

Supplementary data 1 for

Genomic evidence for cryptic speciation in tree frogs from the Apennine Peninsula, with description of *Hyla perrini* sp. nov.

Dufresnes et al., Frontiers in Ecology & Evolution

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Table S1a: Sequences included in the mitochondrial phylogeny.

Species	Country, region	Specimen ID	COI	cytb
<i>Hyla intermedia</i> s. s.	Italy, Calabria	NME0916/01	FJ226788	FJ226877
<i>Hyla intermedia</i> s. s.	Italy, Sicily	Sicily101	FJ226789	FJ226878
<i>Hyla perrini</i> sp. nov.	Switzerland, Ticino	Ti02	FJ226763	FJ226852
<i>Hyla perrini</i> sp. nov.	Switzerland, Ticino	Ti01	FJ226764	FJ226853
<i>Hyla arborea</i>	Germany, Thüringen	BAC5M	FJ226774	FJ226863
<i>Hyla arborea</i>	France, Burgundy	Bo1	FJ226776	FJ226865
<i>Hyla arborea</i>	Greece, Crete	NHMC80.2.7.21	FJ226778	FJ226867
<i>Hyla arborea</i>	Croatia, N-Dalmatia	NMEA0674/00	FJ226781	FJ226870
<i>Hyla arborea</i>	Greece, Peloponese	NMEA0902a/01	FJ226783	FJ226872
<i>Hyla molleri</i>	Portugal, Alentejo	HPA248	FJ226828	FJ226917
<i>Hyla molleri</i>	Spain, Salamanca	Hlimol	FJ226829	FJ226918
<i>Hyla orientalis</i>	Turkey, Thrace	IgneadaI	FJ226765	FJ226854
<i>Hyla orientalis</i>	Azerbaijan, Lenkoran	Hgum	FJ226767	FJ226856
<i>Hyla orientalis</i>	Turkey, Izmir	H204	FJ226769	FJ226858
<i>Hyla orientalis</i>	Ukraine, Kharkov	HLi49	FJ226827	FJ226916
<i>Hyla sarda</i>	France, Corsica	Hsarda1.1	FJ226811	FJ226900
<i>Hyla sarda</i>	Italy, Sardinia	sarda23	FJ226816	FJ226905
<i>Hyla felixarabica</i>	Yemen, Amran	H122	FJ226817	FJ226906
<i>Hyla felixarabica</i>	Yemen, Amran	H155	FJ226818	FJ226907
<i>Hyla felixarabica</i>	Syria, As Suwayda	H10	FJ226771	FJ226860
<i>Hyla savignyi</i>	Cyprus	Cyprus2	FJ226819	FJ226908
<i>Hyla savignyi</i>	Syria, Al Hasakah	H103	FJ226821	FJ226910
<i>Hyla savignyi</i>	Georgia	H394	FJ226822	FJ226911
<i>Hyla savignyi</i>	Iran, Kermanshah	MVZ245918	FJ226823	FJ226912
<i>Hyla savignyi</i>	Iraq, Bagdad	H89	FJ226849	FJ226939
<i>Hyla meridionalis</i>	Italy, Liguria	HM38	FJ226795	FJ226884
<i>Hyla meridionalis</i>	Morocco, Tanger	MVZ186158	FJ226799	FJ226888
<i>Hyla meridionalis</i>	Portugal, Algarve	NMEA0946a/02	FJ226803	FJ226892
<i>Hyla meridionalis</i>	Spain, Andalusia	MVZ186012	FJ226805	FJ226894
<i>Hyla meridionalis</i>	Spain, Canaria	NMEA0299a/97	FJ226807	FJ226896
<i>Hyla meridionalis</i>	Morocco, Marrakesh	MVZ177941	FJ226842	FJ226925
<i>Hyla cf. meridionalis</i>	Algeria, El Tarf	Algeria1	FJ226793	FJ226882
<i>Hyla cf. meridionalis</i>	Tunisia, Jendouba	Tunisia2.1	FJ226809	FJ226898
<i>Hyla japonica</i>	Japan, Hiroshima	HjM1	FJ226839	FJ226923
<i>Hyla cinerea</i>	USA, Georgia	MVZ150007	FJ226785	FJ226874
<i>Hyla squirella</i>	USA, N-Carolina	MVZ145422	FJ226851	FJ226942

Table S1b: Samples included in the nuclear phylogeny. * indicates specimens from the type series of *H. perrini* sp. nov. (PIA10: holotype MZL42340; PIA08 and PIA09: paratypes MZL42342 and MZL42341, respectively).

Species	Country, region	Specimen ID	Origin
<i>Hyla intermedia</i> s. s.	Italy, Tuscany	VLC01	This study
<i>Hyla intermedia</i> s. s.	Italy, Tuscany	VLC02	This study
<i>Hyla intermedia</i> s. s.	Italy, Abruzzo	ROS01	Canestrelli et al., 2007b
<i>Hyla intermedia</i> s. s.	Italy, Abruzzo	ROS03	Canestrelli et al., 2007b
<i>Hyla intermedia</i> s. s.	Italy, Calabria	FIU5	Canestrelli et al., 2007b
<i>Hyla intermedia</i> s. s.	Italy, Calabria	FIU3	Canestrelli et al., 2007b
<i>Hyla intermedia</i> s. s.	Italy, Sicily	NME0915/01	Stöck et al. 2008b, 2012
<i>Hyla perrini</i> sp. nov.	Switzerland, Ticino	PIA08*	This study
<i>Hyla perrini</i> sp. nov.	Switzerland, Ticino	PIA09*	This study
<i>Hyla perrini</i> sp. nov.	Switzerland, Ticino	PIA10*	This study
<i>Hyla perrini</i> sp. nov.	Switzerland, Ticino	PIA01	This study
<i>Hyla perrini</i> sp. nov.	Italy, Lombardy	OLE01	This study
<i>Hyla perrini</i> sp. nov.	Italy, Lombardy	OLE02	This study
<i>Hyla perrini</i> sp. nov.	Italy, Piedmont	TRI01	This study
<i>Hyla perrini</i> sp. nov.	Italy, Piedmont	TRI02	This study
<i>Hyla perrini</i> sp. nov.	Italy, Lombardy	CRE07	Canestrelli et al., 2007b
<i>Hyla perrini</i> sp. nov.	Italy, Lombardy	CRE10	Canestrelli et al., 2007b
<i>Hyla perrini</i> sp. nov.	Italy, Piedmont	TOR02	Canestrelli et al., 2007b
<i>Hyla perrini</i> sp. nov.	Italy, Piedmont	TOR07	Canestrelli et al., 2007b
<i>Hyla arborea</i>	Greece, Crete	CF14	Dufresnes et al. 2013
<i>Hyla arborea</i>	Croatia, S-Dalmatia	DMX14	Dufresnes et al. 2013
<i>Hyla arborea</i>	Croatia, Zagreb	ZF16	Dufresnes et al. 2013
<i>Hyla arborea</i>	Switzerland, Vaud	Abrx657	Dufresnes et al. 2013
<i>Hyla molleri</i>	France, Aquitaine	MOL67	This study
<i>Hyla molleri</i>	France, Aquitaine	MOL74	This study
<i>Hyla molleri</i>	Spain, Madrid	Hmol24	Stöck et al. 2011
<i>Hyla molleri</i>	Spain, Madrid	Hmol25	Stöck et al. 2011
<i>Hyla orientalis</i>	Turkey, Black Sea region (Clade A)	loc32.01	Dufresnes et al. 2016b
<i>Hyla orientalis</i>	Turkey, Black Sea region (Clade B)	loc22.15	Dufresnes et al. 2016b
<i>Hyla orientalis</i>	Turkey, Black Sea region (Clade C)	loc11.14	Dufresnes et al. 2016b
<i>Hyla orientalis</i>	Greece, Thrace (Clade D)	DidM06	Dufresnes et al. 2015a, 2016b
<i>Hyla sarda</i>	France, Corsica	Hsarda13	Dufresnes et al. 2015b
<i>Hyla sarda</i>	France, Corsica	Hsarda27	Dufresnes et al. 2015b
<i>Hyla sarda</i>	France, Corsica	Hsarda47	Dufresnes et al. 2015b
<i>Hyla felixarabica</i>	Jordan	HLi359	Dufresnes et al. 2015b
<i>Hyla felixarabica</i>	Jordan	HLi360	Dufresnes et al. 2015b
<i>Hyla felixarabica</i>	Jordan	HLi364	Dufresnes et al. 2015b
<i>Hyla savignyi</i>	Cyprus	Hsav20	Dufresnes et al. 2015b
<i>Hyla savignyi</i>	Cyprus	Hsavi40	Dufresnes et al. 2015b
<i>Hyla savignyi</i>	Israel, Tel-Aviv	CQ83	This study
<i>Hyla savignyi</i>	Azerbaijan, Ganja-Gazakh	HLi130	Stöck et al. 2012
<i>Hyla meridionalis</i>	Spain, Canaria	Ale01	Dufresnes et al. 2011
<i>Hyla meridionalis</i>	France, Languedoc-Roussillon	TVHM_F25	Dufresnes et al. 2015b
<i>Hyla meridionalis</i>	Portugal, Algarve	NME946/02	Stöck et al. 2008b, 2012
<i>Hyla cf. meridionalis</i>	Algeria, El Tarf	Algerie2	Stöck et al. 2008b, 2012
<i>Hyla cf. meridionalis</i>	Algeria, El Tarf	Algerie3	This study
<i>Hyla cf. meridionalis</i>	Tunisia, Jendouba	Tunisia2.1	Stöck et al. 2008b, 2012
<i>Hyla japonica</i>	Japan, Hiroshima	HjM03	Dufresnes et al. 2016c
<i>Hyla japonica</i>	Japan, Hiroshima	HjF03	Dufresnes et al. 2016c
<i>Hyla japonica</i>	Japan, Akita	AkitaF02	Dufresnes et al. 2016c
<i>Hyla japonica</i>	Japan, Akita	AkitaF03	Dufresnes et al. 2016c

Table S1c: Localities included in the hybrid zone analyses, with respective numbers of samples (n) for RAD and mtDNA data. Numbers correspond to Fig. 3.

	Site	n RAD	n MtDNA	Latitude	Longitude	Origin of samples
1	PIA	16	20	46.14	8.82	Stöck et al., 2011, Dufresnes et al. 2015b
-	LUG	-	3	46.03	8.91	Canestrelli et al., 2007a, 2007b
2	CRE	8	4	45.15	10.02	Canestrelli et al., 2007a, 2007b
3	SGI	11	-	44.92	12.27	This study
4	ALB	9	-	44.51	12.22	This study
-	CAV	-	2	45.13	12.07	Canestrelli et al., 2007a, 2007b
5	PUA	10	8	44.50	12.27	Canestrelli et al., 2007a, 2007b
6	LDC	15	-	44.34	12.31	This study
7	LDS	7	-	44.32	12.34	This study
8	MAG	8	8	44.15	12.08	Canestrelli et al., 2007a, 2007b
-	VER	-	6	43.98	12.42	Canestrelli et al., 2007a, 2007b
9	BAG	7	7	43.83	11.95	Canestrelli et al., 2007a, 2007b
10	SSE	13	-	43.57	12.07	This study
11	SLO	9	5	43.57	13.43	Canestrelli et al., 2007a, 2007b
12	ROS	7	7	42.67	13.98	Canestrelli et al., 2007a, 2007b

Table 1d: Populations sampled for the biometric analyses, with respective number of samples (n) for the morphometric and bioacoustic data.

Species	Country, region	n morphometrics	n bioacoustics	Latitude	Longitude
<i>H. intermedia</i> s. s.	Italy, Tuscany	9	6	43.87	11.34
<i>H. intermedia</i> s. s.	Italy, Lazio	1	-	42.20	11.73
<i>H. intermedia</i> s. s.	Italy, Lazio	2	2	41.38	12.99
<i>H. intermedia</i> s. s.	Italy, Lazio	3	2	42.25	12.63
<i>H. perrini</i> sp. nov.	Italy, Lombardy	10	5	45.59	8.67
<i>H. perrini</i> sp. nov.	Switzerland, Ticino	14	6	46.14	8.82
<i>H. arborea</i>	Switzerland, Vaud	12	4	46.50	6.42
<i>H. arborea</i>	Switzerland, Zürich	10	6	47.43	8.62

Table S2: Variables used in the ecological niche modelling (MaxEnt) and their relative contributions (in %) for each species, obtained from jackknife analysis.

	<i>H. intermedia</i> s. s.	<i>H. perrini</i> sp. nov.
Mean temperature of driest quarter (Bio 9)	36.2	8.0
Altitude	9.1	7.2
Land cover	8.6	3.7
Tree coverage percent	6.7	0.5
Temperature seasonality (Bio 4)	6.6	17.0
Precipitation of coldest quarter (Bio 19)	5.7	5.4
Precipitation of wettest quarter (Bio 16)	5.7	0.2
Precipitation of driest month (Bio 14)	4.5	9.9
Mean diurnal range (Bio 2)	4.2	0.3
Isothermality (Bio 3)	2.6	0.5
Slope	1.6	0.1
Mean temperature of wettest quarter (Bio 8)	1.5	5.3
Exposition	1.5	0.4
Terrain roughness index	1.5	0.8
Habitat homogeneity	1.4	1.1
Annual mean temperature (Bio 1)	1.3	24.6
Precipitation seasonality (Bio 15)	0.9	3.9
Aspect	0.4	0.2
Aridity index	0.0	10.9

Table S3: Measurements of the type series (in mm).

	MZL43340	MZL42341	MZL42342	MZL42343	MZL42344
	Holotype (M)	Paratype (M)	Paratype (M)	Paratype (F)	Paratype (F)
Snout Ventral Length (SVL)	43.3	41.7	41.6	40.6	45.3
Head Width (HW)	14.7	15.5	15.9	14.5	16.6
Head Length	12.8	13.2	13.0	12.6	13.6
Tympanum Diameter (TD)	2.4	2.4	2.0	2.5	2.6
Eye Diameter (ED)	4.3	4.1	4.3	4.2	4.4
Eye-Nostril Distance (EN)	4.0	3.6	4.0	3.9	3.9
Nostril-Snout Distance	4.0	3.7	4.2	3.4	4.2
Inter-Nostril Distance (NN)	3.4	3.5	3.3	3.5	3.7
Hand Length	14.8	14.9	16.1	14.0	15.7
Forelimb Length	21.9	22.8	22.1	21.2	24.4
Foot Length	20.9	20.1	20.7	20.0	21.4
Tarsus	12.8	12.2	11.6	12.0	13.7
Tibia Length	19.7	19.6	18.3	18.5	21.4
Femur Length	19.8	19.5	19.4	19.2	23.2

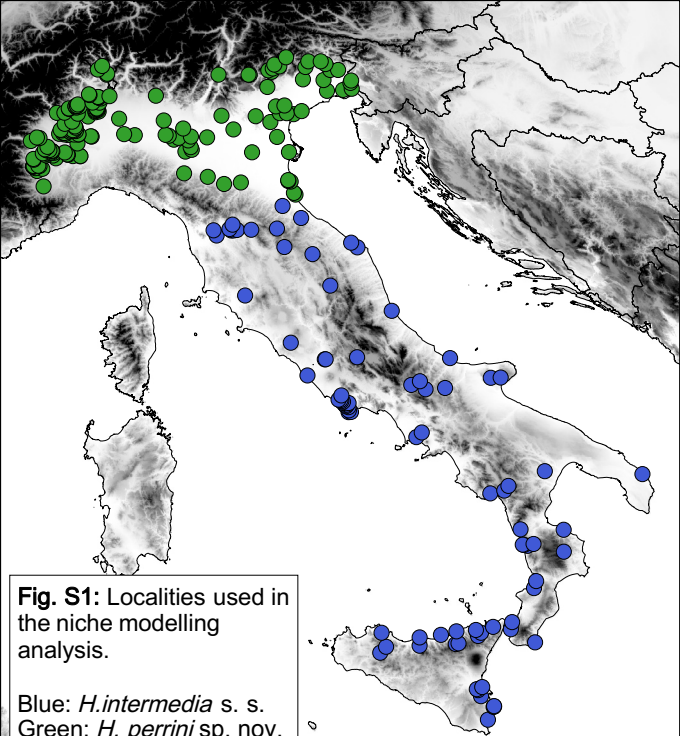


Fig S2: Principal Component Analysis on the phylogenomic data for all Western-Palearctic tree frogs, except the early-diverged *H. meridionalis*

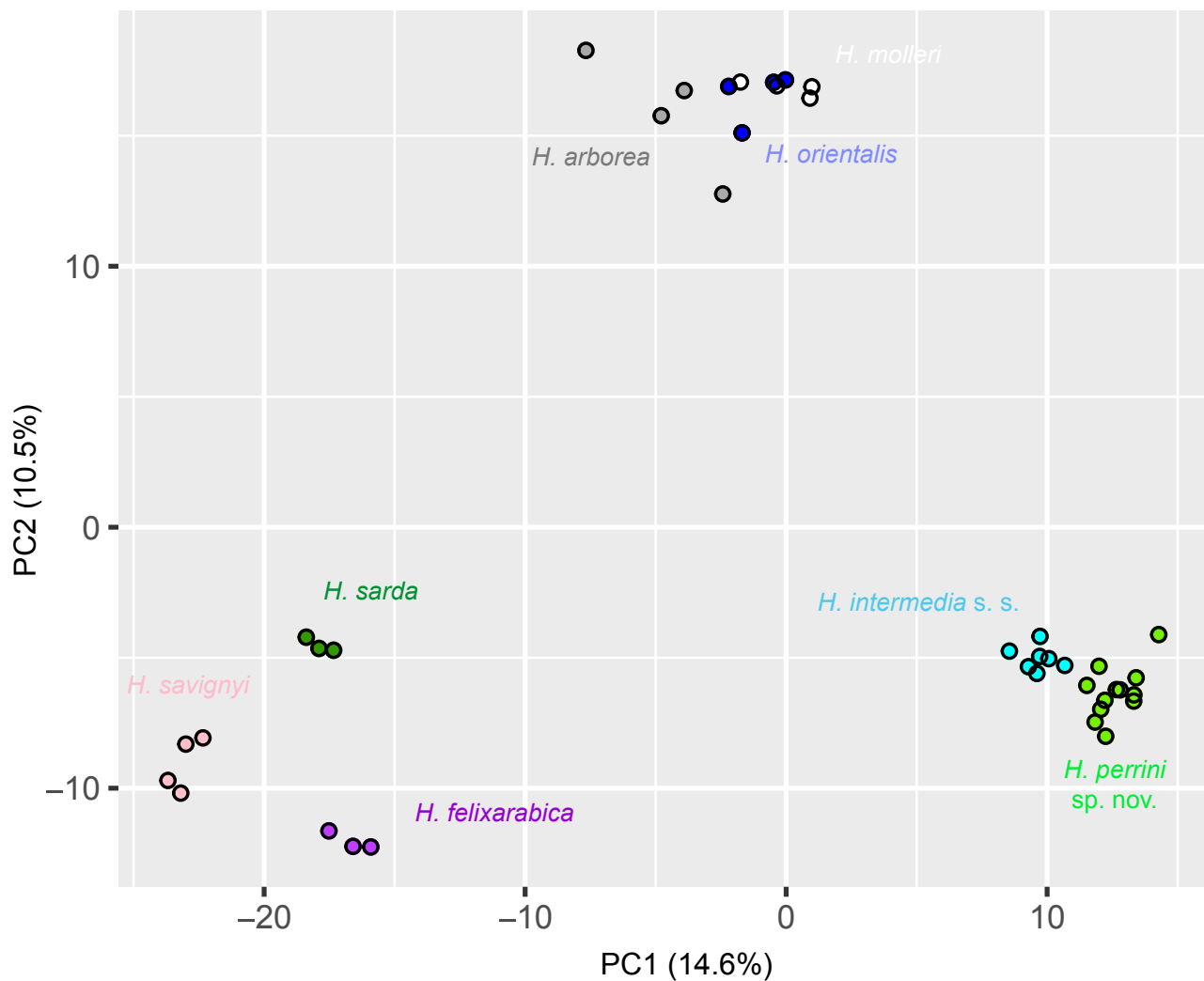


Fig. S3: Comparative morphometrics for *H. arborea* (arb), *H. intermedia* s. s. (int) and *H. perrini* sp. nov. (per). Measurements are corrected by SVL. Variables that showed a species effect in the MANOVA are highlighted by *, and the differing pair(s) of species are shown, when significant (Tuckey tests).

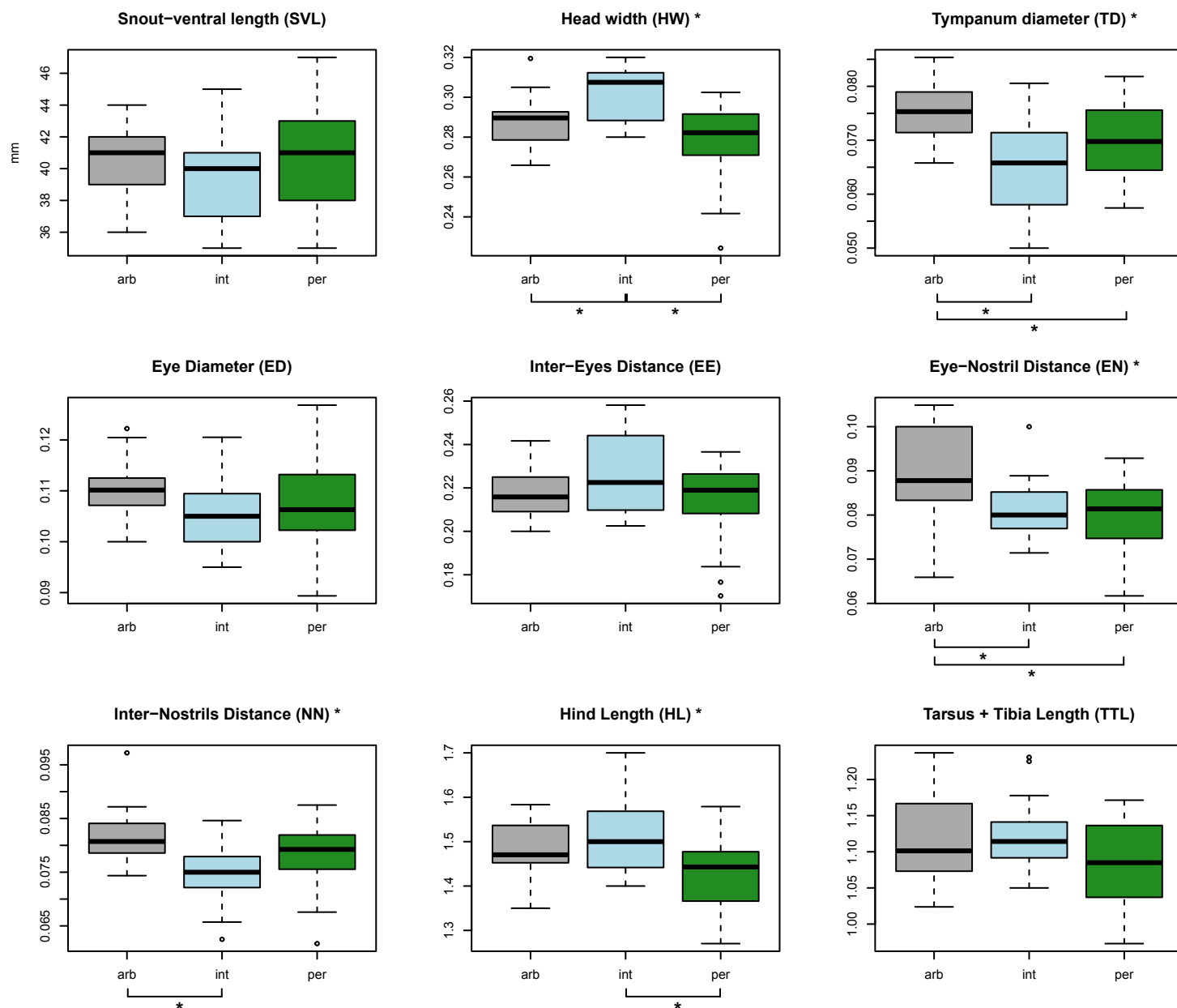


Fig. S4: Comparative bioacoustics for *H. arborea* (arb), *H. intermedia* s. s. (int) and *H. perrini* sp. nov. (per). Variables that showed a species effect in the MANOVA are highlighted by *, and the differing pair(s) of species are shown, when significant (Tuckey tests).

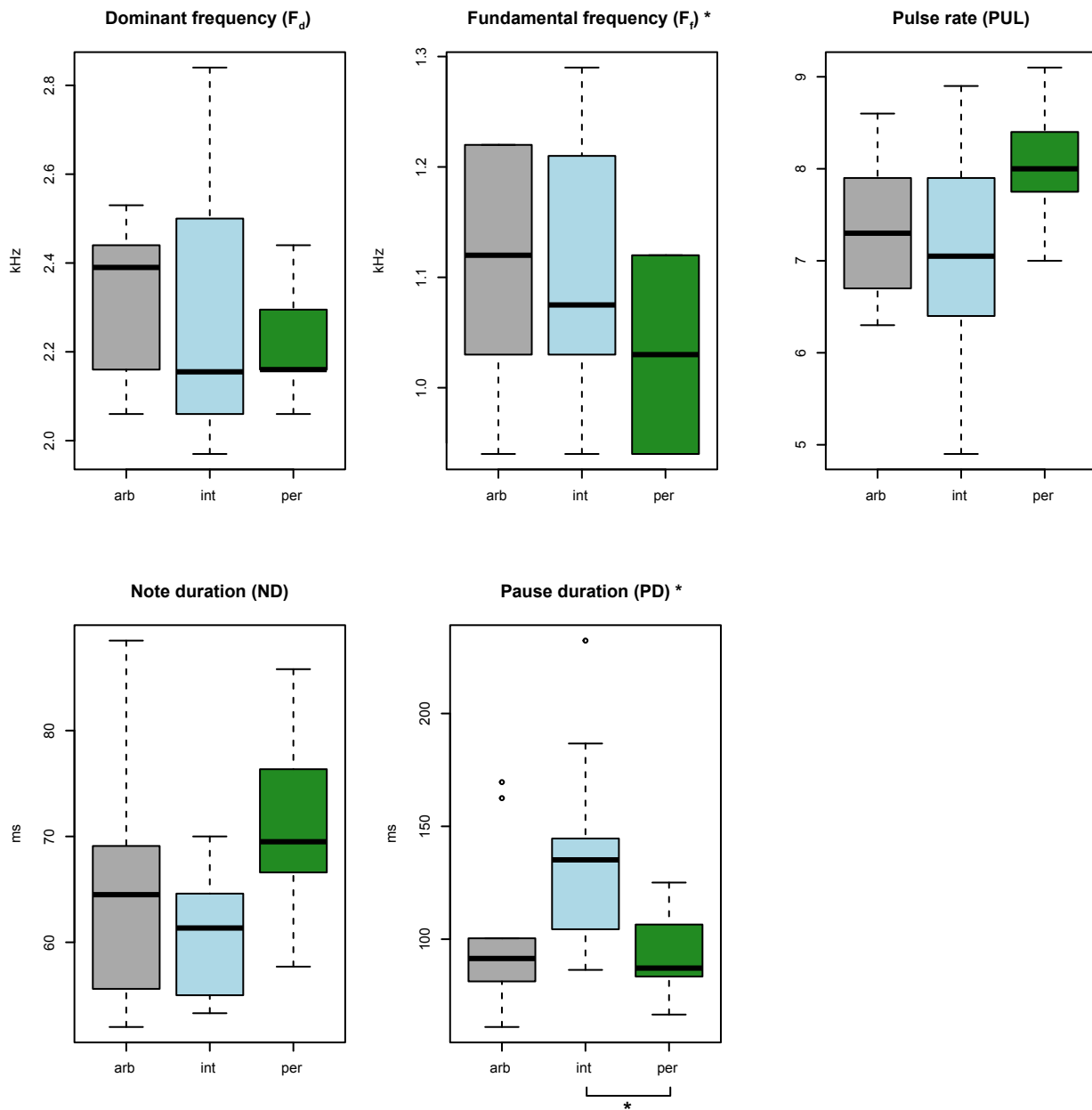


Fig. S5: Dorsal and ventral views of the paratypes of *Hyla perrini* sp. nov., deposited at the Cantonal Museum of Zoology of Lausanne (MZL).

