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A systematic survey on the extralimital populations of *Hyla meridionalis* in Salamanca (Spain)

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Resumen: Se presentan 51 nuevos registros para *Hyla meridionalis* en el suroeste de la provincia de Salamanca (España), recogidos a través de seis transectos de estaciones de escucha realizados en mayo y junio de 2007. No se han detectado zonas de conexión con las poblaciones de esta especie al sur del Sistema Central, al igual que no se ha encontrado el paso por el que debieron atravesar dicho sistema montañoso. Todo esto parece indicar que *H. meridionalis* se encuentra aislada en Salamanca. Se discute si la especie está realmente en expansión o no en dicha área.

Hyla meridionalis (Boettger, 1874) occurs in North-western Africa, Madeira, Canary Island, Iberian Peninsula, Minorca (Balearic Islands), south and south-eastern France and north-western Italy (Tejedo & Reques, 2002; Malkmus, 2004; Sindaco *et al*., 2006). In the Iberian Peninsula, its range is divided in three isolated nuclei: central and south-western parts of the Peninsula, the north-eastern extreme of Spain (Catalunya region), and one population in the Basque Country (Spain).

Recently, several works have reported new records of *H. meridionalis* in the south-eastern part of the Salamanca province (Bueno, 1991; Merchán *et al*., 2004; García, 2007), and in the south-western part of the Madrid province (Aceituno, 2001; Martínez-Solano & Aceituno, 2001). Martínez-Solano & Fernández-González (2003) and Merchán *et al*. (2004) suggested that the species is currently in expansion in both provinces. This has a special relevance for the Salamanca records, as they constitute the only populations located northern to the Central Mountain Range. Until then, this mountain chain was considered an effective geographic barrier (Merchán *et al*., 2004).

According to Recuero *et al*. (2007), the southern Iberian populations come from the western coast of Morocco by natural expansion, and the northern Iberia and southern France populations from northern Morocco by human introduction.

Merchán *et al*. (2004) suggested that the global climatic warming is the most probable cause for the expansion of *H. meridionalis*, notwithstanding alternative explanations, namely, the lack of intense sampling, at least in Salamanca. However, Martínez-Solano & Fernández-González (2003) rejected this possibility because Madrid is considered as a well sampled area (García-París *et al*., 1989).

The main objective of this work was to establish a sampling methodology for evaluating if *H. meridionalis* is currently in expansion in Salamanca. Several years of systematic monitoring are needed for reaching this objective, therefore surveys will be repeated every year in the next five years. This paper presents the results of the first year. A second objective was to determine the contact areas with southern populations (Cáceres and Ávila).

Methods

Sampling methodology was based on listening stations of three minutes for anuran calls. A total of six car transects were performed on 2007 May 7, 8, 9, and 10, and on 2007 June 12 and 13. The transects had

different lengths: transect nº 1, 95 km; nº 2, 106 km; nº 3, 100 km; nº 4, 105 km; nº 5, 78 km; and nº6, 153 km (Figure 1). The listening stations were repeated each 3 - 4 km. The total number of listening stations was 144, distributed as follows: 32 stations on May 7; 29 on May 8; 28 on May 9; 28 on May 10; 17 on June 12; and 10 on June 13. In May, listening stations were recorded in the province of Salamanca around the area where the presence of the species was previously reported (Bueno, 1991; Merchán *et al.*, 2004; García, 2007). In June, the approximated limits of the whole area occupied by *H. meridionalis* were defined. The six sampling nights were moderately warm and without rain, excepting the last one (2007 June 13).

Results

A total of six species of anurans were recorded during surveys (see Appendix and Figure 1): *Alytes* sp. (32 stations), *Bufo calamita* (6), *Pelobates cultripes* (6), *H. arborea* (110), *H. meridionalis* (51) and *Phelophylax perezi* (132). In 82% of stations, *H. meridionalis* were in sympatry with *H. arborea*, and in 98% with *P. perezi*. The name *Pelophylax perezi* is used following Frost *et al.* (2006).

Discussion

H. meridionalis is limited to the north by the division line between the Duero and Tajo basins; to the east by the Duero basin; to the south by the Cuerpo de Hombre river; and to the west by the Alagón river (Figure 1). There is only a record of *H. meridionalis* to the west of the Alagón river, at Valero village, far away from the remaining records (marked with a black arrow in Figure 1). Therefore, the main populations are situated in the Sangusin river basin. Most of the records of *H. meridionalis* are in strict syntopy with *H. arborea*. Except only one record of *H. meridionalis*, all the remaining are in syntopy with *P. perezi*. The syntopy of both species of the genus *Hyla* is frequent and is well documented (Tejedo & Reques, 2002).

Apparently, there is no connection between these records and the southern populations. Three of the six transects (nº 3, 5 and 6) were performed close to the Central Mountain Range and Alagón river (Figure 1), and no records of *H. meridionalis* were collected. However, records of *H. arborea* and *P. perezi* were collected in the same transects, although the presence of amphibians in the south seems to be lower (the topography is more complex and there are less ponds). Moreover, no records were collected in the Alagón river, the natural pass (as Mediterranean corridor) for crossing from the Southern plateau to the Northern plateau. *H. meridionalis* is currently located in a zone with a very difficult access from the south, due to the presence of the highest mountain in Salamanca (Calvitero, 2405 m). If the Central Mountain Range acts as an effective barrier in this area and there are no records close to southern populations, *H. meridionalis* could be considered isolated. Then, if *H. meridionalis* is assumed to be isolated in Salamanca, is still possible to consider that the species is currently in expansion? Two answers are possible:

1) If affirmative, the species should have entered in the Northern plateau through a natural pass, such as the Alagón river. As there are currently no records of *H. meridionalis* there, it is necessary to confirm the presence of the species in the river, south to the Central Mountain Range. If the Alagón river was effectively the natural pass for crossing to the Northern plateau, then the species continued advancing to the north and for a particular cause, it did not remain in the river. If the natural pass was not the Alagón river, then an alternative pass has not been yet detected.

2) If negative, the species presence was not detected before in Salamanca due to lack of sampling. Previous works did not survey this area with listening stations (Sillero *et al.*, 2005 and references therein), therefore it is very difficult to detect *H. meridionalis*, due to its secretive live (García-París, 2004). Independently if *H. meridionalis* is currently or not in expansion, it is necessary to find the present or past pass between both plateaux.

To evaluate which hypothesis is correct, more surveys are necessary throughout the

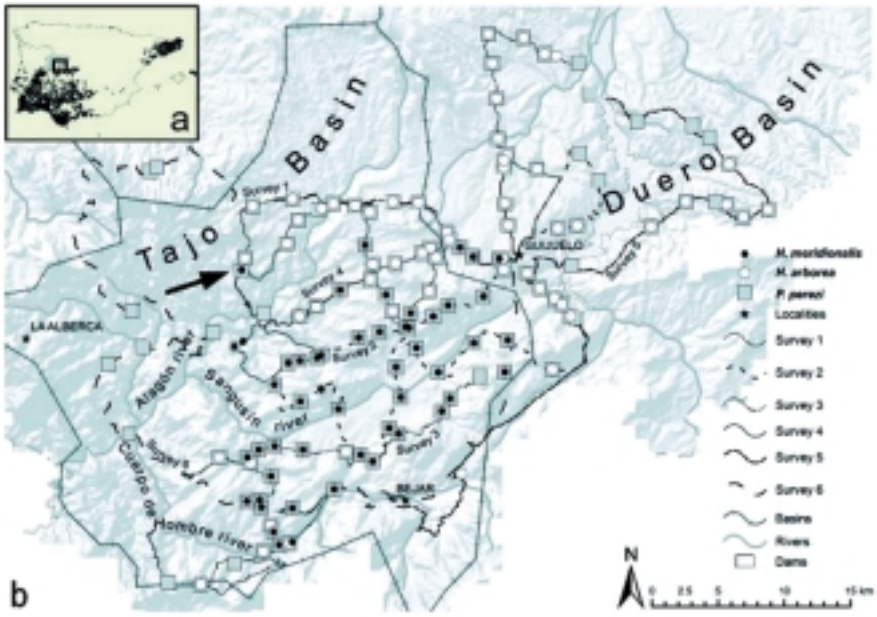

Figure 1. Map a: Location of Salamanca province in the Iberian Peninsula. The study area was located inside the black square. Black dots represent the Iberian distribution of *H. meridionalis* (Pleguezuelos *et al*., 2002; Malkmus, 2004). Map b: New records of *Hyla meridionalis*, *H. arborea* and *Phelophylax perezi* collected in the south-western part of Salamanca province (Spain), from six listening station transects (surveys in the map) on May and June 2007. Records of *Alytes sp*, *Bufo calamita* and *Pelobates cultripes* are not represented (see Appendix 1). The division between the Duero and Tajo basins is represented. The only record of *H. meridionalis* located to the west of the Alagón river is marked with a black arrow. The figure background is an orographic shaded representation from the Shuttle Radar Topography Mission digital elevation model <http://srtm.usgs.gov/> [accessed on 2007 June 20].

following years. Moreover, new surveys should be performed in Madrid province, in order to confirm if the species is effectively in expansion in that area. Also, a predictive model should be produced in order to determine which are the potential areas of expansion and the pass used for crossing the Central Mountain Range. Finally, a genetic analysis is required to confirm that the original population come from the western coast of northern Morocco, as an introduction by human could not be eliminated as alternative hypotheses (notwithstanding improbable).

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Appendix 1. List of species collected in the sourth-western part of the province of Salamanca (Spain), from six listening station transects on 2007 May and June. The abbreviated species' name, the 1x1 km UTM square and the locality name are indicated. Species' names are abbreviated as follows: A, *Alytes* sp; BC, *Bufo calamita*, HA, *Hyla arborea*, HM, *Hyla meridionalis*, PC, *Pelobates cultripes*; and PP, *Pelophylax perezi*.

N	sp	UTM	Locality	N	sp	UTM	Locality
1	A	TK9099	Armenteros	20	A	TK6090	Los Santos
2	A	TK4876	Colmenar de Montemayor	22	A	TK6178	La Calzada de Bejar
3	A	TK4968	Lagunilla	23	A	TK6196	Endrinal
4	A	TK5286	Santibañez de la Sierra	24	A	TK6293	Los Santos
5	A	TK5388	San Esteban de la Sierra	25	A	TK6296	Endrinal
6	A	TK5392	San Miguel de Valero	26	A	TK6380	Navalmoral de Bejar
7	A	TK5474	Aldeacipreste	27	A	TK6793	Fuenterroble de Salvatierra
8	A	TK5488	San Esteban de la Sierra	28	A	TK7078	Fresnedoso
9	A	TK5571	Montemayor del Rio	29	A	TK7292	Guijuelo
10	A	TK5578	Horcajo de Montemayor	30	A	TK7684	Santibañez de Bejar
11	A	TK5588	San Esteban de la Sierra	31	A	TK7895	Aldeavieja de Tormes
12	A	TK5697	Monleon	32	A	TL8900	Armenteros
13	A	TK5788	San Esteban de la Sierra	33	BC	TK5076	Colmenar de Montemayor
14	A	TK5883	Valdefuentes de Sangusin	34	BC	TK5772	Montemayor del Rio
15	A	TK5885	Valdefuentes de Sangusin	35	BC	TK6078	La Calzada de Bejar
17	A	TK5889	San Esteban de la Sierra	36	BC	TK6484	Valverde de Valdelacasa
18	A	TK6078	La Calzada de Bejar	37	BC	TK9096	Armenteros
19	A	TK6081	Valdefuentes de Sangusin	38	BC	TK9296	Armenteros

Appendix 1. Continuación.

N	sp	UTM	Locality
39	HA	TK4968	Lagunilla
40	HA	TK5177	Colmenar de Montemayor
41	HA	TK5286	Santibañez de la Sierra
42	HA	TK5374	Aldeacipreste
43	HA	TK5377	Horcajo de Montemayor
45	HA	TK5392	San Miguel de Valero
46	HA	TK5396	Linares de Riofrio
47	HA	TK5470	Montemayor del Rio
48	HA	TK5472	Aldeacipreste
50	HA	TK5474	Aldeacipreste
51	HA	TK5478	Horcajo de Montemayor
52	HA	TK5571	Montemayor del Rio
53	HA	TK5572	Montemayor del Rio
54	HA	TK5576	Aldeacipreste
55	HA	TK5578	Horcajo de Montemayor
56	HA	TK5583	Cristobal
57	HA	TK5671	Montemayor del Rio
58	HA	TK5674	Aldeacipreste
59	HA	TK5685	Cristobal
60	HA	TK5694	El Tornadizo
61	HA	TK5697	Monleon
62	HA	TK5778	Valdehijaderos
63	HA	TK5782	Horcajo de Montemayor
64	HA	TK5785	Cristobal
65	HA	TK5788	San Esteban de la Sierra
67	HA	TK5795	Monleon
68	HA	TK5883	Valdefuentes de Sangusin
69	HA	TK5885	Valdefuentes de Sangusin
71	HA	TK5889	San Esteban de la Sierra
73	HA	TK5896	Monleon
74	HA	TK5975	La Calzada de Bejar
75	HA	TK5997	Monleon
76	HA	TK6078	La Calzada de Bejar
77	HA	TK6081	Valdefuentes de Sangusin
78	HA	TK6090	Los Santos
80	HA	TK6178	La Calzada de Bejar
82	HA	TK6186	Valdefuentes de Sangusin
83	HA	TK6196	Endrinal
84	HA	TK6277	Navalmoral de Bejar
85	HA	TK6291	Los Santos
86	HA	TK6292	Los Santos
87	HA	TK6293	Los Santos
88	HA	TK6296	Endrinal
89	HA	TK6380	Navalmoral de Bejar
90	HA	TK6387	Valverde de Valdelacasa
91	HA	TK6390	Los Santos
92	HA	TK6479	Navalmoral de Bejar
93	HA	TK6482	Peromingo
94	HA	TK6484	Valverde de Valdelacasa
95	HA	TK6488	Los Santos
96	HA	TK6492	Los Santos
97	HA	TK6497	Endrinal
98	HA	TK6587	Valdelacasa
100	HA	TK6588	Valdelacasa
101	HA	TK6690	Fuenterroble de Salvatierra
102	HA	TK6693	Fuenterroble de Salvatierra
103	HA	TK6697	Casafranca
104	HA	TK6781	Sanchotello
106	HA	TK6793	Fuenterroble de Salvatierra
107	HA	TK6882	Sanchotello
108	HA	TK6889	Valdelacasa
110	HA	TK6894	Fuenterroble de Salvatierra
111	HA	TK6976	Vallejera de Riofrio
112	HA	TK6993	Fuenterroble de Salvatierra
113	HA	TK7086	Fuentes de Bejar
114	HA	TK7093	Fuenterroble de Salvatierra
116	HA	TK7284	Nava de Bejar
117	HA	TK7286	La Cabeza de Bejar
118	HA	TK7292	Guijuelo
121	HA	TK7295	Guijuelo
122	HA	TK7297	Guijuelo
124	HA	TK7299	Berrocal de Salvatierra
125	HA	TK7491	Guijuelo
126	HA	TK7590	Guijuelo
127	HA	TK7599	Pizarral
128	HA	TK7684	Santibañez de Bejar
129	HA	TK7689	Guijo de Avila
130	HA	TK7695	Aldeavieja de Tormes
131	HA	TK7788	Guijo de Avila
132	HA	TK7789	Guijo de Avila
133	HA	TK7895	Aldeavieja de Tormes
134	HA	TK8395	Salvatierra de Tormes
135	HA	TK8697	La Tala
136	HA	TK8997	Armenteros
137	HA	TK9096	Armenteros
138	HA	TK9296	Armenteros
139	HA	TL7107	Pedrosillo de los Aires
140	HA	TL7110	Pedrosillo de los Aires
142	HA	TL7200	Berrocal de Salvatierra
143	HA	TL7202	Berrocal de Salvatierra
144	HA	TL7205	Berrocal de Salvatierra
145	HA	TL7409	Pedrosillo de los Aires
146	HA	TL7508	Pedrosillo de los Aires
147	HA	TL7608	Pedrosillo de los Aires
148	HA	TL8900	Armenteros
149	HM	TK5286	Santibañez de la Sierra
151	HM	TK5292	San Miguel de Valero
152	HM	TK5374	Aldeacipreste
153	HM	TK5377	Horcajo de Montemayor
154	HM	TK5474	Aldeacipreste
156	HM	TK5478	Horcajo de Montemayor
157	HM	TK5571	Montemayor del Rio
158	HM	TK5572	Montemayor del Rio
159	HM	TK5576	Aldeacipreste
160	HM	TK5578	Horcajo de Montemayor
161	HM	TK5583	Cristobal
162	HM	TK5671	Montemayor del Rio
163	HM	TK5674	Aldeacipreste
164	HM	TK5685	Cristobal
165	HM	TK5778	Valdehijaderos
166	HM	TK5782	Horcajo de Montemayor
167	HM	TK5785	Cristobal
168	HM	TK5883	Valdefuentes de Sangusin
170	HM	TK5885	Valdefuentes de Sangusin
171	HM	TK5975	La Calzada de Bejar
172	HM	TK6081	Valdefuentes de Sangusin
173	HM	TK6090	Los Santos
174	HM	TK6178	La Calzada de Bejar
175	HM	TK6186	Valdefuentes de Sangusin
176	HM	TK6277	Navalmoral de Bejar
177	HM	TK6293	Los Santos
178	HM	TK6380	Navalmoral de Bejar
179	HM	TK6387	Valverde de Valdelacasa
180	HM	TK6390	Los Santos
181	HM	TK6479	Navalmoral de Bejar
182	HM	TK6482	Peromingo
183	HM	TK6484	Valverde de Valdelacasa
184	HM	TK6587	Valdelacasa
186	HM	TK6588	Valdelacasa
187	HM	TK6686	Puebla de San Medel
188	HM	TK6781	Sanchotello
189	HM	TK6784	Ledrada

Appendix 1. Continuación.

N	sp	UTM	Locality
190	HM	TK6788	Valdelacasa
191	HM	TK6882	Sanchotello
192	HM	TK6889	Valdelacasa
193	HM	TK6993	Fuenterroble de Salvatierra
194	HM	TK7086	Fuentes de Bejar
195	HM	TK7093	Fuenterroble de Salvatierra
196	HM	TK7190	Guijuelo
197	HM	TK7284	Nava de Bejar
198	HM	TK7286	La Cabeza de Bejar
199	HM	TK7292	Guijuelo
200	PC	TK6588	Valdelacasa
201	PC	TK7888	Guijo de Avila
202	PC	TL7107	Pedrosillo de los Aires
203	PC	TL7409	Pedrosillo de los Aires
204	PC	TL8104	Pelayos
205	PC	TL8802	Galinduste
206	PP	QE5184	Cepeda
207	PP	QE5288	Villanueva del Conde
208	PP	QE5379	Sotoserrano
209	PP	QE5386	Miranda del Castanar
210	PP	QE5399	Navarredonda de la Rinconada
211	PP	TK4699	Navarredonda de la Rinconada
212	PP	TK4768	Lagunilla
213	PP	TK4968	Lagunilla
214	PP	TK5087	Santibañez de la Sierra
215	PP	TK5177	Colmenar de Montemayor
216	PP	TK5269	Lagunilla
217	PP	TK5292	San Miguel de Valero
218	PP	TK5374	Aldeacipreste
220	PP	TK5377	Horcajo de Montemayor
221	PP	TK5392	San Miguel de Valero
222	PP	TK5396	Linares de Riofrio
223	PP	TK5470	Montemayor del Rio
224	PP	TK5472	Aldeacipreste
226	PP	TK5474	Aldeacipreste
227	PP	TK5478	Horcajo de Montemayor
228	PP	TK5488	San Esteban de la Sierra
229	PP	TK5571	Montemayor del Rio
230	PP	TK5572	Montemayor del Rio
231	PP	TK5576	Aldeacipreste
232	PP	TK5578	Horcajo de Montemayor
233	PP	TK5583	Cristobal
234	PP	TK5671	Montemayor del Rio
235	PP	TK5674	Aldeacipreste
236	PP	TK5685	Cristobal
237	PP	TK5694	El Tornadizo
238	PP	TK5697	Monleon
239	PP	TK5778	Valdehijaderos
240	PP	TK5782	Horcajo de Montemayor
241	PP	TK5785	Cristobal
243	PP	TK5788	San Esteban de la Sierra
244	PP	TK5795	Monleon
246	PP	TK5885	Valdefuentes de Sangusin
248	PP	TK5889	San Esteban de la Sierra
249	PP	TK5896	Monleon
250	PP	TK5975	La Calzada de Bejar
251	PP	TK5997	Monleon
252	PP	TK6078	La Calzada de Bejar
253	PP	TK6081	Valdefuentes de Sangusin
255	PP	TK6090	Los Santos
256	PP	TK6178	La Calzada de Bejar
257	PP	TK6186	Valdefuentes de Sangusin
258	PP	TK6196	Endrinal
259	PP	TK6277	Navalmoral de Bejar
260	PP	TK6291	Los Santos
261	PP	TK6292	Los Santos
262	PP	TK6293	Los Santos
263	PP	TK6296	Endrinal
264	PP	TK6380	Navalmoral de Bejar
265	PP	TK6387	Valverde de Valdelacasa
266	PP	TK6390	Los Santos
267	PP	TK6479	Navalmoral de Bejar
268	PP	TK6482	Peromingo
269	PP	TK6484	Valverde de Valdelacasa
270	PP	TK6488	Los Santos
271	PP	TK6492	Los Santos
272	PP	TK6497	Endrinal
274	PP	TK6587	Valdelacasa
275	PP	TK6588	Valdelacasa
276	PP	TK6686	Puebla de San Medel
277	PP	TK6690	Fuenterroble de Salvatierra
278	PP	TK6693	Fuenterroble de Salvatierra
279	PP	TK6697	Casafranca
280	PP	TK6781	Sanchotello
281	PP	TK6784	Ledrada
282	PP	TK6788	Valdelacasa
284	PP	TK6793	Fuenterroble de Salvatierra
285	PP	TK6882	Sanchotello
286	PP	TK6889	Valdelacasa
288	PP	TK6894	Fuenterroble de Salvatierra
289	PP	TK6993	Fuenterroble de Salvatierra
290	PP	TK7084	Ledrada
291	PP	TK7086	Fuentes de Bejar
293	PP	TK7093	Fuenterroble de Salvatierra
294	PP	TK7190	Guijuelo
295	PP	TK7284	Nava de Bejar
296	PP	TK7286	La Cabeza de Bejar
297	PP	TK7292	Guijuelo
300	PP	TK7295	Guijuelo
303	PP	TK7297	Guijuelo
304	PP	TK7299	Berrocal de Salvatierra
305	PP	TK7491	Guijuelo
306	PP	TK7590	Guijuelo
307	PP	TK7599	Pizarral
308	PP	TK7684	Santibañez de Bejar
309	PP	TK7689	Guijo de Avila
310	PP	TK7695	Aldeavieja de Tormes
311	PP	TK7788	Guijo de Avila
312	PP	TK7789	Guijo de Avila
313	PP	TK7792	Cespedosa de Tormes
314	PP	TK7888	Guijo de Avila
315	PP	TK7895	Aldeavieja de Tormes
316	PP	TK7998	Salvatierra de Tormes
317	PP	TK8395	Salvatierra de Tormes
318	PP	TK8697	La Tala
320	PP	TK8997	Armenteros
321	PP	TK9096	Armenteros
322	PP	TK9296	Armenteros
323	PP	TL7107	Pedrosillo de los Aires
324	PP	TL7110	Pedrosillo de los Aires
326	PP	TL7200	Berrocal de Salvatierra
327	PP	TL7202	Berrocal de Salvatierra
328	PP	TL7205	Berrocal de Salvatierra
329	PP	TL7409	Pedrosillo de los Aires
330	PP	TL7508	Pedrosillo de los Aires
331	PP	TL7800	Montejo
332	PP	TL7807	La Maya
333	PP	TL7905	Montejo
334	PP	TL8203	Pelayos
335	PP	TL8603	Galinduste
336	PP	TL8802	Galinduste
337	PP	TL8900	Armenteros