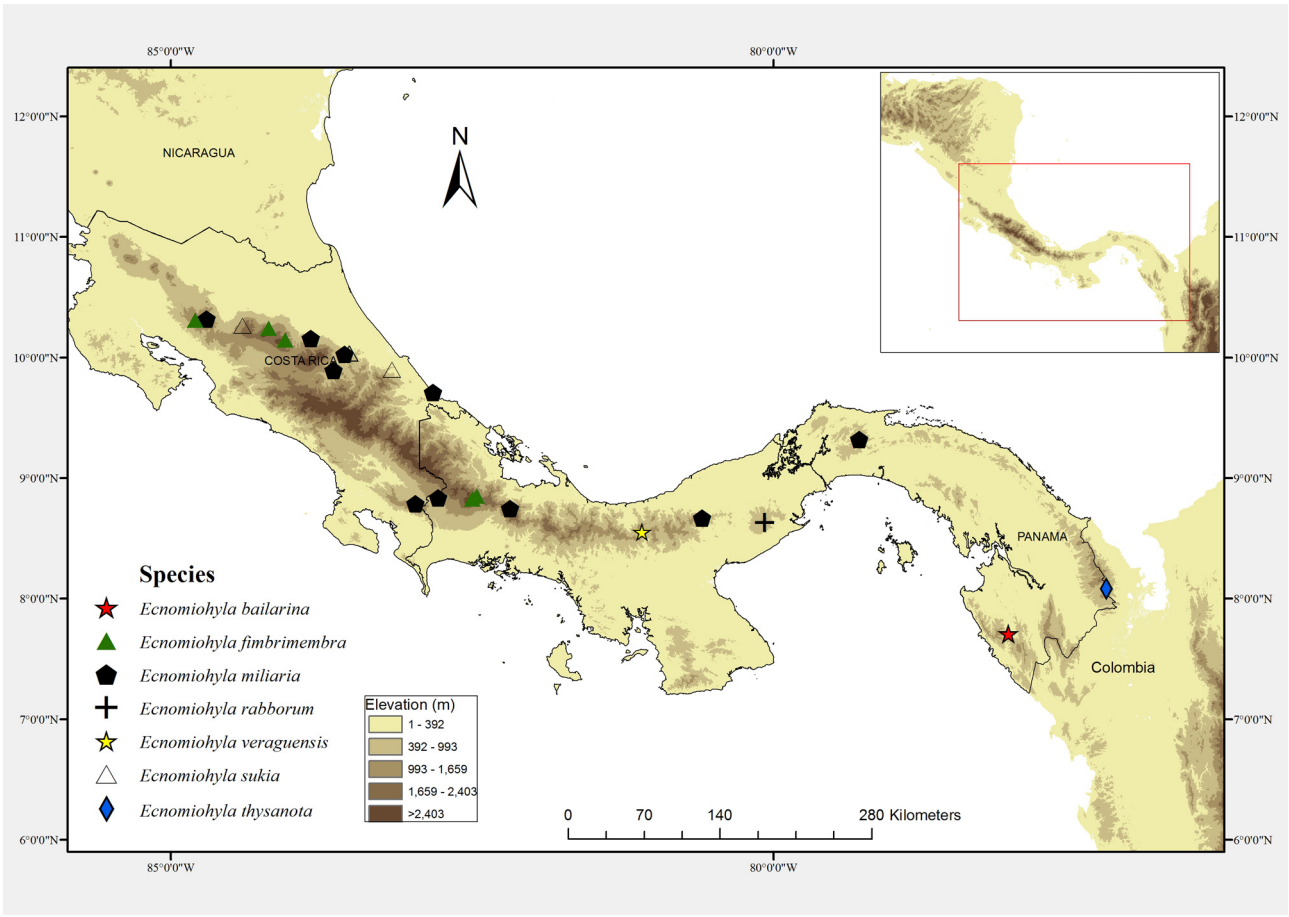


**FIGURE 1.** Distribution of the *Ecnomiohyla* spp. in lower Central America (main map). See Table 1 for detailed information on the localities.

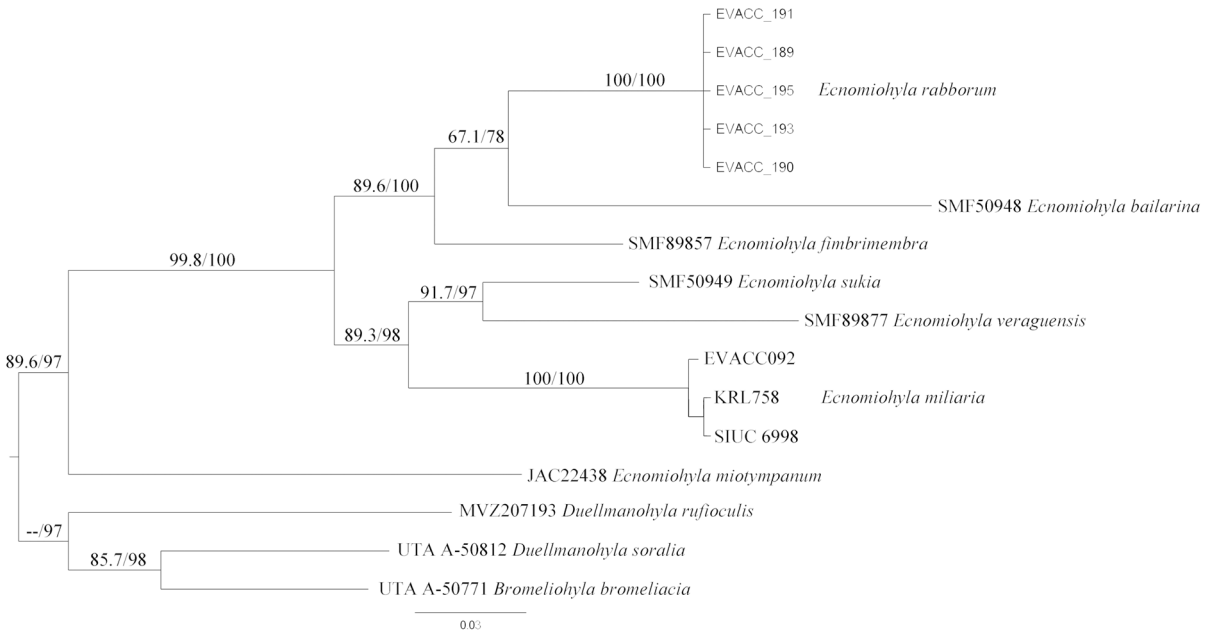


**FIGURE 2.** Maximum likelihood consensus tree of the 16S mtDNA gene, for the *Ecnomiohyla* spp. from lower Central America. Specimen labels refer to collection or museum number. Scale bar refers to number of substitutions per site. Maximum likelihood bootstrap values are shown in front of slash mark, Bayesian posterior probabilities (multiplied by 100) behind slash mark.

**TABLE 1.** Genbank accessions and detailed information on the localities of specimens mentioned in the text.

| Museum number | Accession 16S | Species | Locality | Country | Coordinates N | Coordinates W | Elev. |
|---|---|---|---|---|---|---|---|
| UTA A-50771 | AY843612.1 | *Bromeliohyla bromeliacia* | Huehuetenango, Sierra de los Cuchumatanes, Finca Chiblac (now Aldea Buenos Aires) | Guatemala | | | |
| MVZ 207193 | AY843583.1 | *Duellmanohyla rufioculis* | Guanacaste, Volcan Cacao | | | | |
| UTA A-50812 | AY843584.1 | *Duellmanohyla soralia* | Izabal, Morales, Sierra de Caral, Finca Quebradas-Cerro Pozo de Agua | Guatemala | | | |
| SMF97398 | KF924240 | *Ecnomiohyla bailarina* | North slope of the Jingurudó mountain range, about ca.14.6 Km S from Pavarandó village, Sambú, Comarca Emberá-Wounaam N°2 | Panama | 7.70903 | -78.04882 | 750 |
| SMF 89857 | KF924242 | *Ecnomiohyla fimbrimembra* | Boquete/Bajo Mono Sendero La Cascada | Panama | 8.82629 | -82.49907 | 1820 |
| LACM 149980 | | *Ecnomiohyla fimbrimembra* | Pantanosa Trail, Monte Verde | Costa Rica | 10.310388 | -84.798056 | 1600 |
| LACM 149979 | | *Ecnomiohyla fimbrimembra* | Northern slope of Volcan Barba, Heredia | Costa Rica | 10.144033 | -84.052224 | 1800 |
| FMNH 191784 | | *Ecnomiohyla fimbrimembra* | Isla Bonita, Alajuela, Costa Rica | Costa Rica | 10.241438 | -84.190483 | 1300 |
| CHP1036 | | *Ecnomiohyla fimbrimembra* | Cerro Horqueta, Boquete Panama | Panama | 8.850493 | -82.46196 | 1600 |
| SIUC 6998 | AY843777.1 | *Ecnomiohyla miliaria* | EL Copé, Parque Nacional Omar Torrijos, Loop, Stream 2, Coclé. | Panama | 8.667 | -80.592 | 800 |
| KRL758 | DQ055824 | *Ecnomiohyla miliaria* | EL Copé, Parque Nacional Omar Torrijos, Loop, Stream 2, Coclé. | Panama | 8.667 | -80.592 | 800 |
| EVACC092 | KC014814.1 | *Ecnomiohyla miliaria* | Chagres National Park, Cerro Brewster Stream, Panamá | Panama | 9.31985 | -79.2889 | 818 |
| KU 98451 | | *Ecnomiohyla miliaria* | Santa Clara, Renacimiento, Chiriquí | Panama | 8.834816 | -82.783559 | 1100 |
| KU30404 | | *Ecnomiohyla miliaria* | Cartago: 2.5 km east of Turrialba | Costa Rica | 9.8936 | -83.6521 | 602 |
| USNM 331414 | | *Ecnomiohyla miliaria* | Siquirres | Costa Rica | 10.0269 | -83.5602 | 62 |

**TABLE 1.** (Continued)

| Museum number | Accession 16S | Species | Locality | Country | Coordinates N | Coordinates W | Elev. |
|---|---|---|---|---|---|---|---|
| UCR 4979 | | *Ecnomiohyla miliaria* | Estacion Biologia Las Cruces, Puntarenas | Costa Rica | 8.787262 | -82.972649 | 1200 |
| LACM 150152 | | *Ecnomiohyla miliaria* | Río Peñas Blancas, Alajuela | Costa Rica | 10.319491 | -84.704758 | 800 |
| UCR 12678 | | *Ecnomiohyla miliaria* | Río Blanco area, Provincia Limón. | Costa Rica | 10.157077 | -83.840814 | 450 |
| UMMZ 149201 | | *Ecnomiohyla miliaria* | Comadre de Cahuita, Limón | Costa Rica | 9.710069 | -82.82482 | 20 |
| AMNH 94887 | | *Ecnomiohyla miliaria* | RF fortuna Río Chiriquí | Panama | 8.747156 | -82.186534 | 1100 |
| JAC22438 | AY843645.1 | *Ecnomiohyla miotympanum* | Puebla, Sierra Norte, Cuetzalan, Hotel Villas Cuetzalan | Mexico | | | 1250 |
| SMF94908 | | *Ecnomiohyla miotympanum* | Cuetzalan, Apuleo, hacienda km 7, sierra norte, Puebla | Mexico | | | |
| EVACC191 | KC014813.1 | *Ecnomiohyla rabborum* | El Valle, Rio Maria, Panama | Panama | 8.63312 | -80.0767 | 990 |
| EVACC189 | KC014811.1 | *Ecnomiohyla rabborum* | El Valle, Rio Maria, Panama | Panama | 8.63312 | -80.0767 | 990 |
| EVACC195 | KC014809.1 | *Ecnomiohyla rabborum* | El Valle, Rio Maria, Panama | Panama | 8.63312 | -80.0767 | 990 |
| EVACC193 | KC014807.1 | *Ecnomiohyla rabborum* | El Valle, Rio Maria, Panama | Panama | 8.63312 | -80.0767 | 990 |
| EVACC190 | KC014812.1 | *Ecnomiohyla rabborum* | El Valle, Rio Maria, Panama | Panama | 8.63312 | -80.0767 | 990 |
| SMF94578 | KF924239 | *Ecnomiohyla sukia* | San Carlos, Cerro Chato, Alajuela | Costa Rica | 10.2632 | -84.4052 | 922 |
| UCR 12787 | | *Ecnomiohyla sukia* | Guayacán: Alto Colorado, | Costa Rica | 10.037139 | -83.522889 | 710 |
| UCR 10966 | | *Ecnomiohyla sukia* | 5km from Moravia de Siquirres toward Turrialba | Costa Rica | 10.033333 | -83.516667 | 710 |
| UCR 17024 | | *Ecnomiohyla sukia* | S Río Blanco (town): Fila Asunción | Costa Rica | 9.9 | -83.166667 | 400 |
| USNM 151080 | | *Ecnomiohyla thysanota* | Cerro Malí, 1265 m (holotype of E. thysanota), Darién. | Panama | 8.080757 | -77.235448 | 1265 |
| SMF82418 | | *Ecnomiohyla tuberculosa* | | | | | |
| SMF 89877 | KF924241 | *Ecnomiohyla veraguensis* | Cerro Negro/PN Santa Fe | Panama | 8.5533 | -81.09261 | 540 |

**TABLE 2.** Estimates of evolutionary divergence among 16S mtDNA gene sequences of the *Ecnomiohyla* spp. used in the phylogenetic analysis. Numbers below diagonal are for uncorrected p-distances and numbers above are standard error estimates.

| | Species | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *E. bailarina* (SMF97398) | | 0.02 | 0.02 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 | 0.02 | 0.02 | 0.01 | 0.02 |
| 2 | *E. veraguensis* (SMF89877) | 0.14 | | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 |
| 3 | *E sukia* (SMF94578) | 0.15 | 0.07 | | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 |
| 4 | *E. rabborum* (EVACC191) | 0.11 | 0.10 | 0.10 | | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 |
| 5 | *E. rabborum* (EVACC189) | 0.11 | 0.10 | 0.10 | 0.00 | | 0.00 | 0.00 | 0.00 | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 |
| 6 | *E. rabborum* (EVACC195) | 0.11 | 0.10 | 0.10 | 0.00 | 0.00 | | 0.00 | 0.00 | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 |
| 7 | *E. rabborum* (EVACC193) | 0.11 | 0.10 | 0.10 | 0.00 | 0.00 | 0.00 | | 0.00 | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 |
| 8 | *E. miliaria* (EVACC190) | 0.11 | 0.10 | 0.10 | 0.00 | 0.00 | 0.00 | 0.00 | | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 |
| 9 | *E. miliaria* (SIUC6998) | 0.14 | 0.09 | 0.10 | 0.12 | 0.12 | 0.12 | 0.12 | 0.12 | | 0.00 | 0.00 | 0.01 | 0.02 |
| 10 | *E. miliaria* (KRL 0758) | 0.14 | 0.09 | 0.10 | 0.12 | 0.12 | 0.12 | 0.12 | 0.12 | 0.00 | | 0.00 | 0.01 | 0.02 |
| 11 | *E. miliaria* (EVACCC092) | 0.14 | 0.09 | 0.10 | 0.11 | 0.11 | 0.11 | 0.11 | 0.11 | 0.00 | 0.00 | | 0.01 | 0.02 |
| 12 | *E. fimbrimembra* (SMF89857) | 0.12 | 0.10 | 0.10 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.11 | 0.11 | 0.11 | | 0.02 |
| 13 | *E. miotympanum* (JAC22438) | 0.19 | 0.15 | 0.15 | 0.14 | 0.14 | 0.14 | 0.14 | 0.14 | 0.15 | 0.15 | 0.16 | 0.15 | |

**TABLE 3.** Principal differential traits of the *Ecnomiohyla* species from lower Central America and Colombia.

| Trait | *E. rabborum* | *E. bailarina* sp. n. | *E. fimbrimembra* | *E. miliaria* | *E. sukia* | *E. veraguensis* | *E. phantasmagoria* | *E. thysanota* |
|---|---|---|---|---|---|---|---|---|
| SVL males | 62.8-97.3 | 68 | NA | 86.0-110.0 | 56.7-63.2 | 57.8 | 95 | NA |
| SVL females | 61.3-79.9 | NA | 71.0 – 91.0 | 86.2 | 58.1-68.1 | NA | NA | 95 |
| dorsum | granular | tuberculate | granular | tuberculate | tuberculate | finely tuberculated | tuberculate | granular |
| Cephalic skin co-ossified with skull | - | - | + | - | - | - | - | - |
| Cranial osteoderms | - | + | - | + | + | + | + | - |
| Dorsal osteoderms | - | + | - | + | + | + | + | - |
| Humeral projection in males | + | - | - | - | - | - | - | - |
| Prepollex (males) | blunt | rounded | blunt | recurved | obtuse | recurved | recurved | NA |
| Prepollical bony projection (males) | rounded | bluntly pointed. directed medially | rounded | spine | spadelike, directed laterally | spadelike, directed laterally | spine | NA |
| Keratinized black spines on prepollex and thumbs (males) | + | + | + | - | - | + | - | NA |
| Finger webbing | S | EX | C | EX | EX | EX | C | EX |
| Toe webbing | EX | EX | C | EX | EX | EX | EX | EX |
| Heel | smooth | scalloped fringe | pointed tubercles | pointed tubercles | scalloped fringe | scalloped fringe | pointed tubercles | smooth |
| Color in life | | green with brownish flecks | brown with darker markings | brown to mottled brown and green | | | | uniformly green |

See methods for finger and toe webbing abbreviations; "-"= absent; "+"= present; NA= no specimen available for comparison.

## Results

According to the barcode analysis with ABGD, we found that the comparison of all included *Ecnomiohyla* samples resulted in seven distinct species with a prior intraspecific divergence of 4.9% (Fig. 2). The overall genetic p-distance between the samples was 10.0%. Our two newly sequenced *Ecnomiohyla* specimens, one from western and one from eastern Panama, are genetically distinct and have no morphological characters that would assign them to any previously described species in the genus (Savage & Kubicki 2010; Köhler 2011). The specimen from western Panama (SMF89877) forms a sister clade to *E. sukia* from Costa Rica with an estimated evolutionary divergence of 7% (Table 2). The specimen from eastern Panama (SMF97398) is most closely related to *E. rabborum* and *E. fimbrimembra,* but is genetically distinct by 11 and 12% p-distance in the 16S gene (Table 2), respectively. SMF97398 shows the highest p-distance of 15% to *E. sukia*. *Enomiohyla miotympanum* is revealed as sister taxon to all *Ecnomiohyla* from lower Central America (Fig. 2, Table 2), separated by a p-distance of 14–19%. According to our findings of significant genetic and morphological differences in two of our newly obtained specimens (Table 3), we proceed to describe them as two species new to science.

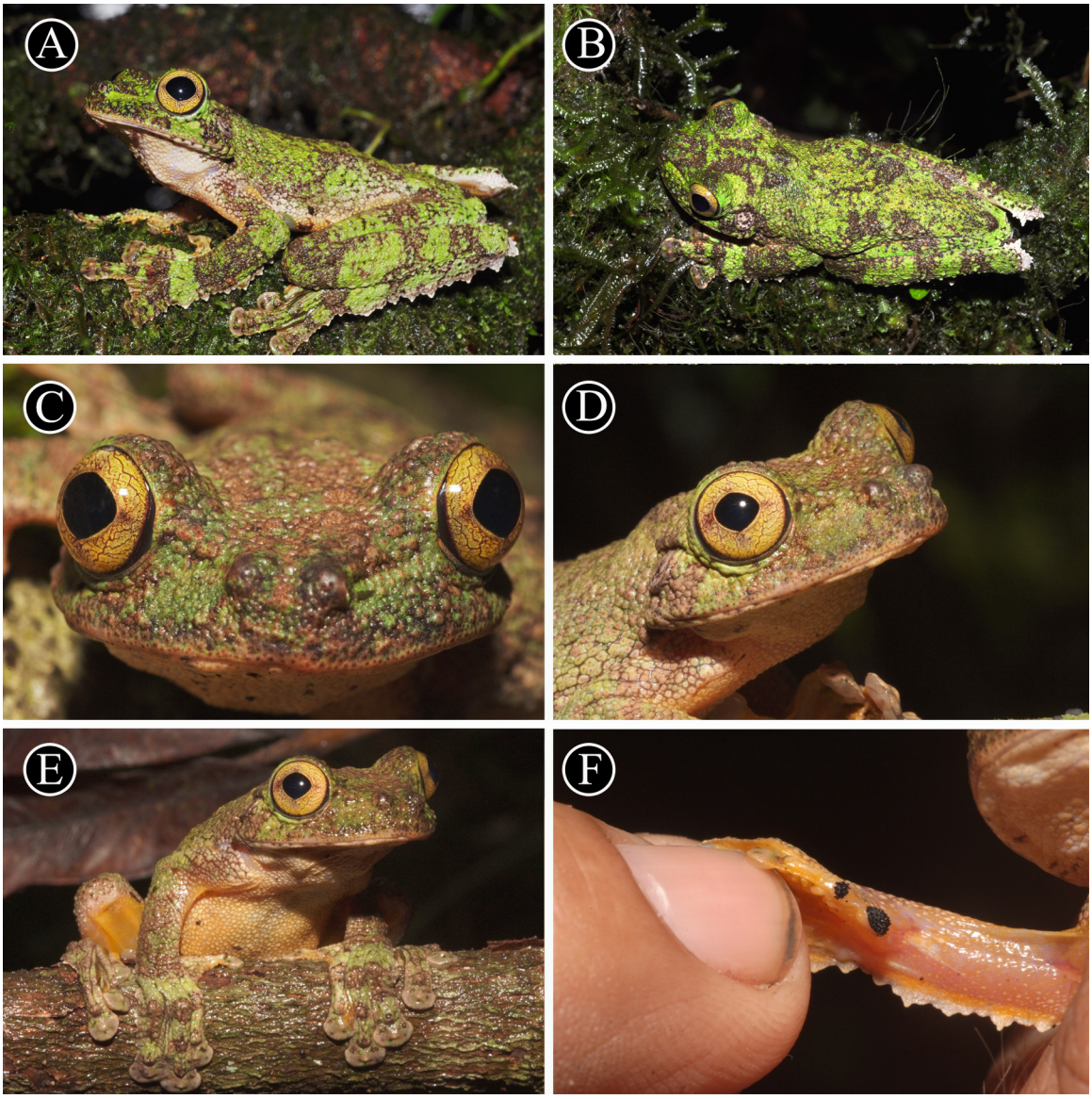


**FIGURE 3.** Photographs of the holotype of *Ecnomiohyla bailarina* in life. A) lateral view; B) dorsal view; C) frontal view; D) profile; E) ventral coloration; F) prepollical spines on right hand.

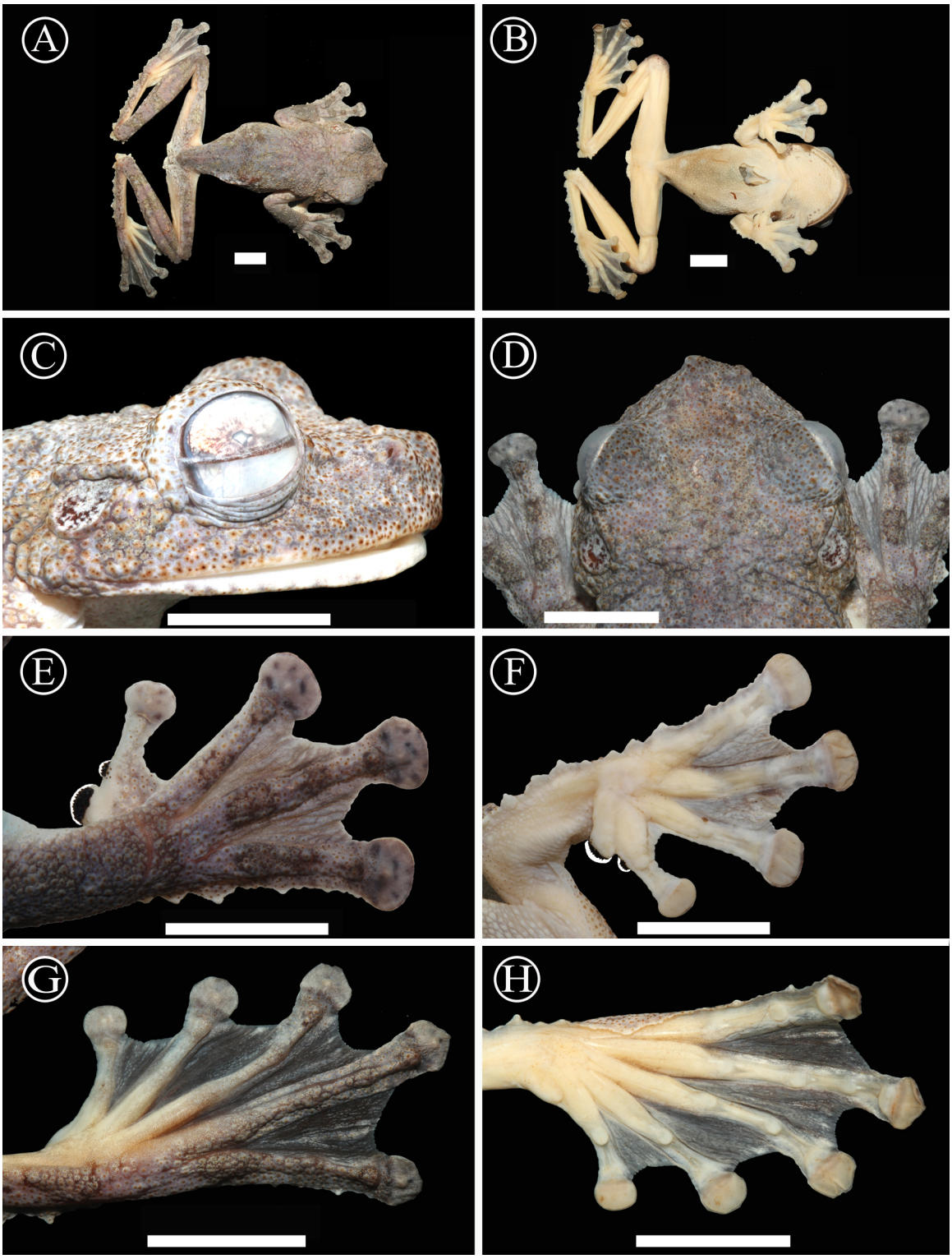


**FIGURE 4.** Photographs of preserved holotype of Ecnomiohyla bailarina. A) dorsal view; B) ventral view; C) head in profile; D) head dorsally; E) right hand dorsally; F) right hand ventrally; G) right foot dorsally; H) right foot ventrally. Scale bars = 10 mm.

***Ecnomiohyla bailarina* sp. nov.**

**Holotype.** SMF97398 (original field number AB297; Fig. 3–4), an adult male from the north slope of the Jingurudó mountain range (Fig. 5), about 14.6 km S from Pavarandó village (7.70903°N, -78.04882°W, 750 m a.s.l.), Sambú, Comarca Emberá-Wounaan N°2, Darién, Panama, collected by Abel Batista and Milan Vesely on 25 September 2011 at 21:27 hrs.

**Diagnosis.** A medium-sized *Ecnomiohyla* (single known specimen is an adult male 68.1 mm in SVL; Figs. 3–4), differing from other known species in the genus by the following combination of characters: 1) finger webbing extensive, web reaching the finger disk on at least one side on two fingers (Fig. 4); 2) toes extensively webbed as well, web reaching the toe disk at least on one side of four toes (Fig. 4); 3) skin on dorsum strongly tuberculate; 4) cranial and dorsal osteoderms present; 5) skin on upper surface of head not co-ossified with underlying cranial elements; 6) humerus without enlarged *crista lateralis*; 7) prepollex distinct, obtuse, with bony prepollical projection rounded distally, bluntly pointed at side adjacent to thumb; 8) two clusters of nuptial spines at the distal end of prepollical tubercle and at the end of the first phalanx of the thumb; 9) a distinct scalloped fringe with pointed tubercles on a ventral surface of heel flaps, continuing almost to the disc of the 5$^{th}$ toe; 10) dorsal coloration in life green with scattered brownish or black flecks.

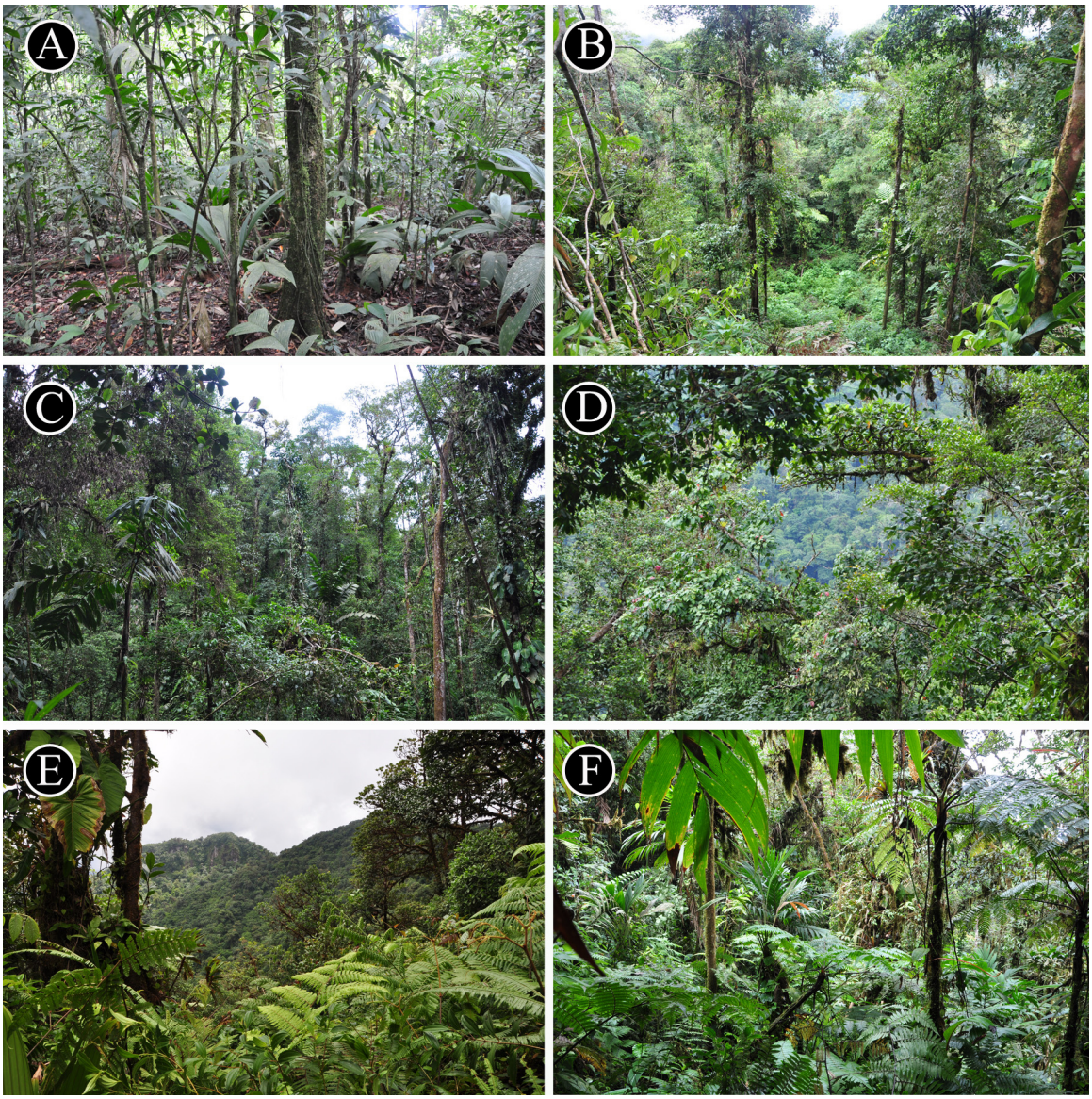


**FIGURE 5.** Habitat of *Ecnomiohyla bailarina* A) understory area where holotype was caught; B-C) forest structure from an open area; D) canopy forest; E) Cerro Bailarín, view from a ridge to 900 m a.s.l.; F) understory at Cerro Bailarín.

**Comparison with other species of *Ecnomiohyla*.** *Ecnomiohyla bailarina* can be distinguished from other species of *Ecnomiohyla* by the following characters (with contrasting features for *E. bailarina* in parentheses, see Table 3 for more details): *Ecnomiohyla minera, E. thysanota* (see Fig. 6) and *E. rabborum* are easily distinguished from the new species by having smooth heels without a scalloped fringe (triangular serrate fringe with pointed tubercles on a ventral surface of heel flaps); *E. rabborum* and *E. minera* are further distinct in having a humeral projection in males (no humeral projection); *E. rabborum* has a substantial webbing on one finger only, reaching

base of disk on one finger (webbing extensive reaching base of disk on two fingers); *E. echinata, E. fimbrimembra, E. minera, E. salvaje* and *E. valancifer* lack of cranial or dorsal osteoderms (well developed cranial and dorsal osteoderms); the type locality of the only known specimen of *E. thysanota,* a female collected at Cerro Malí, Darién (Duellman 1966), is only 100 km northeast of the type locality of *E. bailarina*, but *E. thysanota* lacks cranial and dorsal osteoderms (well developed cranial and dorsal osteoderms, see Figs. 3−4, and 6), skin on dorsum is granular (strongly tuberculate), coloration in life is reported to be uniformly green (green with scattered brown or blackish flecks); in addition, these potentially sympatric species would probably differ also in size, as the *E. thysanota* specimen is a female that is much larger (95 mm vs 68.1 mm SVL, see Table 3) than our male *E. bailarina*; males in *Ecnomiohyla* spp. tend to be bigger or at least the same size as females (Table 3, Savage & Kubicki 2010), and hence, an adult male *E. thysanota* is presumed to be considerably larger than the adult male holotype of *E. bailarina; E. fimbrimembra* (see Fig. 9), *E. miliaria*, and *E. phantasmagoria* also lack a fringe on heels (present), but have pointed heel tubercles; in addition, males of *E. miliaria* and *E. phantasmagoria* have a sharp prepollical spine directed laterally (prepollical spine vestigial, bluntly pointed and directed to the thumb); *E. fimbrimembra* and *E. salvaje* have the skin on the head co-ossified with the cranium, (Fig. 9 E–H) (skin not co-ossified with cranium); males of *E. miliaria, E, phantasmagoria, E. sukia, E. tuberculosa* and *E. valancifer* have no nuptial black spines on prepollex (numerous small black keratinized spines present on prepollex); *E. miotympanum* lacks of scalloped dermal fringes on the outer margin of the forearm and foot, large digital disks, and enlarged prepollices (present in *E. bailarina*); *E. tuberculosa* does not have a prepollical projection in adult males (prepollical projection present); in *E. sukia*, the prepollical spine has a similar size and direction, but is rather spade-like, not forming a sharp spine as in *E. bailarina*; *E. veraguensis* (sp. nov., see below) can be distinguished from *E. bailarina* by having only a few large, widely spaced nuptial black keratinized spines, dorsolaterally on the base of the pollex and none on the prepollex in adult males (thickly clustered smaller spines on prepollex and pollex; Fig. 10); further, it has a finely tuberculated dorsum (strongly tuberculated dorsum), and keratinized tubercles on the ventral side of the scalloped fringe on the heels are absent (present in *E. bailarina*).

**TABLE 4.** Measurements and morphological proportions for the holotypes of the new *Ecnomiohyla* species described herein.

| Trait | Measurements (mm) | | Trait | Proportions (%) | |
|---|---|---|---|---|---|
| | *E. veraguensis* | *E. bailarina* | | *E. veraguensis* | *E. bailarina* |
| SVL | 57.8 | 68.1 | IND/SVL | 9.7 | 8.2 |
| HL | 20.2 | 22.0 | HL/SVL | 34.9 | 32.3 |
| HW | 23.6 | 24.1 | HW/SVL | 40.8 | 35.4 |
| IOD | 14.7 | 14.1 | HL/HW | 85.6 | 91.3 |
| ED | 6.0 | 6.8 | IOD/SVL | 25.4 | 20.7 |
| TD | 3.6 | 4.2 | ED/SVL | 10.4 | 10.0 |
| HAL | 20.8 | 21.2 | TD/SVL | 6.2 | 6.2 |
| FAL | 11.8 | 16.7 | HAL/SVL | 36.0 | 31.1 |
| IND | 5.6 | 5.6 | FAL/SVL | 58.4 | 75.9 |
| TL | 32.8 | 35.8 | TL/SVL | 56.7 | 52.6 |
| FL | 28.0 | 27.6 | FL/SVL | 48.4 | 40.5 |
| 3FW | 2.2 | 3.1 | 3FW/SVL | 3.8 | 4.6 |
| 3FD | 3.4 | 4.4 | 3FD/SVL | 5.9 | 6.5 |
| 4TW | 2.0 | 3.4 | 4TW/SVL | 3.5 | 5.0 |
| 4TD | 2.7 | 3.4 | 4TD/SVL | 4.7 | 5.0 |
| 3TW | 2.1 | 2.9 | 3TW/SVL | 3.6 | 4.3 |
| 3TD | 2.7 | 3.1 | 3TD/SVL | 4.7 | 4.6 |
| BW | 30.5 | 19.7 | BW/SVL | 52.8 | 28.9 |

**Description of the holotype.** An adult male, as indicated by the presence of keratinized nuptial spines. Measurements of the holotype are shown in Table 4. Head rounded in dorsal view, slightly wider than long (HL/HW = 91.3%); snout truncate in dorsal and lateral views; nearly terminal nostrils directed laterally; top of head flat; canthus rostralis concave; loreal region concave; skin on dorsal surface of head and body tuberculate, tubercles formed by osteoderms; tubercles on upper lip, loreal and supraorbital area tipped with tiny blunt keratinous spines; lower eyelid with transparent upper part; a well-developed supratympanic fold running from midpoint of posterior margin of eye above the upper margin of tympanum, slightly curved around its upper posterior edge, tympanum prominent, opaque, smooth, 51.5% of ED, separated from eye by 3.20 mm; upper surfaces of body and limbs tuberculate, intermixed with scattered larger tubercles, cluster of tubercles above the insertion of arms; a triangular serrate-like fringe extends from the elbow along the ventrolateral margin of the forearm and continues along the outer edge of Finger IV to the base of the disk; serrate fringe largest on forearm, less evident serration along fingers; hands moderate in length (HAL/SVL = 31.1%); Finger lengths I<II<IV<III, terminal disk on Finger I 79% of diameter of disks on Fingers II–IV; which are almost the same size as tympanum (3FD/TD 1.04 times); distal subarticular tubercles on Fingers I–III large, rounded; bifid at Finger IV, larger than proximal subarticular tubercles on Fingers III–IV; indistinct supernumerary tubercles; prepollex enlarged and rounded; bony prepollical projection rounded distally, bluntly pointed at side adjacent to thumb; two clusters of nuptial spines at the distal end of prepollical tubercle and at the end of the first phalanx of the thumb; fingers extensively webbed, web extending to base of disk on at least two fingers; webbing formula: **I** $1^{3/4}$–2 **II** $^{3/4}$–$1^{1/2}$ **III** $1^{1/2}$–$1^{1/4}$ **IV**; legs relatively long and slender (TL/SVL = 52.6%), heels of adpressed limbs overlapping about 1/4 length of tibia, thigh 30.00 mm long; distinct fleshy, triangular serrate like fringe begins on heel by a striking flap and extends along ventrolateral margin of tarsus and outer margin of Toe V to base of disk; scallops deeply incised and pointed, largest on tarsus, smaller along toe; small tubercles with keratinized tips present on dorsal and ventral surface of fringe on heel; tarsal fold and outer metatarsal tubercle absent, inner metatarsal tubercle moderately large (same size as 3TD), ovoid, flat, and spadelike distally; toe lengths I<II<III=V<IV; disks on toes 75% of diameter of those on fingers, equal on Toes III–V, decreasing in size on toes II–I; subarticular tubercles rounded; supernumerary tubercles indistinct; toes extensively webbed, webs extending to base of disks on at least four toes; webbing formula: **I** $^{3/4}$–$1^{1/4}$ **II** $^{3/4}$–$1^{1/4}$ **III** $^{3/4}$–1 **IV** $1^{1/4}$–$^{3/4}$ **V**; gular area and venter strongly granulate, fine granulation on undersides of arms and proximal thighs, smooth skin on anterior surfaces of thighs and ventral parts of legs; cloacal opening directed posteriorly at mid-level of thighs, two distinct granular dermal folds under the vent; tongue slightly cordiform; vomerine ridges transverse, narrowly separated medially, placed between the posterior margins of the moderately large ovoid choanae; vomerine teeth 12–13; vocal slits not present.

**Coloration of holotype in life (Fig. 3).** Dorsal ground colour Light Grass Green (color 109 of Köhler 2012) with irregular Vandyke Brown (281) flecks scattered all over the head and body giving the animal a "moss cryptic" appearance; Raw Umber (22) bands present on dorsal surfaces of arms and legs, edges of scalloped fringes on arms and fleshy flaps on heels Cream Color (12); toe webbing Tawny Olive (17); tops of some dorsal granules and tubercles Orange-Rufous (56). After metachrosis (day and night coloration), ground coloration faded to Pale Emerald Green (141), brown areas to Dark Salmon color (59), pattern did not change; throat, chest, venter and ventral surfaces of arms and legs Cream Color (12) grading into Salmon (83) ventrolaterally and Orange Yellow (8) on anterior surface of thigh; a few small dark blotches on the edge of lower lip; iris Light Yellow Ocher (13), finely reticulated with Dark Brownish Olive (127); tympanum Pale Mauve (204) with scattered irregular Vinaceous Pink (245) blotches.

**Coloration in preservative (Fig. 4).** Dorsal surfaces Glaucous (272) with Sepia (279) mottling on upper surfaces of hind limbs; tympanum Pratt's Payne's Gray (293) with scattered irregular Maroon (39) blotches, cloacal region Pratt's Payne's Gray (293) dorsally and Cream (12) ventrally; posterior surfaces of thighs Light Yellow Ocher (13); ventral surfaces of body and limbs Cream (12); toe webbing Amber (51).

**Distribution and natural history.** *Ecnomiohyla bailarina* is known only from the type locality, in the eastern Panamanian montane forest (Fund & Hogan 2012; Fig. 5 A-D). The potential area of distribution of *E. bailarina* comprises the vicinities of Jingurudó and Sapo mountain ranges, between 400 to 1400 m a.s.l. (Fig. 1). Although the type locality is in a primary forest, there are some open areas with successional secondary forest. The area is on a ridge, so the trees could be affected by strong winds. In the surroundings we saw four fallen large trees probably overthrown by the wind that left clearings in the otherwise pristine forest. The largest trees in this area reached more than 20 m in height having branches in the canopy covered by bromeliads and other epiphytes (e. g., orchids

and Lorantaceae), Tree trunks were almost bare or with just a little epiphytic growth. In the understory, palms and vines were predominant. The holotype was found on a ridge in a water conserving posture (see Fig 1B in Pough *et al.* 1983) on the bark of a small tree (Fig. 5A), approximately 1.5 m above the ground. The day before the night of the capture was dry except for a drizzle that had fallen in the afternoon between 14:00–15:00 hrs. During the encounter, a slight breeze was blowing. Other amphibian species observed in the area that day were: *Colostethus* aff. *pratti* (Boulenger, 1899), *Craugastor opimus* (Savage and Myers, 2002), *Pristimantis cruentus* (Peters, 1873), *P. taeniatus* (Boulenger, 1912), *Rhinella alata* (Thominot, 1884), and *Sachatamia ilex* (Savage, 1967).

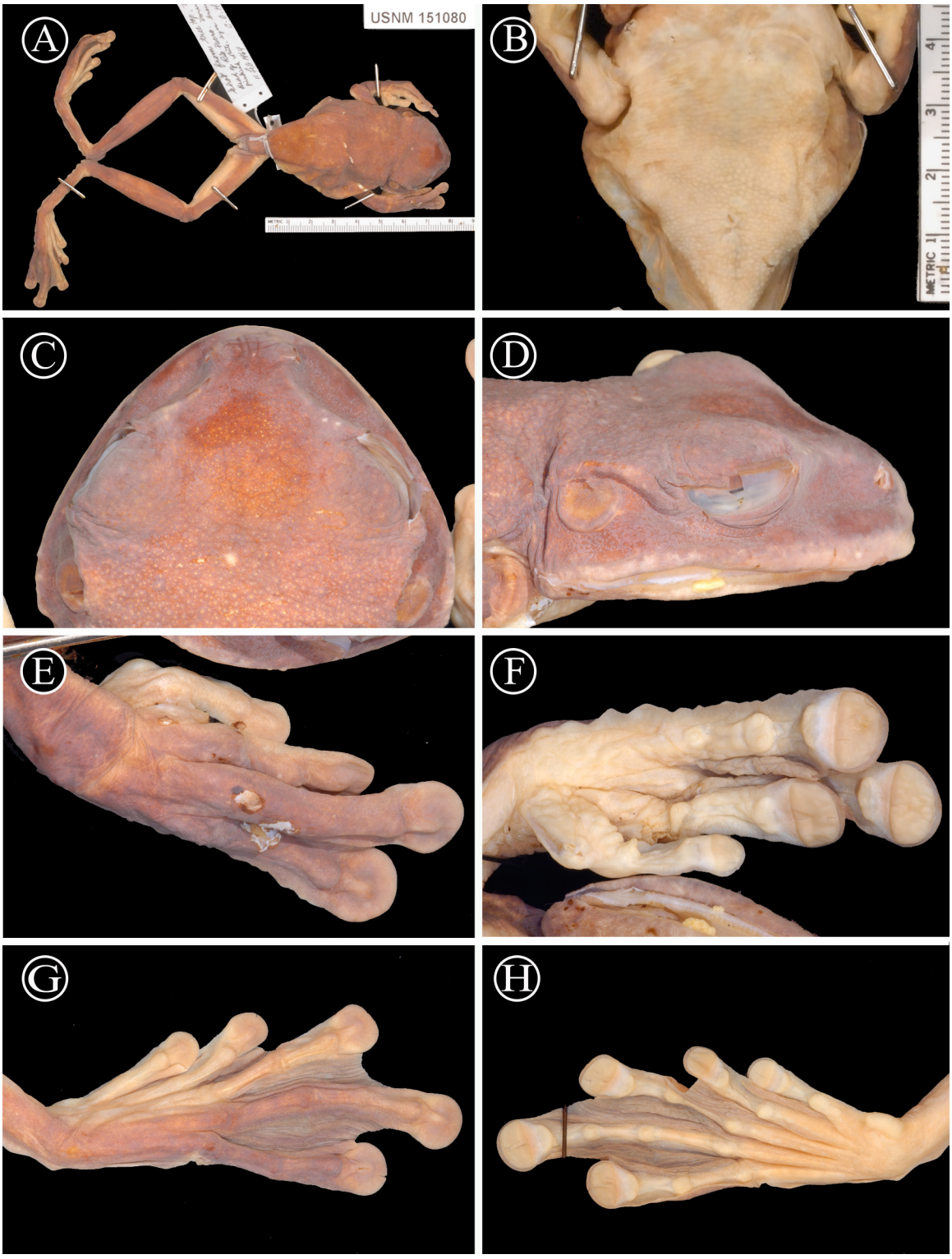


**FIGURE 6.** *Ecnomiohyla thysanota*, Holotype (USNM151080), preserved specimen. A) dorsal view; B) ventral view; C) head dorsally; D) head in profile; E) right hand dorsally; F) right hand ventrally; G) right foot dorsally; H) right foot ventrally.

**Etymology.** The name *bailarina* is a noun in apposition in reference to the hill where the specimen was found. The indigenous people of the Embera call it "Cerro Bailarín", in addition, the English translation of "bailarina" is ballerina, so the name also refers to the resemblance of the fringes on arms and feet of the frog to the tutu skirt that a ballerina wears.

**Conservation status.** The secretive habits of *Ecnomiohyla bailarina* make the assessment of the population size difficult, as in other *Ecnomiohyla* species. Considering that the status of the *E. bailarina* population is unknown, the data deficient (DD) criterion, according the IUCN (IUCN 2013), seems appropriate for this species, until data on its population trend become available. Moreover, due to fact that *E. bailarina* and *E. thysanota* occur in a region affected by social problems and political conflicts along the border between Panama and Colombia, it is unlikely that there will be sufficient opportunity to visit the region to assess population sizes in the near future.

### *Ecnomiohyla veraguensis* sp. nov.

*Ecnomiohyla rabborum*–Köhler 2011: p. 224 Fig. 537; p. 226 Fig. 541 b.

**Holotype.** SMF89877 (original field number AH210) an adult male (Figs. 7–8) collected near Cerro Negro (8.5533 °N, -81.09261 °W, 540 m a.s.l.), Santa Fé National Park, Veraguas, Panama, on 31 March 2009 at 12:00 hrs, collected by Smelin Abrego, Arcadio Carrizo, Andreas Hertz, and Sebastian Lotzkat.

**Diagnosis.** A medium-sized species of *Ecnomiohyla.* The single known specimen is an adult male, 57.8 mm in SVL) differing from other known species of the genus by following combination of characters: 1) finger webbing extensive, web touching the finger disk on at least one side on Fingers II–IV; 2) toes extensively webbed; web reaching the toe disk at least on one side on four toes; 3) skin on dorsum finely tuberculate with scattered minute keratin tipped tubercles posteriorly; 4) cranial and dorsal osteoderms present; 5) skin on upper surface of head not co-ossified with underlying cranial elements; 6) humerus without enlarged *crista lateralis*; 7) prepollex distinct, recurved, with distinct bony prepollical projection, spadelike and directed laterally; 8) 6–8 widely spaced, keratinized black spines present bordering the outer side of the thumb; 9) a distinct scalloped fringe without pointed tubercles on its ventral surface, arising at the heel and continuing on the outer side of Toe V and reaching almost to the disk of Toe V (Fig. 8); 10) dorsal coloration in life smoke gray, with upper surface of forearms bearing a suggestion of lime green (Fig. 7).

**Comparison with other species of *Ecnomiohyla*.** *Ecnomiohyla veraguensis* can be distinguished from other species of *Ecnomiohyla* by the following characters (with contrasting features for *E. veraguensis* in parentheses; see Table 3 for more details): *E. echinata, E. minera*, *E. rabborum, E. salvaje, E. thysanota,* and *E. valancifer* can be distinguished from the new species by the lack of cranial and dorsal osteoderms (both present); *E. rabborum* and *E. minera* are further distinct in having a humeral projection in males (no humeral projection); *E. rabborum* has substantial finger webbing, web reaching base of disk on one finger (extensive webbing, web touching the finger disk on at least one side on Fingers II–IV); *E. fimbrimembra* (Fig. 7 E-F), *E. miliaria* and *E. phantasmagoria* lack scalloped fleshy fringes on heels and have pointed heel tubercles instead (scalloped fleshy fringes present, no heel tubercles); *E. fimbrimembra* and *E. salvaje* have the skin on the head co-ossified with the cranium, (skin not co-ossified with cranium); males of *E. miliaria* and *E. phantasmagoria* have a sharp prepollical spine protruding from the prepollex (prepollex recurved, no protruding spine); *E. bailarina* has a strongly tuberculate dorsum (finely tuberculate) and two clusters of numerous, small nuptial spines at the distal end of the prepollex and the base of the pollex (only 6–8 larger, widely spaced nuptial spines along the outer side of the pollex; Fig. 10); *E. tuberculosa* lacks an enlarged prepollical bony projection or keratinized black spines on the prepollex in adult males (enlarged prepollical bony projection and 6–8 widely spaced, keratinized black spines presents); *E. miotympanum* lacks of scalloped dermal fringes on the outer margin of the forearm and foot, large digital disks, and enlarged prepollices (present in *E. veraguensis*); in terms of general appearance, *E. sukia* is most similar to the new species, but differs by a genetic distance in the 16S gene of 7% and the lack of nuptial spines in adult males (6–8 widely spaced nuptial spines on the outer side of the pollex; see Fig. 9 (B, D) and 10); further, *E. sukia* lacks keratin tipped tubercles on the dorsum (presence of keratin tipped tubercles on the dorsum).

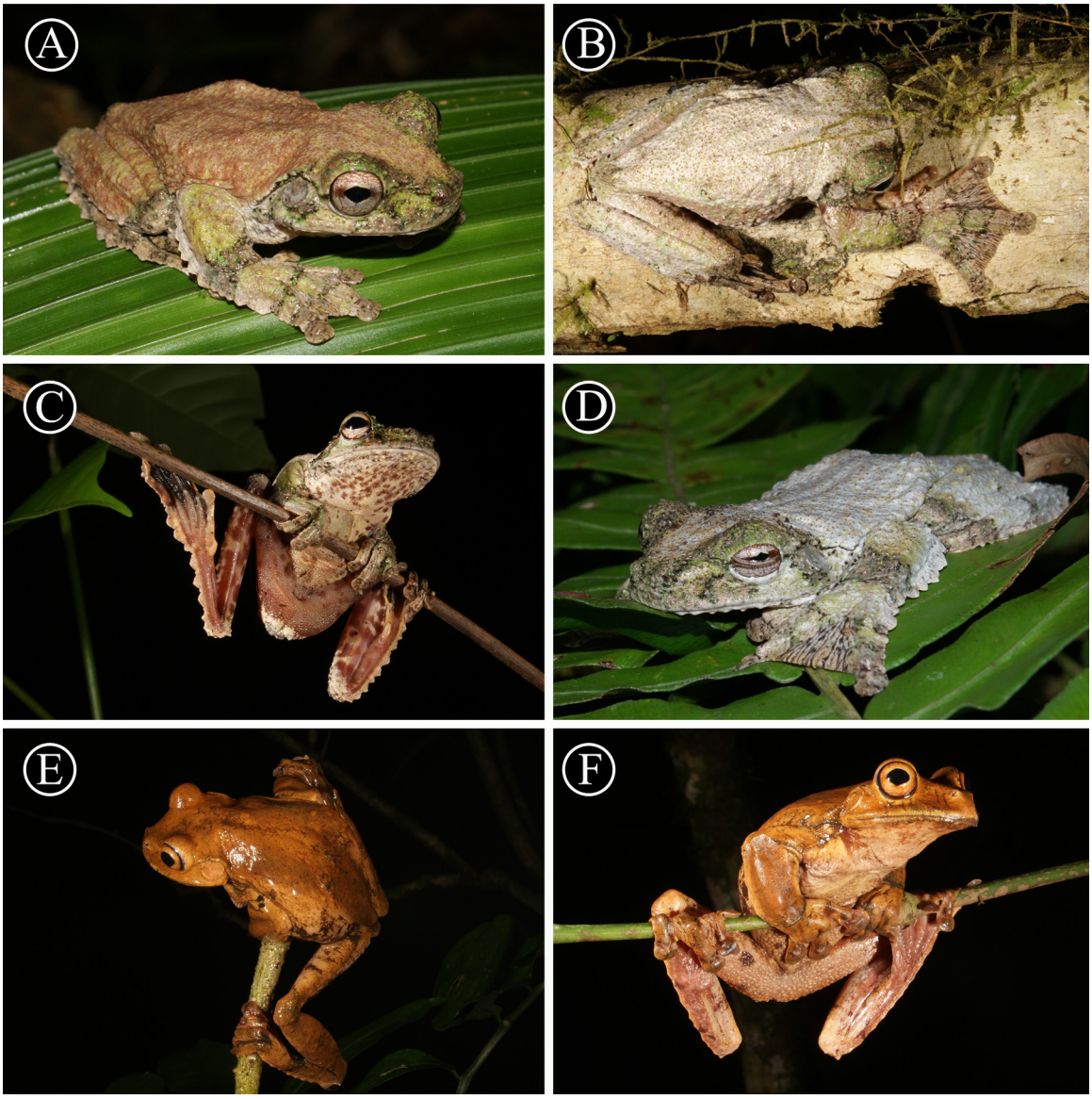


**FIGURE 7.** Photographs of the holotype of *Ecnomiohyla veraguensis* in life. A) lateral view (night time); B) dorsal view (day time); C) frontal view; D) at the moment of encounter; E-F) voucher specimen of *E. fimbrimembra* (SMF89857).

**Description of the holotype.** An adult male, as determinated by the presence of nuptial spines, and vocal slits. Measurements of the holotype are indicated in Table 4. Head rounded in dorsal view, wider than long (HL/HW= 85.6%); snout truncate in dorsal and lateral views; nostrils directed laterally; top of head flat; canthus rostralis concave; loreal region concave; a well-developed supratympanic fold running from above the upper margin of tympanum, slightly curved around its upper posterior edge; tympanum prominent, smooth, same color as dorsum, and 60.0% of ED; separated from eye by 3.04 mm; upper surface of body finely tuberculated, scattered tubercles present on dorsal surfaces of the limbs; arms robust, hypertrophied; a fleshy scalloped fringe extends from the elbow along the ventrolateral margin of forearm and continues along the outer edge of Finger IV to base of disk; scallops of fringe largest on forearm, weak scallops along finger; hands moderate in length (HAL/SVL= 36.0%); finger lengths I<II<IV<III, terminal disk on Finger I 70% of diameter of disks on Fingers II–IV, which are slightly smaller in size than tympanum (3FD/TD= 94%); distal subarticular tubercles on fingers rounded and elevated; a row of supernumerary tubercles present under the first phalanges on Fingers II–III; palmar tubercles rounded and low; prepollex enlarged and recurved, bony prepollical projection spadelike, directed laterally at side adjacent to thumb; 6–7 nuptial spines along the outer side of the pollex; fingers extensively webbed, web extending to base of disks on at least two fingers; webbing formula: **I** $1^{3/4}$–2 **II** $^{3/4}$–$1^{1/4}$ **III** $1^{1/4}$–$^{3/4}$ **IV**; legs relatively long and slender (FL/SVL= 48.4%), heels of adpressed limbs overlapping about 1/3 length of tibia, thigh 26.70 mm long; distinct fleshy,

scalloped fringe begins on heel and extends along ventrolateral margin of tarsus and outer margin of Toe V to base of disk; scallops sinuously serrated, widest on tarsus, smaller along toe; tarsal fold slightly evident; outer metatarsal tubercle barely distinct, inner metatarsal tubercle large (1.12 times 3TD), ovoid, slightly elevated, and spadelike distally; toe lengths I<II<III>V<IV; disks on toes 80–91% of diameter of those on fingers, disk on Toe IV same size as disks on Toes III and V, decreasing in size on Toes II–I; subarticular tubercles rounded and slightly elevated; a row of 5–10 supernumerary tubercles barely distinct under the proximal phalanges on toes; extensive toe webbing , webs extending to base of disks on at least four toes; webbing formula: **I** $^{3/4}$–$1^{1/2}$ **II** $^{3/4}$–$1^{1/4}$ **III** $^{3/4}$–$^{3/4}$ **IV** $^{1/4}$–$^{3/4}$ **V**; gular area and venter granulate, fine granulation on undersides of arms and proximal thighs, smooth skin on anterior surfaces of thighs and ventral parts of legs; cloacal opening directed posteriorly at mid-level of thighs, a distinct granular dermal fold under the vent. Tongue slightly cordiform, broader at the base; vomerine ridges large and transverse, well separated medially, placed between the posterior margins of choanae; vomerine teeth 10–14; paired vocal slits extending posteriorly from posterior lateral base of tongue toward angle of jaws.

**Coloration of holotype in life (Fig. 7).** Coloration in life was recorded at daytime: Dorsal ground color Smoke Gray (44); snout, canthus rostralis, and supraorbital regions Brownish Olive (29) suffused with Olive Green (Auxiliary 47); upper surfaces of forearms with a suggestion of Lime Green (59); dorsal surfaces of finger webbing like dorsal coloration on body, but toe webbing Vandyke Brown (121); ventral surfaces of chin and body Cream Color (54), spotted with Raw Sienna (136); ventral surfaces of hindlimbs True Cinnamon (139); ventral coloration of toe and finger webbings Vandyke Brown (121).

**Coloration in preservative (Fig. 8).** Dorsal surfaces Grayish Horn Color (268); snout, canthus rostralis, and supraorbital regions Medium Plumbeus (294); darker bars on upper surfaces of limbs Medium Plumbeus (294); cloacal region Pale Buff (1), suffused with Medium Plumbeus (294); groin and posterior surfaces of thighs mottled with Maroon (39) on a Pale Buff (1) ground; ventral regions Pale Buff (1); chin suffused with Maroon (39); toe and finger webbing Burnt Umber (48).

**Distribution and natural history.** *Ecnomiohyla veraguensis* is known only from the type locality in the Isthmian-Pacific moist forests (Fund & Hogan 2012). The holotype was found at noon on a sunny day at the end of the dry season. Relative air humidity at the moment of encounter was 68% at a temperature of 21.8 °C and it was slightly windy. The frog was sitting in a water conserving posture (Fig. 7D) on a fern leaf approximately 0.5 m above the ground, next to a water tube that is used by local people to obtain drinking water. Other amphibian species that were observed at Cerro Negro on this expedition conducted between March 31 and April 03 2009 include *Atelopus varius* (Lichtenstein & Martens, 1856), *Bolitoglossa colonnea* (Dunn, 1924), *Craugastor gollmeri* (Peters, 1863), *C. megacephalus* (Cope, 1875“1876“), *Diasporus citrinobapheus* Hertz, Hauenschild, Lotzkat & Köhler, 2012, *Lithobates warszewitschii* (Schmidt, 1857), *Pristimantis caryophyllaceus* (Barbour, 1928), *P. cerasinus* (Cope, 1875 “1876“), *P. cruentus*, *P. museosus* (Ibáñez, Jaramillo & Arosemena, 1994), *P. pardalis* (Barbour, 1928), *Rhaebo haematiticus* Cope, 1862, and *Sachatamia albomaculata* (Taylor, 1949). Since *E. veraguensis* is only known from a single specimen from a single locality, the distribution is unknown. It is expected to occur along mid-elevations of the Serranía de Tabasará.

**Etymology.** The species name is derived from the province name Veraguas where the holotype was found, with the Latin suffix *-ensis* donating a place or locality. The species name has been chosen to accentuate the particular role the province of Veraguas plays in terms of amphibian conservation. It is the only Panamanian province with Atlantic and Pacific coasts, thus encompassing a great variety of habitats for many amphibian species.

**Conservation status.** As other *Ecnomiohyla* species, *E. veraguensis* could be considered as a rare species, due to the habitats it uses, this fact makes it difficult to assess its populations. Like *E. bailarina*, the data deficient (DD) criterion, according the IUCN (IUCN 2013), seems appropriate for *E. veraguensis* too, until data on its population trend become available.

## Discussion

We describe *Ecnomiohyla bailarina* based on both molecular and morphological data. This spectacular species appears to be very distinct from all other known members of the genus. The type locality of *E. bailarina* is relatively close to that of *E. thysanota* (Fig. 6), and our first assumption in the field was, that they could be conspecific. However, after comparing pictures of the *E. thysanota* holotype with our specimen we easily detected

several substantive differences in morphology, mainly demonstrated by the presence of cranial and dorsal osteoderms in *E. bailarina* and not in *E. thysanota*, the different fringe shape on the heel as well as a different skin texture, which argue for two distinct species despite of the lack of molecular genetic data from *E. thysanota*.

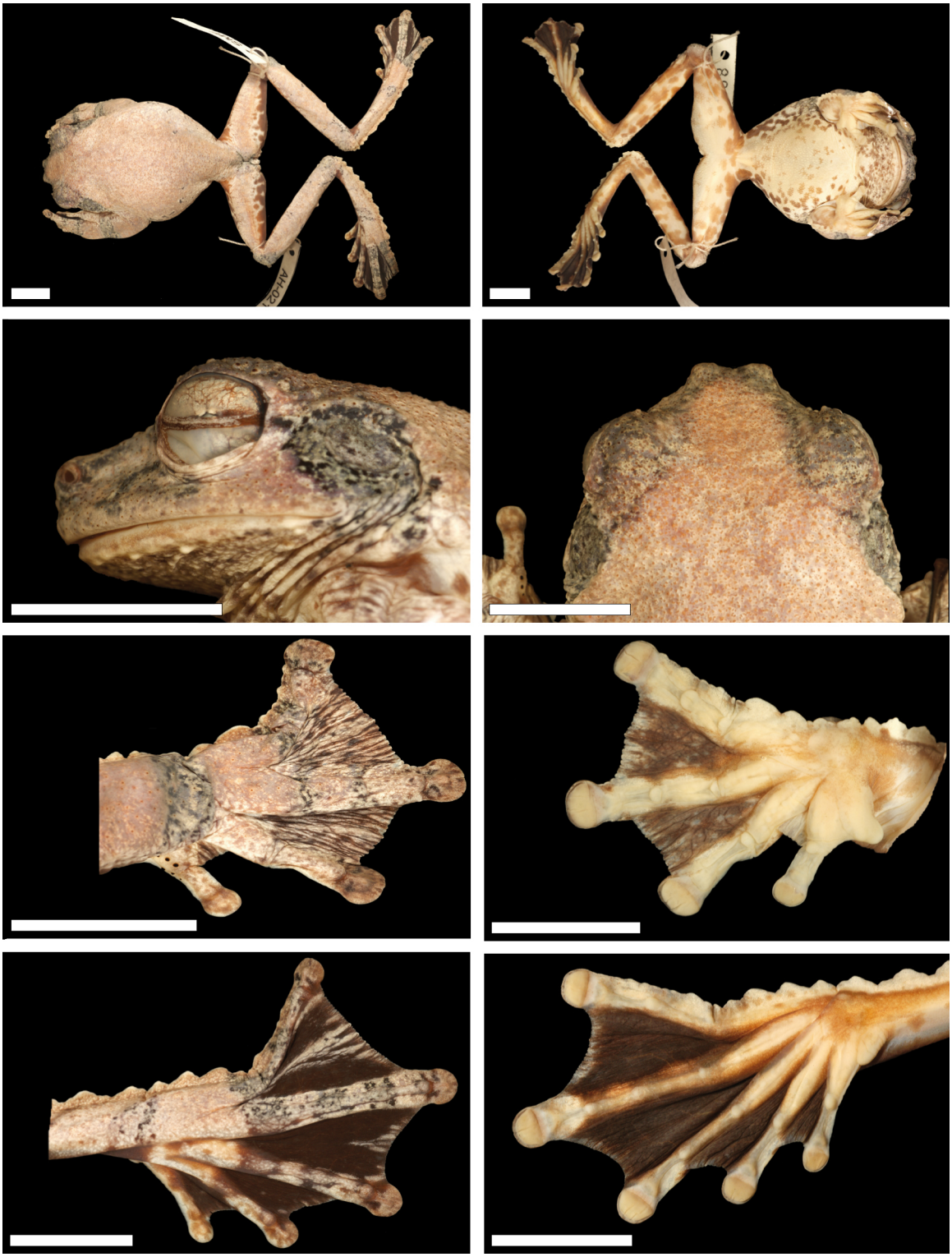

**FIGURE 8.** Holotype of *Ecnomiohyla veraguensis* in preservation. A) dorsal view; B) ventral view; C) head in profile; D) head dorsally; E) right hand dorsally; F) right hand ventrally; G) right foot dorsally; H) right foot ventrally. Scale bars= 10 mm.

*Ecnomiohyla veraguensis* is similar to *E. sukia* in overall appearance and both species clusters as sister clades in the 16S tree. However, the genetic distance between them is 7% and thus far above the threshold of 3%, that is commonly used to identify potential candidate species with 16S mtDNA barcoding in the tropics (Vences *et al*.

2005; Fouquet *et al*. 2007; Jansen *et al.* 2011; Crawford *et al*. 2010, 2013). Additionally, *E. sukia* lacks nuptial spines in all examined males (Fig. 10D; Brian Kubicki pers. comm. 2011). Although the appearance of spines and tubercles could be influenced by seasonality in some other species (Mendelson *et al.* 2008), this seems not to be the case in *E. sukia*, for which two adult males have been examined, and one was kept in captivity for four years without evidence of developing any nuptial spines (Savage & Kubicki 2010). Moreover, the holotype of *E. sukia*, an adult male, was collected on 25 March 1999, thus in the same season (see ETESA 2009, and IMN 2009) as the holotype of *E. veraguensis*, but showed no nuptial spines.

So far, this is the most comprehensive phylogenetic study of the genus *Ecnomiohyla* based on molecular data. DNA sequences of few species of fringed frogs were already used in several older large-scale phylogenies (Faivovich at al. 2005, Wiens et al. 2010; Pyron & Wiens 2011) to uncover the phylogenetic relationships inside the Hylidae, but without comments to on relations inside the genus. Herein we used sequence data for six of the seven *Ecnomiohyla* species known from lower Central America and for *E. miotympanum*. Since our motivation is to identify species delimitations through genetic barcoding, we used only the widely accepted mitochondrial 16S marker (Monaghan *et al.* 2009; Vieites *et al.* 2009). Deeper analyses, including nuclear markers to support the phylogenetic relationships between the species are certainly needed.

Anyway, we found incongruence between our molecular phylogenetic analysis and the three morphological groups suggested by Savage & Kubicki (2010). In their concept *Ecnomiohyla bailarina* would clearly meet the Group 2 criteria, whereas *E. veraguensis* meets the criteria of Group 3, assuming that nuptial spines are not necessarily absent, but only largely reduced. Our phylogenetic analyses revealed two major clades in the genus. One clade contains only *E. miotympanum* and stands opposed to the rest of the species in the genus. The latter is divided into two subclades, separated by a well-supported node in both trees (bootstrap value, bs: 99.8; posterior probability, pp: 100): Sub-clade 1 contains *E. rabborum*, *E. bailarina*, and *E. fimbrimembra*; and subclade 2 contains *E. sukia*, *E. veraguensis*, and *E. miliaria*. Thus, *E. bailarina* appears to be closer related to *E. rabborum* (Group 1), than to *E. fimbrimembra* (Group 2). Our subclade 2 also contains all available species assigned to Group 3 by Savage & Kubicki (2010) (Fig. 2). As a consequence of these results, we modify the groups proposed by Savage & Kubicki (2010) into two consequent groups, characterized as follows (for the remaining species, see below): In members of the *E. fimbrimembra* species group (Group 1), male frogs may have a bony humeral projection or not, but always have conspicuous cluster of black keratinized nuptial spines on thumb and prepollex, as demonstrated in species *E. bailarina, E. echinata, E. fimbrimembra, E. minera*, *E. rabborum*, and *E. salvaje.* While in the *E. miliaria* species group (Group 2), male frogs have neither humeral projection nor black nuptial spines on the prepollex. A few nuptial spines may be present on the thumb, but if this is the case these are fewer than ten, usually light brown (not black) and widely spaced (not building a cluster); this group contains *E. miliaria*, *E. phantasmagoria*, *E. sukia*, *E. valancifer*, and *E. veraguensis* (Fig. 10).

In our phylogeny *E. miotympanum* is the only member of an own species group within *Ecnomiohyla* and appears as a sister clade to other *Ecnomiohyla* what is strongly supported (bs: 89.6; pp: 97). Thus the molecular monophyly of all members of the genus we have data for, is confirmed here. Other recent studies suggested *Ecnomiohyla* may not be monophyletic with regard to *E. miotympanum*, even though this was not strongly supported (Wiens *et al.* 2010; Pyron & Wiens 2011). While our taxon sampling of *Ecnomiohyla* species is large, the molecular dataset is restricted to a single marker, so a deeper analysis is still needed. However, as a consequence from our results we continue to treat *E. miotympanum* as a member of the genus *Ecnomiohyla* for the moment. Savage & Kubicki (2010) pointed out that *E. tuberculosa* should not be included in the genus as it lacks the enlarged prepollex and prepollical bony projection, which is present in all other species in the genus. Pro tem, we are including *E. tuberculosa* within the genus, until further data becomes available. Further, we could not assign *E. thysanota* to one of the species groups, since the male of this species is not known yet and molecular data is lacking.

In Table 4, we have summarized the morphological characteristics of the two species described here compared to the other *Ecnomiohyla* species that are present in Lower Central America (Table 3). The genus is distributed as follows: In Lower Central America, Colombia and Ecuador, the fringe-limbed frog representatives are *E. bailarina, E. fimbrimembra, E. miliaria, E. phantasmagoria, E. rabborum*, *E. sukia, E. thysanota, E. tuberculosa,* and *E. veraguensis* (Ortega-Andrade *et al.* 2010; Savage & Kubicki 2010; Köhler 2011; Ron 2012; this paper); in Nuclear Central America the fringe-limbed frogs are *E. echinata, E. miliaria, E. minera*, *E. salvaje*, and *E. valancifer*; The only known species reaching North America in eastern and central Mexico is *E. miotympanum.* The most widespread species seems to be *E. miliaria*, which is found in Nuclear and Lower Central America, distributed

from south-eastern Honduras to central Panama (Köhler 2011). However, it seems likely that *E. miliaria* represents more than one species (Solís *et al.* 2010). One evidence for this assumption is that there are two specimens from Panama assigned to *E. miliaria*, one from the Reserva Forestal Fortuna dam site (Myers & Duellman 1982) and one from El Copé (Savage & Kubicki 2010), which indeed are different to *E. miliaria* from Nuclear Central America (revised by Savage & Kubicki 2010), *E. sukia* or *E. veraguensis* (different in skin texture and fringe shape, see Fig. 13 in: Myers & Duellman 1982).

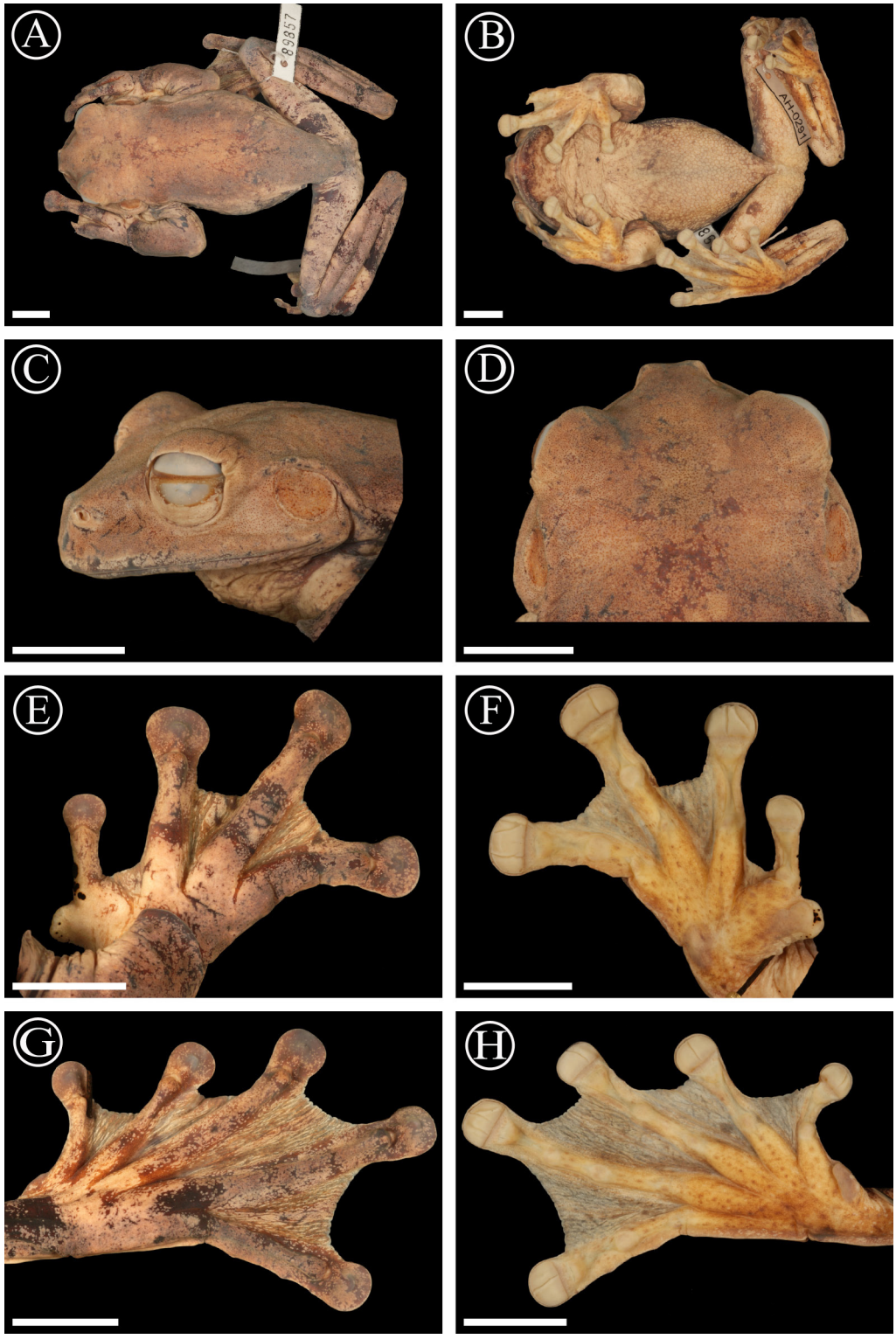


**FIGURE 9.** *Ecnomiohyla fimbrimembra* (SMF89857), preserved male specimen from Panama. A) dorsal view; B) ventral view; C) head in profile; D) head dorsally; E) right hand dorsally; F) right hand ventrally; G) right foot dorsally; H) right foot ventrally. Scale bars= 10 mm.

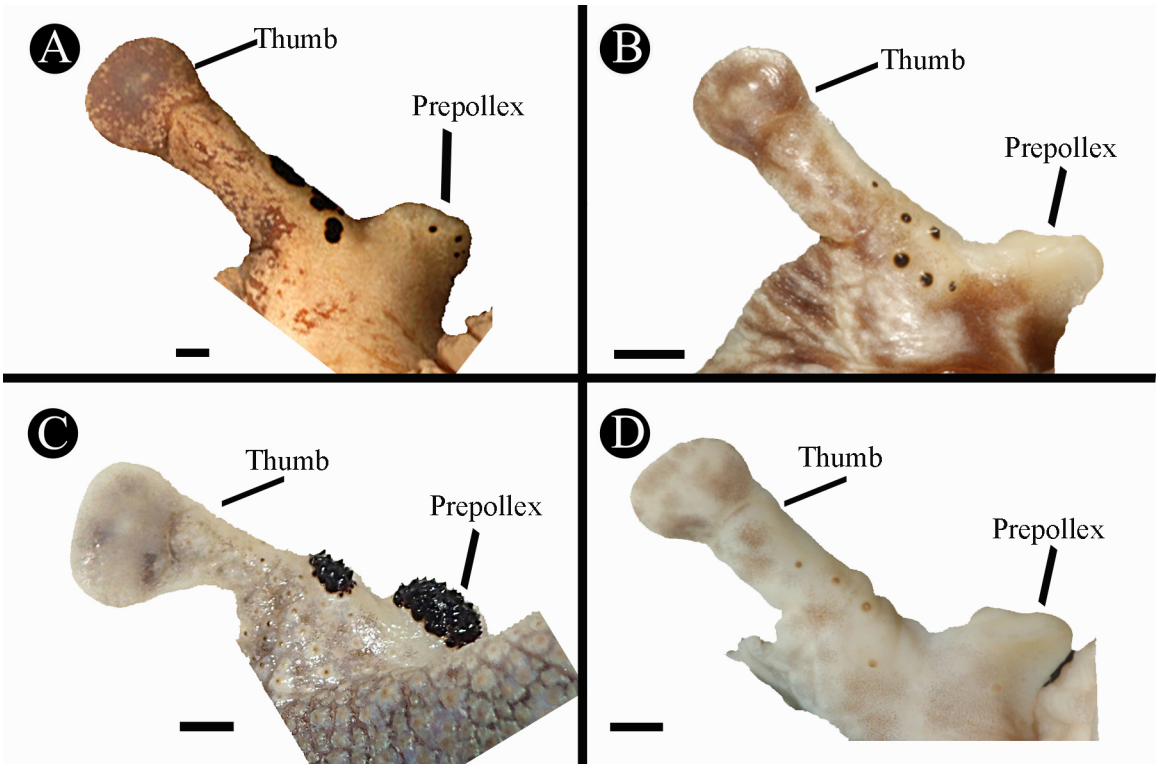


**FIGURE 10.** Details of the thumb and the keratinized black spines on prepollex, A) *Ecnomiohyla fimbrimembra* (SMF89857); B) *E. veraguensis* (SMF89877); C) *E. bailarina* (SMF97398); D) *E. sukia* (SMF94578). Scale bars= 1 mm

## Key to the species of the genus *Ecnomiohyla*.

1a. No scalloped dermal fringes on the outer margin of the forearm and foot . . . . . *E. miotympanum*
b. Scalloped dermal fringes on the outer margin of the forearm and foot present . . . . . 2
2a. Males without an enlarged prepollex and prepollical bony projection . . . . . *E. tuberculosa*
b. Males with an enlarged prepollex and prepollical bony projection . . . . . 3
3a. Webbing between Finger II–IV not extending beyond penultimate subarticular tubercle on Finger III (Fig. 9F); supratympanic fold continuing posteriorly behind tympanum to terminate above axilla (Fig. 9C); dermal fringe along lateral edge of forearm and tarsus narrow and not or only weakly scalloped; dorsal skin smooth to minutely granular; skin of dorsal surface of head co-ossified with skull . . . . . *Enomiohyla frimbrimembra*
b. Webbing between Finger II–IV extending well beyond penultimate subarticular tubercle on Finger III; supratympanic fold not continuing posteriorly behind tympanum to terminate above axilla; dermal fringe along lateral edge of forearm and tarsus usually prominent and scalloped; dorsal of variable texture; skin of dorsal surface of head co-ossified with skull or not . . . . . 4
4a. Dorsum uniform green in life; skin of dorsal surface of head not co-ossified with skull, and granular; heel without tubercles but with a well-defined, scalloped dermal fold (Fig. 6A) . . . . . *Ecnomiohyla thysanota*
b. Dorsum brown, reddish brown, or brown with green or darker brown markings or mottling; dorsum granular or tuberculate; skin of dorsal surface of head in adults co-ossified with skull or not; condition of heel variable . . . . . 5
5a. Dorsum tuberculate; osteoderms usually present . . . . . 6
b. Dorsum granular or smooth; without osteoderms . . . . . 12
6a. Humeral projection present; heel without tubercles; prepollex in adult males with scattered small black spines . . . . . *Ecnomiohyla minera*
b. No humeral projection; heel with one or several tubercles or, if without tubercles then with a scalloped fringe; prepollex in adult males with or without small black spines. . . . . 7
7a. Dorsum tuberculate without cranial or dorsal osteoderms; heel with one large tubercle; males with spade-like prepollex and flattened prepollical bony projection. . . . . *Ecnomiohyla valancifer*
b. Dorsum tuberculate with cranial or dorsal osteoderms; heel with one or several pointed tubercles or, if without tubercles then with a scalloped fringe; males with variable prepollex and prepollical bony projection . . . . . 8
8a. Webbing on fingers not reaching the base of disk on any digit . . . . . *Ecnomiohyla phantasmagoria*
b. Webbing on fingers reaching to base of disk on two to four but not all digits . . . . . 9
9a. Heel with one or several pointed tubercles; black keratin tipped tubercles over most of flanks and venter; prepollical bony projection in males terminating in a sharp spine in adults . . . . . *Ecnomiohyla miliaria*

b. Heel without tubercles but with a scalloped fringe; without black keratin tipped tubercles over most of flanks and venter; prepollical bony projection in males, variable . . . . . 10

10a. Males without keratinized black spines on prepollex; without black keratin tipped tubercles on dorsum (Fig. 10D). . . . . *Ecnomiohyla sukia*

b. Males with keratinized black spines on prepollex; black keratin tipped tubercles over most of dorsum . . . . . 11

11a. Dorsum strongly tuberculated; two clusters of nuptial spines at the distal end of prepollical tubercle and the base of prepollex in males (Fig. 10C) . . . . . *Ecnomiohyla bailarina*

b. Dorsum slightly tuberculated; without nuptial spines arranged in clusters, instead 6-7 nuptial spines scattered along the pollex (Fig. 10B) . . . . . *Ecnomiohyla veraguensis*

12a. Cephalic skin co-ossifed with skull; webbing on fingers extensive, reaching to the base of disk on two to four but not all digits; toe webbing full, reaching to the base of disks on all digits . . . . . *Ecnomiohyla salvaje*

b. Cephalic skin not co-ossifed with skull; finger webbing usually not reaching to the base of disk and if, then only on one digit; toe webbing never reaching to the base of disk on all digits. . . . . 13

13a. Dorsum smooth; humeral projection absent in males; heel with few small tubercles; SVL of adult females 60.2 mm, SVL of adult males 57 mm . . . . . *Ecnomiohyla echinata*

b. Dorsum granular; humeral projection present in males; heel smooth; SVL of adult females 61.3–79.9 mm, SVL of adult males 62.8–97.3 mm . . . . . *Ecnomiohyla rabborum*

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