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Formal description of the new tree frog species inhabiting Northern Italy and Southern Switzerland

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Abstract

Several lines of evidence support that north-Italian and Ticinese tree frogs, recently called *Hyla perrini*, represent a distinct species, formerly considered as *Hyla intermedia*. However, a nomenclatural clause for electronic publishing of species names according to the Code of International Nomenclature caused that the new name has been unavailable so far. Hence, here we formally describe *Hyla perrini*.

Keywords: Amphibians, Taxonomy, *Hyla perrini*, Nomenclature.

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Résumé

Plusieurs analyses génétiques ont montré que les rainettes nord-italiennes et tessinoises, récemment nommées *Hyla perrini*, représentaient une espèce distincte, auparavant considérée comme *Hyla intermedia*. Cependant, une clause nomenclaturale liée aux publications de noms d'espèces dans des revues électroniques, énoncée par le Code Internationale de Nomenclature, rend le nom invalide pour le moment. Afin d'y remédier, nous décrivons ici formellement *Hyla perrini*. Un résumé étendu en français se trouve en fin d'article.

Mots-clés : Amphibiens, taxonomie, *Hyla perrini*, nomenclature.

INTRODUCTION

In a recent issue of the peer-reviewed scientific online-only journal *Frontiers in Ecology & Evolution* (DUFRESNES *et al.*, 2018), we provided two lines of genetic evidence for species-level divergences between tree frog populations (*Hyla*), distributed in the southern and northern parts of the Apennine Peninsula (DUFRESNES *et al.* 2018). First, their divergence, as estimated from phylogenetic relationships of thousands of genetic markers, dates back to the middle Pliocene, i. e. 3.5 million years. Second, based on population genomics data, their hybrid zone, spanning across the northeastern Adriatic coast in Emilia Romagna, is restricted to less than a hundred kilometers, a relatively short distance for such a mobile species, suggesting partial reproductive isolation. Both of these features support distinction at the species level, as claimed for similar sibling amphibian taxa currently bearing a specific status.

Hence, our paper called for a taxonomic revision of Apennine tree frogs. Through a thorough nomenclatural search (see below), we concluded that no name has been available for North-Italian tree frogs. As a consequence, we described them under a new name, *Hyla perrini*, a tribute to Nicolas Perrin, professor emeritus from the University of Lausanne.

However, although our description (DUFRESNES *et al.* 2018) provided all preconditions to describe the new species, including paragraphs on evolutionary divergence, genome characteristics, intraspecific variation, nomenclatural history, etymology, geographic distribution, threats, etc., it was brought to our attention that one criterion from the Code of Zoological Nomenclature was not met. The registration in the Official Register of Zoological Nomenclature (ZooBank) postdated by several hours the publication date of the species description (DUFRESNES *et al.* 2018). Namely, Article 8.5.3.3 of the Code states “An error in stating the evidence of registration does not make a work unavailable, provided that the work can be unambiguously associated with a record created in the Official Register of Zoological Nomenclature **before the work was published.**”. Because our work (DUFRESNES *et al.* 2018) was technically published earlier than the Zoobank registration therein, the name *Hyla perrini* has become unavailable (*nomen nudum*), pending a description with a proper Zoobank registration predating the publication and/or a printed species description.

Therefore, we here formally describe the new species.

***HYLA PERRINI* N. SP. (text partially cited from DUFRESNES et al. 2018)**

Identity and diagnosis

Populations of this species have previously been considered as *H. intermedia*, due to phenotypic similarity and phylogenetic relationships. Based on mtDNA variation, *H. perrini* was previously called “clade N” by CANESTRELLI *et al.* (2007a, 2007b) and “new taxon 2” by STÖCK *et al.* (2008, 2012). *Hyla perrini* is a medium-sized tree frog, morphologically similar to the parapatric *H. intermedia* s. s. (its sister species) and *H. arborea*. According to the populations analyzed by DUFRESNES *et al.* (2018), it subtly differs from *H. intermedia* s. s. by a narrower head and shorter hind legs, yet without diagnostic differences. Like most European hylids, *H. perrini* displays a green or brownish back coloration and an immaculate belly, separated by a lower black and upper white lateral stripe ending near the groin in an inguinal loop of varying shapes. Other typical characteristics include a horizontal pupil, adhesive disks on fingers and toes, and a yellowish vocal sac on the male throat. The advertisement calls have a similar pattern and structure as *H. intermedia* and *H. arborea*: singular notes made of 7-9, partially fused pulses, totaling ~60-80 ms, interrupted by pauses of 80-120 ms, emitted over the 0.5-5 kHz range, with main bursts of energy at 0.95-1.05 kHz (fundamental frequency) and 2.0-2.4 kHz (dominant frequency); a sonogram and a spectrogram of *H. perrini* from the type locality is provided in DUFRESNES *et al.* (2018, Fig7B), as well as an audio file (Audio S1 in DUFRESNES *et al.* 2018). To our knowledge, the tadpoles have not been systematically compared but are supposedly similar between these species (DUFRESNES in press): spiracle sinistral; cloaca dextral; pointy tail tip and high upper fin reaching the eyes, with a single line of dark patches on the tail muscle; tooth composition 2/3. *Hyla perrini* has evolved since the upper Pliocene, 2.5-3.5Mya, and can be distinguished from its relatives, notably *H. intermedia* s. s., by concordant differences at genetic markers, i. e. ~ 9 % of mitochondrial divergence (*COI*, *cyt-b*), and ~ 0.3 % of nuclear divergence (RADtags).

Holotype

MZL42340, adult male collected by Christophe Dufresnes on April 7th 2018 at Piazzogna, canton of Ticino, Switzerland (46.1361° N, 8.8206° E), subsequently deposited at the Cantonal Museum of Zoology of Lausanne, Switzerland. Full details, including measurements and photos are available in DUFRESNES *et al.* (2018).

Paratypes

The type series includes four paratypes, two males (MZL42341 and MZL42342) and two females (MZL42343 and MZL42344), collected on April 7th 2018 and May 8th 2018, respectively, by the same collector at the same locality. Full details, including measurements and photos are available in DUFRESNES *et al.* (2018).

Karyotype

Like other European hylids, *Hyla perrini* has two sets of 12 chromosomes (2n = 24). A karyotype and idiogram (specimen K51), most likely from this species, is shown by ANDERSON (1986; p. 61, as “*H. arborea*”; cf. ANDERSON 1991), who analyzed eight metaphases of voucher HGD74640 (Florida State Museum), listed as “? Po River” (p. 347) yet elsewhere detailed “unknown locality in Italy”. Importantly, ANDERSON (1986) found a secondary constriction in

the long arm of chromosome 10 (ANDERSON 1986: Fig. 2; see ANDERSON 1991), interpreted as the position of the nucleolous organizing region (NOR), which is similar to that in the closely related *H. arborea* (SCHMID 1978). In *H. perrini*, sex determination is under genetic control by chromosome 1 (LG1, STÖCK *et al.* 2011, DUFRESNES *et al.* 2015; as *H. intermedia*), most likely at the *dmrt1* gene (BRESLFORD *et al.* 2016).

Nomenclatural history

The nomenclatural history of the new species is tightly intertwined with that of *H. intermedia*. BOULENGER (1882: p. 381) first described *Hyla arborea* var. *intermedia* based on two syntypes: BMNH 52.12.11.55 from Bononia (i.e. Bologna, at the south-eastern edge of the range of the new species), and BMNH 82.7.13.6 from Palermo, Sicily. However, by lectotype designation of BMNH 82.7.13.6, DUBOIS (1995) made this latter specimen the only name bearer of the epitheton *intermedia*, and also restricted its type locality to “Palermo”, making *intermedia* inapplicable to North-Italian tree frogs. In the same work, DUBOIS (1995) synonymized *H. intermedia* (i. e. the South-Italian taxon) with *H. arborea* (LINNAEUS, 1758). Using allozymes, NASCETTI *et al.* (1995) found tree frogs from the entire Italian Peninsula to be distinct from *H. arborea*, and described them as *Hyla italica*. However, they conducted two additional nomenclatural acts. First, they stated for another old name coined for Italian tree frogs, *Hyla variegata* (RAFINESQUE, 1814; type specimens not designated or known to exist; cf. FROST 2018), an homonym of *Rana variegata* (BONNATERRE, 1789), hence making it unavailable. Second, they designated a neotype (MZUF 20365) with its type locality near Palermo (Sicily; ca. 37° 00' N, 14° 20' E) and called it *H. italica* as a replacement name (*nomen novum*) for *H. variegata*. By this action and type locality, *H. italica* became a junior subjective synonym of *H. intermedia* (as stated by DUBOIS 1995), and thus inapplicable to our new species.

Therefore, to the best of our knowledge, there has been no available scientific name for North Italian tree frogs (see also FROST 2018). In this region, several other authors (cf. BONATO *et al.* 2007) have previously used either *H. arborea* or, like DE BETTA (1857, p. 265), *Hyla viridis* (= *Rana arborea* LINNAEUS, 1758; cf. STEJNEGER, 1907), for the herpetofauna of Veneto and Tirol Provinces. Phylogenetic studies did not apply any name other than *H. intermedia* (CANESTRELLI *et al.* 2007a, 2007b) or “*Hyla* new taxon 2” (“n. t .2”) (STÖCK *et al.* 2008, 2012).

As stated (DUFRESNES *et al.* 2018), the name *Hyla intermedia* applies for the southern lineage (*H. intermedia* s. s.), and we have proposed a new name for the tree frogs from Northern Italy.

Distribution

Hyla perrini is endemic to the Po Plain and adjacent valleys in Northern Italy and the Swiss canton of Ticino. Its range borders *H. arborea* in northeastern Italy / Slovenia, where the two species meet in a narrow contact zone along the Isonzo River; it borders and hybridizes with *H. intermedia* s. s. on the northeastern edge of the Apennines in Emilia Romagna (DUFRESNES *et al.* 2018). A now-extinct population was introduced in the 1950s from Ticino to the Grangettes natural reserve on the eastern shores of Lake Lemman (DUBEX *et al.* 2018). The species is found from sea level up to about 1 500 m in Italy and 1 100 m in Switzerland.

Etymology

As proposed in DUFRESNES *et al.* (2018), we name this new species *Hyla perrini* after Nicolas Perrin, professor of ecology, who recently retired from the University of Lausanne (Switzerland). As a vernacular name in English, we recommend *H. perrini* to be called the “Po’s tree frog”, which reflects its geographic distribution. Different translations are available in DUFRESNES *et al.* (2018).

ZooBank Registration

We hereby state that the present paper has been registered to the Official Register of Zoological Nomenclature (ZooBank) under LSID: urn:lsid:zoobank.org:pub:8601B626-BDFC-4D2B-B0F0-37DF71482BCD. The new species name *Hyla perrini* has been registered under LSID: urn:lsid:zoobank.org:act:AA7557C0-1496-4140-A6B2-98368355A52B.

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RÉSUMÉ ÉTENDU

La rainette italienne, distribuée en Italie et dans le Tessin en Suisse, était considérée jusqu’à ce jour comme une seule et même espèce, *Hyla intermedia*. Des travaux de phylogéographie basés sur l’ADN mitochondrial soupçonnaient tout de même des différences génétiques entre le nord et le sud de la chaîne des Apennins. Dans une récente étude (DUFRESNES *et al.*, 2018), nous avons conduit des analyses de génomique, basées sur des milliers de marqueurs génétiques et afin de comprendre la diversité des rainettes italiennes. Ces analyses nous ont permis de faire deux découvertes importantes. Premièrement, les populations du nord et du sud de la chaîne des Apennins forment deux lignées bien distinctes, qui divergent depuis au moins trois millions d’années. Deuxièmement, ces lignées se rencontrent le long de la côte adriatique en Émilie-Romagne, et forment une zone hybride relativement étroite (moins de 100 km), suggérant un certain degré d’isolement reproducteur. En effet, sachant que les rainettes font partie des amphibiens les plus mobiles (jusqu’à 1,5 km par an), et que ces lignées sont vraisemblablement en contact depuis la fin de la dernière glaciation, on s’attendrait à un mélange des génomes sur une zone géographique plus étendue si ceux-ci se mélangeaient sans incompatibilités. En comparaison, le degré de divergence et la largeur de la zone hybride sont similaires à ce qu’on observe chez d’autres couples d’espèces proches d’amphibiens. Aussi, nous avons conclu que ces lignées représentaient deux espèces distinctes. Comme « *Hyla intermedia* » a originellement été attribué aux populations du sud de l’Italie, ce nom s’applique en priorité au taxon sud-Apennins. Aucun nom ne semble jamais avoir été proposé pour les populations du nord, et nous avons donc choisi un nouveau nom pour le taxon nord-Apennins: *Hyla perrini*, en hommage à Nicolas Perrin, professeur emeritus de l’université de Lausanne, qui a notamment travaillé sur ces rainettes. Afin de tester si *Hyla intermedia* et *Hyla perrini* présentaient des

critères autres que génétiques, nous avons également comparé leurs chants de reproduction et leur morphométrie, sans trouver de véritables différences, et avons donc conclu que ces deux rainettes étaient des espèces cryptiques. Un holotype mâle (MZL42340, figure 1) et quatre paratypes (deux mâles et deux femelles : MZL42341, MZL42342 et MZL42343, MZL42344 respectivement) ont ainsi été déposés au Musée cantonal de zoologie de Lausanne dans le cadre de la description de l'espèce. Cependant, quelques jours après la publication de cette étude, le Comité Internationale de Nomenclature Zoologique (CINZ) nous a informés que nous n'avions pas enregistré notre publication (ainsi que le nom associé) à temps dans le registre officiel de nomenclature zoologique (ZooBank), ce qui est désormais nécessaire pour les publications électroniques. Aussi, le nom de l'espèce n'a pas pu être validé par le Code International de Nomenclature (CIN) en l'état. L'objet de cet article est ainsi de décrire l'espèce formellement, en l'associant cette fois au registre ZooBank.



Figure 1: The holotype of *Hyla perrini* (MZL42340) before its capture at the type locality / L'holotype d'*Hyla perrini* avant sa capture à la localité type. Photo credit / credit photo : C. Dufresnes.

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