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Locality number (as Fig. 1)	GenBank Acc.-No. D-loop (or partial)	GenBank Acc.-No. 16S	GenBank Acc.-No. Tropomyosine	GenBank Acc.-No. RAG 1	Sample-ID	Voucher (if available)	N	Taxon	Locality	LAT	LONG	ELEVATION (if known)
1	DQ629730	-	-	-	371	-	1	B. boulengeri	Morocco, Ait Baha, E. Recuero leg.	30.130	-9.080	
2	DQ629718	-	-	-	112	MTD 45286	1	B. boulengeri	Morocco, High Atlas, D. Frynta leg.	32.427	-5.156	
3	DQ629704	-	-	-	179	CUP AMPH\MOR\01	1	B. boulengeri	Morocco, High Atlas, D. Frynta leg.	33.427	-5.150	1438
4	DQ629720	-	-	-	163	ZFMK 49652	1	B. boulengeri	Algeria, Ghardaia, W. Bischoff, U. Joger leg.	32.483	3.667	
5a	-	-	-	-	160	ZFMK 37856	1	B. balearicus	Spain, Balearic islands, Mallorca, Cape Andraixs, C.A. Raehmel leg. 1982	39.500	3.000	
5b	EU497593	-	-	-	81B		1	B. balearicus	Spain, Balearic islands, Mallorca, Artà, Charca temporal de Sa Vaca, J. Muntaner leg.	39.690	3.330	
5c	EU497594	-	-	-	82B		1	B. balearicus	Spain, Balearic islands, Mallorca, Fornalutx, Alberca de Bàlitx, J. Muntaner leg.	39.780	2.760	
5d	EU497595	-	-	-	83B		1	B. balearicus	Spain, Balearic islands, Mallorca, Sineu, Acequia carretera de Sineu a Petra, J. Muntaner leg.	39.640	3.030	
5e	EU497596 EU497597	-	-	-	84B, 85B		2	B. balearicus	Spain, Balearic islands, Mallorca, Llucmajor, Charca temporal de Son Cànaves, J. Muntaner leg.	39.480	2.880	
6	EU497577 EU497578 EU497579	EU497477 EU497478 EU497479	-	-	65B, 66B, 67B	-	3	B. balearicus	Spain, Balearic islands, Menorca, Torrellafuda, J. Pretus and A. Sicilia leg.	39.951	3.935	
7	EU497574 EU497575 EU497576	EU497474 EU497475 EU497476	-	-	62B, 63B, 64B	-	3	B. balearicus	Spain, Balearic islands, Menorca, Rafalet, J. Pretus leg.	39.841	4.298	
8	EU497568 EU497569 EU497570 EU497571 EU497572 EU497573	EU497468 EU497469 EU497470 EU497471 EU497472 EU497473	-	-	56B, 57B, 58B, 59B, 60B, 61B	-	6	B. balearicus	France, Corse, Cap Corse, Ersa, Barcaggio, M. Delaugerre leg.	43.050	9.408	
9	DQ629731	-	-	EU497604 (Bufo175)	175	ZSM 6/2004	1	B. balearicus	France, S-Corsica, near Bonifacio, F. Glaw, K. Schmidt, leg.	41.383	9.150	
10	EU497493 EU497494 EU497495 EU497496 EU497497 EU497498 EU497499 EU497500	-	EU497628, EU497629 (Bufo 395), EU497630 (Bufo 402)	EU497608 (Bufo 402)	395, 396, 397, 398, 399, 400, 402, 403	-	8	B. balearicus	Italy, NE-Sardinia, E of Monticanaglia, H. Veith leg.	41.061	9.531	
11	EU497563 EU497564 EU497565 EU497566 EU497567	EU497463 EU497464 EU497465 EU497466 EU497467	-	-	51B, 52B, 53B, 54B, 55B	-	5	B. balearicus	Italy, Sardinia, Sassari, Monte Nurra, G. Sotgiu leg.	40.723	8.342	
12	EU497562	EU497462	-	-	50B	-	1	B. balearicus	Italy, Sardinia, Oristano, Cabras, Stagno di Mistras, L. Bassu leg.	39.912	8.458	
13	EU497523 EU497524 EU497525	EU497423 EU497424 EU497425	-	-	11B, 12B, 13B	-	3	B. balearicus	Italy, Turine, Poirino, R. Sindaco leg.	44.922	7.822	
14	EU497518 EU497519 EU497520 EU497521 EU497522	EU497418 EU497419 EU497420 EU497421 EU497422	-	-	6B, 7B, 8B, 9B, 10B	-	5	B. balearicus	Italy, Pavia, San Martino Siccomario, F. Bernini leg.	45.155	9.142	
15	EU497526 EU497527	EU497426 EU497427	-	-	14B, 15B	-	2	B. balearicus	Italy, Pisa, Tenuta di San Rossore, A. Sicilia leg.	43.721	10.309	
16	EU497528 EU497529	EU497428 EU497429	-	-	16B, 17B	-	2	B. balearicus	Italy, Rome, Laurentina, A. Romano leg.	41.645	12.548	
17	EU497530 EU497532	EU497430 EU497432	-	-	18B, 20B	-	2	B. balearicus	Italy, Brindisi, Villa Castelli, T. Fattizzo leg.	40.578	17.461	
18	DQ629733	-	EU497622, EU497623 (Bufo 189)	EU497605 (Bufo 189)	189	NME 913/01	1	B. balearicus	Italy, W coast, Calabria, Paola, A. Nöllert leg.	39.350	16.033	
19	EU497533 EU497534 EU497535 EU497536 EU497537	EU497433 EU497434 EU497435 EU497436 EU497437	-	-	21B, 22B, 23B, 24B 25B	-	5	B. balearicus	Italy, Reggio Calabria, Condofuri, Amendolea, A. Sicilia leg.	37.983	15.894	
20	DQ629732	-	EU497620, EU497621 (Bufo 188)		188	NME 912/01	1	B. balearicus	Italy, Sicily, N of Francavilla di Sicilia, stream valley, T. Zavianni, A. Nöllert leg.	37.900	15.133	
21	DQ629726 DQ629727 DQ629728 DQ629729 EU497501 EU497502 EU497503 EU497504	-	EU497626 (Bufo 324), EU497627 (Bufo 325)	EU497609 (Bufo 325)	323, 324, 325, 326, 420, 421, 422, 423	ZMB 69556, MVZ 250741, MVZ 250742, MVZ 250743	8	B. siculus	Italy, Sicily, E of Lentini, near mouth of San Leonardo River, 500 m from coast to inland, M. Lo Valvo leg.	37.333	15.067	5
22	EU497543 EU497544 EU497545 EU497546 EU497547	EU497443 EU497444 EU497445 EU497446 EU497447	-	-	31B, 32B, 33B, 34B, 35B	-	5	B. siculus	Italy, Sicily, Agrigento, Macalube di Aragona, A. Sicilia leg.	37.377	13.596	

	GenBank Acc.-No.	GenBank Acc.-No.	GenBank Acc.-No.	GenBank Acc.-No.					**ADDITIONAL FILE 1, Page 2 of 2**			
23	EU497505 EU497538 EU497539 EU497540 EU497541 EU497542	EU497438 EU497439 EU497440 EU497441 EU497442	-	-	26B, 27B, 28B, 29B, 30B, 424	ZFMK 85896	6	B. siculus	Italy, Sicily, Palermo, Monte Pellegrino, A. Sicilia, M. Lo Valvo leg.	38.170	13.351	
23a	EU497598	-	-	-	86 B	MZPA A95	1	B.siculus	Italy, Palermo, La Fossa, A. Sicilia leg.	38.211	13.290	
24	EU497507 EU497508 EU497509 EU497510 EU497511 EU497548 EU497549 EU497550 EU497551 EU497552	EU497448 EU497449 EU497450 EU497451 EU497452	-	-	415, 416, 417, 418, 419, 36B, 37B, 38B, 39B, 40B	-	10	B. siculus	Italy, Ustica island, Gorgo di San Bartolicchio, A. Sicilia, M. Lo Valvo leg.	38.700	13.172	
25	EU497553 EU497554 EU497555 EU497556	EU497453 EU497454 EU497455 EU497456	-	-	41B, 42B, 43B, 44B	-	4	B. siculus	Italy, Favignana island, Cala Rossa, A. Sicilia leg.	37.921	12.360	
26	EU497512 EU497557 EU497558 EU497559 EU497560 EU497561	EU497457 EU497458 EU497459 EU497460 EU497461	EU497631, EU497632 (Bufo 425)	EU497612 (Bufo 425)	45B, 46B, 47B, 48B, 49B, 425	MVZ 250744	6	B. cf. boulengeri	Italy, Lampedusa island, Contrada Poggio Monaco, G. Nicolini and Mario Lo Valvo leg.	35.508	12.600	
27	EU497580 EU497581 EU497582	EU497480 EU497481 EU497482	-	-	68B, 69B, 70B	-	3	B. boulengeri	Tunisia, Cap Bon, Lebna, A. Sicilia leg.	36.728	10.931	
28	EU497583 EU497584	EU497483 EU497484	-	-	71B, 72B	-	2	B. boulengeri	Tunisia, El Kef, A. Sicilia leg.	36.166	8.700	
29	DQ629721	-	EU497616, EU497617 (Bufo 166)	EU497613 (Bufo 166)	166	MVZ 235680	1	B. boulengeri	Tunisia, Nefta oasis, Tawzar (=Tozeur) Governorate, T. Papenfuss leg.	33.917	8.133	45
30	EU497585 EU497586 EU497587	EU497485 EU497486 EU497487	-	-	73B, 74B, 75B	-	3	B. boulengeri	Tunisia, Kerkennah islands, Chergui, Remla, A. Sicilia leg.	34.704	11.200	
31	EU497588 EU497589	EU497488 EU497489	-	-	76B, 77B	-	2	B. boulengeri	Tunisia, Kerkennah islands, Chergui, El Kraten, A. Sicilia leg.	34.820	11.258	
32	DQ629719	-	-	-	165	ZFMK 14704	1	B. boulengeri	Tunesia, Djerba island, Kiehlmann leg. 1974	33.800	10.900	
33	EU497590 EU497591 EU497592	EU497490 EU497491 EU497492	-	-	78B, 79B, 80B	-	3	B. boulengeri	Tunisia, Tataouine, A. Sicilia leg.	32.903	10.416	
34	DQ629717	-	-	-	140	-	1	B. boulengeri	Libya, Al' Fjayj, Sabah Province, D. Frynta leg.	26.533	13.317	
35	DQ629705 DQ629706 DQ629707	-	-	-	139, 107, 114	-	2	B. boulengeri	Libya, Gabroon Lake, D. Frynta leg.	26.800	13.533	
36	DQ629710 DQ629711 DQ629712	-	EU497614, EU497615 (Bufo 109)	EU497607 (Bufo 109)	109, 131, 138	MTD 45036, 45281	3	B. boulengeri	Libya, Shahhat (Ancient Cyrene), Binghazi Province, D. Frynta leg.	32.817	21.867	
37	DQ629714 DQ629715	-	-	-	108, 110	MTD 45280, 45282	2	B. boulengeri	Egypt, Matrouh, via E. J. Bentley	30.000	28.000	
38	DQ629708 DQ629709	-	-	-	146, 147	ZFMK 77600, 77601	2	B. boulengeri	Egypt, Oasis Dakhla (Dakhilah, Al Wahat ad), N. Lutzmann leg.	25.553	28.948	
39	DQ629713	-	-	-	105	MTD 45277	1	B. boulengeri	Egypt, 70 km S Alexandria, via J. Bentley	31.000	30.000	
40	DQ629716	-	-	-	159	ZFMK 50909	1	B. boulengeri	Egypt, Alexandria, El Menoufia (via U. Sinsch), 1989	30.500	31.000	
41	DQ629687	-	-	-	21	-	1	B. viridis	Italy, Padua, University of Würzburg 1995 leg.	45.417	11.883	
42	EU497514 EU497516 EU497517	EU497415, EU497416,EU497417	-	-	3B, 4B, 5B	-	3	B. viridis	Italy, Trieste, San Dorligo della Valle, A. Sicilia leg.	45.621	13.869	
43	EU497515	EU497414	-	-	2B	-	1	old isolate	Croatia, Cres island, Belej, Museum of Natural History of Trieste coll.	44.766	14.429	
44	EU497513	EU497413	-	-	1B	-	1	old isolate	Croatia, Krk island, Stara Bas_a, Museum of Natural History of Trieste coll.	44.957	14.688	
45	DQ629686	-	EU497618, EU497619 (Bufo 168)	EU497603 (Bufo 168)	168	MVZ 164718	1	B. viridis	Austria, MVZ frozen tissue collection (FC 13312), 3.2 km E Podersdorf Burgenland, Austria; R. D. Sage leg.	47.850	16.833	
46	DQ629678	-	-	-	265	HNHM 2004.94.2	1	B. viridis	Hungary, Central Hungary, Orgovany, May 2004, L. Forro leg.	46.750	19.467	
47	DQ629722	-	-	-	149	ZFMK 62479	1	B. variabilis	Greece, Epirus, S Igoumenitsa, Patraia, W. Böhme leg. 1996	39.500	20.266	
48	DQ629630	-	-	-	99	-	1	B. variabilis	Greece, Peloponnes, J. Plötner leg.	37.516	22.367	
49	DQ629675	-	-	-	187	NME 901/01	1	B. viridis	Greece, Peloponnes, Kióna, E-Bank Stymphalian Lake, leg. A. Nöllert, 10 April 1996	37.850	22.450	
50	DQ629654	-	-	-	186	NME 900/01	1	B. viridis	Greece, Alepochori, motorway to Vactioni, leg. A. Nöllert, 2 April 1996	38.133	23.000	
51	DQ629655 DQ629656	-	-	-	133, 134	NME A 1037/03 (2nd + 3rd individual)	2	B. viridis	Greece, Crete, Omalos, U. Scheidt leg.	35.333	23.900	
52	DQ629657 DQ629658	-	-	-	135, 136	-	2	B. viridis	Greece, Crete, Aradena village, 19 April 2003, leg. U.Scheidt	35.200	24.083	
53	DQ629621 DQ629624	-	-	-	236, 237	MVZ 230206, 230207	2	B. variabilis	Turkey, Cicekli Köyü, 7 km E (by road) Ula Mugla Prov., T. Papenfuss leg.	37.066	28.500	
54	DQ629623	-	EU497624, EU497625 (Bufo 238)	EU497606 (Bufo 238)	238	MVZ 230208	1	B. variabilis	Turkey, Osman Gazi, Bursa, Bursa Prov., T. Papenfuss leg.	40.167	29.083	
55	EU497599 EU497600	-	-	-	87B, 88B	-	2	B. balearicus	Italy, Macerata, Morrovalle, M. Marconi leg.	43.280	13.586	
56	EU497601 EU497602	-	-	-	89B, 90B	-	2	B. balearicus	Italy, Macerata, Porto Recanati, N. Polini leg.	43.427	13.658	
						TOTAL:	148					
	-	-	-	EU497610	169	MVZ 186039	1	B. calamita	Spain, Cadiz Prov., Andalusia, 3.1 km S Benalup de Sidonia on road to Vejer de La Frontera, J.A. Visnaw leg.	36.333	5.817	
	-	-	-	EU497611	177905	MVZ 177905	1	Bufo bufo	Morocco, Marrakesh Prov., Oukaimeden, Stephen D. Busack, J. A. Visnaw	31.206	-7.864	2650
	-	-	EU497633	-	116	MTD 45287	1	B. raddei	China, Xinjiang, Kuku-Nor, J. Martens leg.	37.000	100.333	
	DQ629618	-	-	-	59	MTD 43944	1	B. surdus	Iran, Baluchestan, Deh Barez, D. Frynta leg.	27.450	57.317	350

Additional file 2

Nomenclature

(a) *Proposed vernacular names for* Bufo siculus *n.sp.*

English: Sicilian green toad, Italian: Rospo smeraldino siciliano, German: Sizilianische Wechselkröte, French: crapaud vert de Sicile, Spanish: Sapo verde siciliano.

(b) *Etymology*

The Sicels (or Siculi) was one of the three tribes that originally inhabited Sicily and which gave Sicily the name it has held since antiquity. The Greek word *sikelos* ['native of Sicily'] became *sicolos* then *siculus* in Latin. We use *siculus* as a Latin adjective "siculus, -a, -um" = Sicilian, i.e. from Sicily.

(c) *On synonymy and appropriateness of the new name* Bufo siculus

Camerano's [96] taxonomic philosophy included the categories of species and subspecies (as exemplified by the name: "*Rana esculenta* Linn. subsp. *lessonae* var. nigro vittata", p. 190) and the hierarchical order and definitions of "variation", "variety", "subspecies" and "species" (op. cit., p.191). Camerano (p. 233 [96]) expressly gave his green toad variety names infrasubspecific rank (Art. 45.6.4. [97]), when he wrote: "In terms of the coloration, I believe one can establish the following main varieties" [emphasis added] of green toads in Italy, of which he observed some simultaneously in several regions of Italy (e.g., in Piedmont: var. *crucigera* and var. *F. lessona*). Therefore, these unequivocally intrasubspecific entities maintain this rank (and cannot be deemed subspecific, although published before 1961, Article 45.6.4. [97]). Camerano [96] not only quoted but also coined himself two such infrasubspecific entities, *concolor* for green toads from Piedmont, and *maculata*. On the latter he comments (transl. from Italian): "This variety is very frequent in all Italian localities; sometimes some of the spots on the back join and look like they give rise to longitudinal stripes (this fits some individuals from Modica - Sicily)". The stated occurrence all over Italy along with other such varieties reinforces its infrasubspecific status. In any case, according to ICZN (Art. 52 [97]) the name *maculata* is also invalid for this taxon since

it was a primary junior homonym of *Bufo maculatus* [98] at the time of Camerano's description [96] of *Bufo viridis* Var. *maculata*. Furthermore, to our knowledge, the type series is either lost or its location remains unclear (F. Andreone, curator of the Turin Museum, pers. comm.; cf. [99]). Therefore, we did not only coin the new name *Bufo siculus* but we also based it on a new type specimen (we could not acknowledge Camerano's work by designation of a Sicilian specimen from the syntype series of *Bufo viridis* Var. *maculata* as the holotype of *B. siculus*; as otherwise possible according to Paragraph 72.7 [97]).

(d) *On the applicability of the name* Bufo balearicus *Boettger, 1880*

The name coined by Boettger [45] for green toads from the Balearic Island of Mallorca seems currently the oldest available name clearly identifiable with this taxon, since sequences from Mallorca and Menorca appear nested in the clade that also contains the sequences from Sardinia, Corsica and most of the Apennine Peninsula. An old name (*B. viridis* var. *lineatus* [100]), coined for green toads from the Venice region (i.e., between our loc. 41 and 42), is a junior subjective synonym of *B. viridis* [101], as this phylogeographic group is present in that area. Although detailed descriptions of green toads from the range of what we consider *B. balearicus* date at least as far back as Cetti's [102] work, we did not find older scientific names for it, since neither Cetti [102], nor Gené [103] or de Betta [104] coined available scientific names for green toads.

Additional file 3

Biogeographic comments on green toads on Circum-Sicilian islands

Green toads have been detected on the circum-Sicilian islands of Lipari, Salina, Ustica, Favignana, and Marettimo, and are known to have been introduced on Vulcano and Isola Grande dello Stagnone, but are probably not naturalized [41, 105, 106, 107]. A new report of a possible introduction on Pantellaria Island, of unknown origin, has been made [108]. No tissue sample for DNA analyses was collected, so the origin cannot be determined at this time. Gasc *et al.* [109] mapped green toads on Malta "after 1970". However, Savona Ventura (in litt.) reported that exclusively Pleistocene fossils have been found on Malta [110], perhaps from a colonization event out of Sicily during low sea levels, since the submarine plateau between Malta and Sicily is shallower than –95 m.

Additional file 4

Morphological description of the holotype

P215, an adult female; for morphometric data see Table 5 (below), a large sized green toad; head distinctly shorter than wide, rather flat; snout slightly conical from lateral and dorsal view, not protruding; edges of interorbital space slightly wedge-shaped with the wider part orally, not much smaller than upper eyelid and little wider than internarial distance; nostril closer to tip of snout than to eye; tympanum large, distinct, vertical axis about more than half of eye diameter, anterior margin of tympanum closer to tip of snout than the posterior corner of eye, parotids almost adjoining the eyelid and adjacent to the upper margin of the tympanum; parotids wider than half their length, posterior part of the parotids slightly narrower and rounded, lower lateral edges of parotids reach the level of the upper third of the tympanum, small glands on the whole parotid surface; fingers not webbed, quite thick, their relative length (longest to shortest): 1, 3, 2, 4; tips of fingers rounded, not enlarged, subarticular tubercles prominent, single; two palmar tubercles, the inner about three times larger than the outer, numerous small tubercles covering the palms; hind limbs robust and relatively long, leg length more than 1.5 times tibia length, relative length of toes (longest to shortest): 4, 3, 2, 5, 1; tips of toes rounded, not enlarged, toes with dermal fringes, webbing only between the most proximal parts of toes, numerous rounded tubercles along the soles, subarticular tubercles single, inner metatarsal tubercle prominent, longish, about three times longer than wide; outer metatarsal tubercle only half as long as outer, longish; tarsal fold weak; dorsal and lateral skin with flat glandular warts of various size, lateral warts larger, snout and region between eyes smoother, dorsal surface of forelimbs and hindlimbs as well as ventral parts of forelimbs smooth; throat and belly without warts, but slightly warty; ventral parts of thighs with granular skin texture; coloration changed in preservation, in life darkly brownish olive spots of more than twice the eye diameter but with irregular shape (indentations) covered about three quarters of the dorsal skin, interspaces light (yellowish to greenish); ventral parts uniformly yellowish-whitish with numerous small grayish spots, especially between the hind limbs and towards the lateral margins.

Table 5: Morphometric data for the holotype and two adult paratypes.

	Sex	SVL	HL	PL	PW	HDT	VDT	ED	HW	IND	NED	TL	LL	LFT	LMT	IOW
P215	f	85.5	17.5	20.0	11.1	4.2	5.5	9.4	29.6	5.7	4.6	30.3	51.4	7.1	4.9	6.0
MZPA A95	f	69.7	16.4	15.6	8.7	4.1	5.0	7.5	26.4	5.5	3.5	27.0	45.7	6.6	4.1	5.2
ZFMK 8778	m	66.5	14.6	15.4	7.8	2.7	3.0	7.5	23.7	4.0	5.0	25.0	41.8	5.0	3.8	4.0

Abbreviations as in [111]: SVL: snout-urostyle length, HL: head length, PL: length of parotid gland, PW: width of parotid gland, HDT: horizontal diameter of tympanum, VDT: vertical diameter of tympanum, ED: horizontal diameter of eye, HW: head width, IND: internarial distance, NED: distance between nostril and anterior corner of eye, TL: length of tibia, LL: length of leg, LFT: length of first toe, LMT: length of inner metatarsal tubercle, IOW: interorbital width, f: female, m: male.

Additional file 5

Permits

For this work, the following collection permits were kindly provided from the: Ministero dell'Ambiente e della Tutela del Territorio of Italy (DPN/2D/2005/12107) to M. Arculeo; Arrêté préfectoral (n°05-0336, 24 February 2005), Préfecture de Corse, France, to M. Delaugerre; Autoritzacio especial per a caça cientifica (Num. 11742/2006, 20 June 2006), Govern de les Illes Balears, Spain, to M. Stöck; the Ufficio legislativio e legale della Regione Siciliana Italy, (2005.49.3082, 15 November 2005) and Ente Gestore Rangers d'Italia (722/05, 22 August 2005) to M. Lo Valvo.