A new species of Nobellia (Amphibia: Strabomantidae) from the Río Mandurícuaro Reserve on the Pacific slopes of the Ecuadorian Andes

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ABSTRACT
With the third most biodiverse amphibian fauna in the world, Ecuador has bolstered this claim with a particularly high rate of species descriptions in recent years. Many of the species being described are already facing anthropogenic threats despite being discovered within privately protected reserves in areas previously not sampled. Herein we describe a new species of terrestrial frog in the genus Nobellia from the recently established Río Mandurícuaro Reserve, Imbabura, Ecuador. Nobellia warleyae sp. nov. differs from its congeners by having a dorsum finely shagreen; tips of fingers I and IV slightly acuminate, Fingers II and III acuminate, without papillae; distal phalanges of the hand slightly T-shaped; absence of distinctive suprainguinal marks; venter yellowish-cream with minute speckling and throat with irregular brown marks to homogeneously brown. We provide a detailed description of the advertisement call of the new species and present an updated phylogeny of the genus Nobellia. In addition, we emphasize the importance of the Río Mandurícuaro Reserve as a conservation area to threatened fauna.

Ecuador is the third country most diverse in amphibians, and the description of species in the last decade has increased considerably, evidencing the presence of new species in areas protected from human activities. Among the species that have been found in recent years is the Noblella species of the Andes. Nobellia warleyae sp. nov. differs from its congeners by having a dorsum finely shagreen, tips of fingers I and IV slightly acuminate, Fingers II and III acuminate, without papillae; distal phalanges of the hand slightly T-shaped; absence of distinctive suprainguinal marks; venter yellowish-cream with minute speckling and throat with irregular brown marks to homogeneously brown. We provide a detailed description of the call of the new species and present an updated phylogeny of the genus Nobellia. In addition, we emphasize the importance of the Río Mandurícuaro Reserve as a conservation area to threatened fauna.

KEYWORDS
Western Andean slopes; terrestrial frog; phylogeny; conservation

Palabras claves: estribaciones occidentales de los Andes; ranas terrestres; filogénesis; conservación

Introduction

The genus Nobellia Barbour [1] currently contains 15 described species. Approximately half of these ground-dwelling, terrestrial-breeding frogs have been described in the last decade alone [2]. The genus is distributed from Colombia to Bolivia on both versants of the Andes, with the exception of N. myrmecoides, which occurs in the lowlands of the Amazon basin from southeastern Colombia to Bolivia, and Nobellia losamigos, which occurs along the eastern slopes of the Andes and adjacent Amazonian lowlands in southeastern Peru [2]. Members of Nobellia are some of the smallest frogs (SVL < 25 mm), with N. pygmaea being the smallest known species in the Andes [3,4]. Some of the external morphological characteristics that distinguish the genus are small size, head narrower than body, terminal discs of fingers and toes not expanded, conspicuous tarsal tubercle, and phalangeal reduction in Finger IV (in several species). Nevertheless, there are no known morphological synapomorphies for the genus. See Hedges et al. [5] for additional characters.

Although Nobellia is a morphologically homogenous taxon, it is not monophyletic in molecular phylogenies [4,6,7]. The absence of genetic information for the type species of Nobellia and the morphologically similar Psychrophynella adds ambiguity when allocating newly described species into a genus [4,6,7].
Although not rare in collections (e.g. Ecuadorian museums have good representation of the genus, mainly from the eastern slopes), the taxonomy of the genus is complex because of the morphological similarity among populations [8]. The majority of Noblella spp. have been described from the Amazonian versant of the Andes, with just two species known from the Pacific slopes of the Andes, namely N. coloma (northwest) and N. heyeri (southwest) [9,10]. Herein, we describe a new species of Noblella from the Río Manduríacu Reserve situated on the Pacific slope of the Ecuadorian Andes, a region facing rapidly advancing mining activities and, thus, a precarious future. The new species is morphologically and phylogenetically similar to N. coloma; however, it is easily distinguished from the latter by having distal phalanges of the hand slightly T-shaped (distinctly T-shaped in N. coloma), absence of distinctive suprainguinal marks (present in N. coloma), ventral yellowish-cream with minute speckling (orange with minute brown and white spots in N. coloma), and throat with irregular brown marks to homogeneously brown (orange with minute white spots in N. coloma).

Materials and methods

**Taxonomy and species concept**

We follow the generic and family names proposed by De la Riva et al. [8] and Heinicke et al. [11], respectively. We adopted the unified species concept [12,13] for recognizing species, which considers that independent evolutionary lineages exhibit consistent diagnostic traits (i.e., morphological, behavioral, genetic). Specimens examined are listed in Appendix I and institutional acronyms are those of Frost [2].

**Field work**

We carried out exhaustive samplings in the Río Manduríacu Reserve in search of amphibians and reptiles of importance for conservation, with the purpose of promoting conservation efforts in the network of reserves managed by the EcoMinga Foundation, especially in light of the mining threat at this particular reserve [4,14–16]. Samples were collected within the Río Manduríacu Reserve between 2012 and 2019 during the following periods: November 7–8, 2012; May 13–15, 2013; February 21–22, 2014; October 17–30, 2016; April 9–12, 2017; January 20–30, 2018; February 5–13, 2018; February 28 to 12 March 2019 (see Guayasamin et al. [14]; note the corrected year for sampling in April from 2018 to 2017). The most recent fieldwork in Feb–March, 2019, was conducted by SK, RJM, SJT, JC, José María Loaiza, and three assistants. The primary sampling methods were visual encounter surveys and general area searches. Visual encounter surveys were conducted from 1900 h and 0200 h along forest trails, streams, and a dirt road extending from the community to the Río Manduríacu Reserve, and encompassed primary, secondary, and riparian forest, as well as forest clearings. General area searches were performed by day and night, and were often utilized when habitat complexity did not permit linear transects. Data collection included the following information: species ID, date, time, habitat type, microhabitat, perch height/diameter (when applicable), behaviour, and geographic coordinates. A Garmin GPSmap 62s was used to demarcate transects and record coordinates for specimens.

**Evolutionary relationships**

We generated mitochondrial sequences (16S) for individuals of the new species of Noblella (ZSFQ 550, 551, 2502), as well as for N. coloma (QCAZ 32702, 40579). Extraction, amplification, and sequencing protocols are as described in Guayasamin et al. [17]. The obtained sequences were compared with closely related taxa (see [4,6,7,18]) downloaded from GenBank (https://www.ncbi.nlm.nih.gov/genbank/). We generated five new sequences (three for the new species and two for Noblella coloma), which were compared with 44 sequences (43 species) downloaded from Genbank (https://www.ncbi.nlm.nih.gov/genbank/). All Genbank codes are in Figure 1. Sequences were aligned using MAFFT v.7 (Multiple Alignment Program for Amino Acid or Nucleotide Sequences: http://mafft.cbrc.jp/alignment/software/) [19], with the Q-INS-i strategy. MacClade 4.07 [20] was used to visualize the alignment and minimize gap openings; characters where the alignment was ambiguous were excluded. Maximum likelihood (ML) trees were estimated using GARLI 2.01 (Genetic Algorithm for Rapid Likelihood Inference [21]), which uses a genetic algorithm that finds the tree topology, branch lengths, and model parameters that maximize lnL simultaneously [21]. Individual solutions were selected after 10,000 generations with no significant improvement in likelihood, with the significant topological improvement level set at 0.01; the final solution was selected when the total improvement in likelihood score was lower than 0.05, compared to the last solution obtained. Default values were used for all other GARLI settings, as recommended by the developer [21]. Bootstrap support was assessed via 1,000 pseudoreplicates under the same settings used in tree search. GenBank accession numbers are included in Table S1.

**Morphological data**

Before the preservation of specimens, tissue of liver and leg muscle was extracted and deposited in Eppendorf tubes with 95% ethanol. Individuals were euthanized with benzocaine atomizer and then fixed with either 10% formaldehyde or 95% ethanol; all individuals were preserved in 75% ethanol. The specimens were deposited at the Museo de Zoología COCIBA-USFQ (ZSFQ) of the Universidad San Francisco de Quito, Ecuador. Several species of Noblella were examined (see Appendix I, Figure 1).
Figure 1. Phylogeny of Noblella (gray box) showing the relationships of Noblella worleyae sp. nov. The phylogeny was based on mitochondrial (16S) DNA sequences. For each individual, the corresponding museum catalog number (when available) and GenBank accession number are shown.

We measured all collected specimens with a digital caliper (Mitutoyo ABSOLUTE 500–195-20) to the nearest 0.01 mm, following the descriptions provided by Guayasamin and Terán-Valdez [9]: snout to vent length; tibia length; foot length; head length; head width; interorbital distance; upper eyelid width; internarial distance; eye-noristil distance; snout-eye distance; diameter of the eye; diameter of tympanum; eye-tympanum distance; forearm length; and hand length. Sexual maturity was assessed by the presence of vocal sac and/or vocal slits for adult males and the presence of convoluted oviducts for adult females.

We cleared and stained the hands and feet of specimens with Alcian Blue following the protocols proposed by Taylor and Van Dyke [22]. Illustrations were made with the help of an Olympus S2X16 stereo dissecting microscope with an Olympus DP73 camera included. We follow the osteological terminology for hands and feet proposed by Duellman and Trueb [23], Fabrezi [24,25], and Trueb [26,27].

Bioacoustics

Advertisement call recordings were made by José Vieira and Jorge Brito with an Olympus LS-10 Linear PCM Field Recorder and a Sennheiser K6–ME 66 unidirectional microphone. The calls were recorded in WAV format with a sampling rate of 44.1 kHz/second with 16 bits/sample and analyzed with Raven Pro version 1.5 [28]. All calls are stored at the Laboratorio de Biología Evolutiva at Universidad San Francisco de Quito (LBE-USFQ). Measurements of acoustic variables were obtained as described in Köhler et al. [29]. A call is defined as the collection of acoustic signals emitted in sequence and produced in a single exhalation of air. A note is defined as a temporally distinct segment within a call; notes are separated by a silent interval. Pulsed notes are defined as: notes having one or more clear amplitude peaks but are not separated by a fully silent interval. Tonal notes are defined as: notes that maintain relatively constant amplitude throughout the call. A call series (or call group) is defined as a sequence of calls that is separated from other such groups by periods of silence much longer than the inter-call intervals, which are stable or changing in a predictable pattern (see [29]).

Results

Phylogenetic relationships and genetic distances

The resulting mitochondrial tree of Noblella and outgroups is shown in Figure 1. Noblella, as currently defined, is not monophyletic. The new species is inferred as sister to N. coloma. The two species have a considerable genetic distance between them (uncorrected p = 0.066).

Systematics accounts

Noblella worleyae new species Figure 2–6

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Proposed standard English name. Worley’s Leaf FrogProposed standard Spanish name. Cutín Noble de Worley

Holotype

ZSFQ 551 (Figures 3–4), adult female, collected at Río Manduricuα Reserve (0.312057°N, 78.854330°W; 1184 m, Figure 2), Cantón Cotacachi, Imbabura Province, by Ross Maynard, Paul S. Hamilton, Scott J. Trageser and José Vieira on 8 February 2018.

Paratypes (4 males, 2 females). ZSFQ 552, adult female, collected at Río Manduricuα Reserve (0.314256°N, 78.865672°W; 1597 m), Cantón Cotacachi, Imbabura province, Ecuador, by Ross Maynard and Paul S. Hamilton on January 29th, 2018; ZSFQ 550, adult male, collected at Río Manduricuα Reserve (0.317069°N, 78.870297°W; 1701 m), Cantón Cotacachi, Imbabura province, Ecuador, by Ross Maynard, Paul S. Hamilton, and José Vieira on
recovered as a close relative of other Noblella species (Figure 1) and also matches the diagnosis of Noblella by Hedges [5], as follows: head no wider than body; cranial crests absent; tympanic membrane differentiated (except in N. duellmani); dentigerous processes of vomers absent; terminal discs on digits not or slightly expanded; discs and circumferential grooves present (except in N. naturetrekii and N. worleyae sp. nov.); distal phalanges narrowly T-shaped; Finger I shorter than, or equal in length to, Finger II; most species with phalangeal reduction in Finger IV (two phalanges in N. carrascocica, N. lochites, N. myrmecoides, N. personina, N. peruviana, N. madreselva, N. naturetrek, and N. ritarsinaquiae); Toe III shorter than Toe V (except in N. naturetrekii and N. worleyae sp. nov.); tips of at least Toes III–IV acuminate; subarticular tubercles not protruding; dorsum pustulate or shagreen; venter smooth; SVL less than 22 mm.

**Diagnosis**

The new species (Figures 3–6) presents the following characteristics: (1) skin of dorsum finely shagreen; (2) tympanic annulus and membrane visible externally, supratympanic slightly visible; (3) snout rounded in dorsal and lateral view (eye-nostril distance 55% of eye diameter, Figure 3); (4) dentigerous processes of vomers absent; (5) fingers not expanded distally, tips of Fingers I and IV slightly acuminate, Fingers II and III acuminate, without papillae (Figure 3); Finger I shorter than Finger II (Figure 3); nuptial pads not visible; circumferential grooves absent; (6) distal phalanges slightly T-shaped; phalangeal formula of hands: 2, 2, 3, 3 (Figure 6); (7) supernumerary palmar tubercles present, mostly at the base of the digits; subarticular tubercles rounded, proximal tubercles prominent; diminutive rounded ulnar tubercles present; (8) one elongated and subconical tarsal tubercle, two tarsal tubercles (inner tubercle 2–2.5x the size of the outer), small pigmented supernumerary tarsal tubercles, toes slightly expanded and slightly acuminate on Toes I and V, and cuspidate tips on Toes II–IV, papillae absent (Figure 3); (9) Toe V shorter than

11 February 2018; ZSFQ 345, adult male, collected in the Rio Manduriacu Reserve, Cantón Cotacachi, Imbabura province, Ecuador (0.310502°N, 78.856872°W; 1203 m), by Ross Maynard and Paul S. Hamilton on October 19th, 2016; ZSFQ 2502, 2504 adult males, collected at Rio Manduriacu Reserve (0.309800°N, 78.857298°W; 1206 m), Cantón Cotacachi, Imbabura province, Ecuador, by Jorge Brito M., Jaime Culebras, and Sebastián Kohn on 9 April 2017; ZSFQ 2503 adult female, collected at Rio Manduriacu Reserve (0.310643°N, 78.856117°W; 1222 m), Cantón Cotacachi, Imbabura province, Ecuador, by Jorge Brito M., Jaime Culebras, and Sebastián Kohn on 10 April 2017.

**Generic placement**

The new species is placed in the genus Noblella based on morphological and molecular features. The taxon is
Toe III, distal portions of circumferential grooves present on Toes II–V, phalangeal formula of feet: 2, 2, 3, 4, 3 (Figure 6); (11) in life, dorsum brown to dark brown and densely splashed with light brown, brownish-gray, or turquoise, presence of a middorsal line continuing along the posterior lengths of hind legs cream to light brown; flanks light brown to dark brown with scattered irregular white to turquoise marks; venter yellowish-cream with minute speckling; throat with irregular brown to homogeneously brown marks (Figure 5); (12) female SVL 18.1–19.1 mm (n = 3, mean = 18.7); male SVL 15.5–17.9 mm (n = 4, mean = 16.6).

Comparisons
Noblella worleyae is closely related and morphologically similar to N. coloma. Nevertheless, distal phalanges of the feet and hands of the new species are only slightly expanded laterally, as opposed to a distinct T-shape in N. Coloma [9], Figure 6). Moreover, the new species has a yellowish-cream venter with minute speckling (venter orange with minute brown and white spots in N. coloma), throat with irregular brown marks to homogeneously brown, and lacks suprainguinal marks (present in N. coloma).

Figure 5. Dorsal and ventral color patterns of Noblella worleyae sp. nov. in life. (A, D) Dorsal pattern and ventral pattern of ZSFQ 2504, paratype, adult male, SVL = 15.5 mm; (B, E) dorsal pattern and ventral pattern of ZSFQ 2502, paratype, adult male, SVL = 15.9 mm; (C, F) dorsal pattern and ventral pattern of ZSFQ 550, paratype, adult male, SVL = 17.3 mm; (G, J) dorsal pattern and ventral pattern of ZSFQ 552, paratype, adult female, SVL = 19.1 mm; (H, K–L) dorsal pattern and ventral pattern of ZSFQ 550, paratype, adult male, SVL = 17.3 mm; (I) dorsal pattern of an uncollected specimen. Photographs by Jaime Culebras (a, b, d, e), José Vieira (c, f), Ross Maynard (g–k) and Scott Trageser (l).

Figure 6. Right hand (A) and foot (B) in dorsal view of Noblella worleyae sp. nov. ZSFQ 345, paratype, adult male, SVL = 17.9 mm.

Other species of Noblella that have three phalanges on Finger IV are N. duellmani [30], N. heyeri [10], N. lynchii [31], N. pygmaea [32], and N. thiuni [33]. All of them, except N. heyeri, are restricted to the Amazonian slopes of the Andes. Moreover, N. worleyae has a tympanic annulus and membrane visible externally (absent in N. duellmani, tympanic membrane not differentiated in N. thiuni), dorsum finely shagreen (smooth in N. heyeri, pustular in N. lynchi, and tubercular in N. pygmaea).

Noblella worleyae can be distinguished from N. carrascoicola [34], N. lochites [35], N. losamigos [36], N. myrmecoides [35], N. personina [37], N. peruviana [38], N. madreselva [39], N. naturetekii [4], and N. ritarasquinae [40] by having three phalanges on Finger IV instead of two. Additionally, as mentioned before, N. worleyae, N. coloma, and N. heyeri are the only species in the genus found on the Pacific slope of the Andes.

Description of the Holotype
Adult female (ZSFQ 551); head longer than wide; snout round in dorsal and lateral views; canthus rostralis straight; loreal region slightly concave; upper eyelid 47% of interorbital distance; eye-nostril distance 66% of eye diameter; tympanum visible externally, tympanic membrane differentiated from surrounding skin; supratympanic fold slightly visible. Dentigerous processes of vomers absent. Skin of dorsum finely shagreen; venter smooth; few diminutives rounded ulnar tubercles; palmar tubercle oval, about 0.5x the size of thenar tubercle; supernumerary palmar tubercles present, mainly at base of digits; proximal subarticular tubercles prominent, rounded; fingers not expanded distally, tips of Fingers I and IV slightly acuminate, Fingers II and III acuminate, without papillae, circumferential grooves absent; relative lengths of fingers I < II < IV < III.
One elongated and subconicalタルサルタベルケル, 二つのタルサルタベルケル（内側タルサルタベルケル 2 x the size of external); proximal subarticular tubercules well defined; small pigmented super-numerary tubercles. Toes slightly expanded and slightly acuminate on Toes I and V, cuspidate tips on Toes II–IV, distal portions of circumferential grooves visible; relative lengths of toes I < II < V < III < IV. For measurements of type series (mm) see Table 1.

Color of holotype in life
Dorsum brown, densely splashed with light brown; cream middorsal stripe along posterior lengths of hind legs; flanks light brown with scattered irregular white marks; venter yellowish-cream with minute speckling; throat with big irregular brown marks. Iris reddish-brown with some dark brown reticulations.

Color of holotype in ethanol (Figure 4)
Dorsum brown (flecked with white by preservation effects and manipulation); cream middorsal stripe, extending from scapular level to cloaca and then along the posterior surface of hind limb. Sides of head homogeneously dark brown, labial bars absent, with a faded post-typanic fringe; flanks flecked with cream. Venter and ventral surfaces of legs white with scattered diminutive brown dots; throat brown with some irregular white marks. Forelimbs ventrally brown with a longitudinal white stripe along ulna, dorsally brown. Hind limbs like dorsum.

Variation of color patterns (Figure 4–5)
Dorsal surfaces are predominately brown (ZSFQ 550) to dark brown (ZSFQ 2502), densely splashed with light brown (ZSFQ 550, 552), brownish-gray (ZSFQ 2502), or turquoise (ZSFQ 2504), with or without a cream to light brown middorsal line continuing along the posterior edge of hindlimbs. Flanks light brown (ZSFQ 550) to dark brown (ZSFQ 2502–2504) with scattered irregular marks white (ZSFQ 550–552) to light turquoise (ZSFQ 2502–2504). Venter yellowish-cream with minute speckling: throat with irregular brown marks (ZSFQ 550) to homogeneously brown (ZSFQ 345, 551, 552, 2502–2504).

In preservative, dorsum dark brown, with a cream middorsal line continuing along the posterior edge of hindlimbs; middorsal line weakly defined in ZSFQ 550. Venter cream (ZSFQ 550–551, 2502) to brownish cream (ZSFQ 552, 2504); throat brown or cream with brown marks (ZSFQ 550).

Osteology of hands and feet
The hand and foot phalangeal formula are standard: 2-2-3-3 and 2-2-3-4-3, respectively (Figure 6). The relative length of fingers is: I < II < IV < III, and that of toes is: I < II < V < III < IV. The carpus is composed of a radiale, ulnare, Carpal 1, and a large postaxial element assumed to represent a fusion of Carpals 2, 3, and 4 (Figure 6). The prepollex is composed of one proximal bone and an elongated distal cartilage. The terminal phalanges are slightly T-shaped. The prehallux is represented by a small, rounded, proximal bone, and a distal irregular element (Figure 6).

Call description (Figure 7)
Advertant calls were recorded 22:00 h on 9 April 2017 (air temperature not recorded). Adult male ZSFQ 2502 (recording code: LBE-C-049). The call sounds like a short chirp. Each call is composed by a single short, pulsed note, with a duration of 0.032–0.044 s (mean = 0.039 ± 0.005; n = 4). Each note has about 10 to 15 pulses, which are difficult to differentiate. Time between calls is 22.5–31.7 s (mean = 26.6 ± 4.681; n = 3). The dominant frequency is located at a relatively wide range at 3,363–4,172 Hz. One harmonics is present at 7,399–7,695 Hz (n = 4).

Distribution and Natural History
Nobellia worleyae is a leaf litter specialist and has only been found in either primary forest or moderately disturbed old growth secondary forest. It has been recorded from six nearby localities within the Rio Manduriacu Reserve, Cantón Cotacachi, Imbabura province, Ecuador, at elevations between 1,184 m and 1,701 m (Figure 2). Surveys conducted on the east side of the Rio Mandiracu (opposite from the reserve) as well as those in and around the community of Santa Rosa de Mandurica (ca. 3 km south of Río Mandurica Reserve boundary) have yielded no records, although survey effort outside of the reserve is less extensive than that within the reserve. We have made eight field trips between 2012 and 2019, with 59 effective field days, with an average of five people actively searching for amphibians and we have been able to find a total of seven individuals. The new species seems to be endemic to this region, which is an area with high diversity and endemicism of anurans [14,41]. The habitat ecosystem corresponds to Low Montane Evergreen Forest of western slopes of the Andes [42]. The total restricted area that covers these localities is 389,248 m².

Nobellia worleyae is one of the few diurnal anurans at the Río Manduriacu Reserve. Calls were frequently heard from dawn (6:00 h) to dusk (19:00 h), but frogs stop vocalizing when approached. Individuals were found in areas with dense leaf litter and other decaying material, and at the base of large trees. The species is evasive and individuals
quickly immersed between leaves and roots as an escape method. The new species is one of the more abundant amphibians at Río Manduriacu Reserve, as groups of individuals were frequently heard calling by day throughout much of the sloped reserve during expeditions. In fact, during a morning survey (6h20 am) a total of 13 males were heard calling along a 100-m transect (JBM, field notes). The new species did not call with heavy rain. During sampling days in April 2017, air temperature inside the forest was 12–26°C (thermometer placed 120 cm above ground level at the base of a tree). Individuals have also been observed after sunset while conducting night transects, as sampling in October 2016, and January–February, 2018, yielded eight records between 20:00 and 23:05 h. However, these individuals were presumably flushed from their resting place within the leaf litter as surveyors passed by.

**Etymology**
The specific name is a noun in the genitive case and is a patronym for Dr. Elizabeth K. Worley (1904–2004), Professor in the Biology Department at Brooklyn College, naturalist, science communicator, educator, and mentor.

**Discussion**
Our phylogeny shows similar relationships within the genus *Noblella* as those inferred in previous studies [4,6–8]. *Noblella* is a non-monophyletic taxon, as the genera *Noblella* and *Psychrophrynella* are nested and because the northern *Noblella* do not share the most recent common ancestor with the southern clade of *Noblella* and *Psychrophrynella*. Further studies are necessary to solve the polyphyly of the genus, especially with the inclusion of genetic toptypical material of *Noblella peruviana* and *Psychrophrynella bagrecito*. The description of this new species adds to our understanding of the phylogenetic relationships within *Noblella* and the changes needed to render *Noblella* monophyletic.

The description of this new frog species from the Río Manduriacu Reserve is yet another study demonstrating the importance of the reserve as a critical conservation area for amphibian diversity [14]. However, the expansion of large-scale mining concessions in northwestern Ecuador is a major threat to the future of this region [14,43]; illegal mining activities have been conducted within Río Manduriacu Reserve, as well as nearby areas [14,44]. The western Andean slopes of Ecuador is comprised of important micro-regions of small vertebrate endemism, which are restricted to areas with good-quality forest and with little to no anthropogenic activity (e.g. [14,45,46,47]). Thus, projects that threaten these Andean forests must be regulated and authorized within the framework of the Ecuadorian Constitution. Moreover, a program for conservation actions aiming to protect the unique biodiversity is also needed for the Ecuadorian Andes. Such an approach is already being advanced, mostly with the participation of non-profit institutions that aim to protect priority and vulnerable forests for biodiversity conservation, such as the EcoMinga Foundation [4,14,15]. Nonetheless, ongoing research, long-term conservation funding, and assurance that protected areas will not be undermined by extractive companies are necessary to ensure the survival of species with high endemism such as *N. worleyae* and its fragile habitats.

**Author contributions**
Carolina Reyes-Puig performed the experiments, contributed reagents/materials/analysis tools, authored drafts of the paper, approved the final draft.
Ross Maynard performed the experiments, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.
Scott Trageser performed the experiments, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.
José Vieira performed the experiments, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.
Paul Hamilton is Executive Director of The Biodiversity Group, which initiated herpetofauna study at Manduriacu. He obtained funding for fieldwork and lab work, developed field survey protocols, supervised fieldwork, contributed to fieldwork, approved the final draft.

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Figure 7. Call of the paratype (ZSFQ 2502) of *Noblella worleyae* sp. nov. The recording was made at 22:00 on April 9th, 2017 (air temperature not recorded). (A) Amplitude; (B) Frequency.
Ryan L. Lynch performed the experiments, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.
Sebastián Kohn: Fieldwork planning, logistics and funding, present and active at both expeditions where individuals were collected, specimen transportation, acquisition of legal paperwork for specimen transportation, approved the final draft.
Jaime Culebras performed the experiments, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.
Jorge Brito performed the experiments, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.
Juan M. Guayasamin performed the experiments, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Appendix I

**Noblella coloma**: Ecuador: Pichincha: QCAZ 7277, 7412, 8701, 11,614, 26,307, 32,702, Reserva Ecológica Río Guajillo; 1800–2000 m. **Noblella beyeri**: Ecuador, Loja: QCAZ 31,470, 31,471, 31,473, Loja–Zamora road; 2385 m. QCAZ 22,501, Zamora-Huáico; 2000 m. **Noblella lochites**: Ecuador, Napo: ZSFQ 346, Archidona, Reserva Narupa, 1176 m; ZSFQ 347, Reserva Narupa, 1152 m; ZSFQ 348, Reserva Narupa, 1167 m; **Zamora Chinchipe**: ZSFQ 1119,
Yantzaza, Concesión La Zarza, 1385 m; ZSFQ 1124, Concesión La Zarza, 1357 m; ZSFQ 1186, ZSFQ 1187, ZSFQ 1188, Yantzaza, Río Blanco, 1654 m; ZSFQ 1188, Río Blanco, 1830 m. *Nobella cf. lochites*: Ecuador, Zamora Chinchipe: ZSFQ 3262–326, Yantzaza, Estación Experimental El Padmi UNL, 775 m. *Nobella myrmecoides*: Ecuador, Napo: ZSFQ 670, Mera, Parque Nacional Llanganates, 1325 m; ZSFQ 671, Parque Nacional Llanganates, 1352 m; ZSFQ 672, Parque Nacional Llanganates, 1327 m. *Nobella cf. myrmecoides*: Ecuador, Tungurahua: ZSFQ 1341, Río Negro, Reserva Río Zuñag, 1269 m.