Acta Herpetologica 15(2): 125-128, 2020
DOI: 10.13128/a_h-9206

Confirming Lessona's brown frogs distribution sketch: *Rana temporaria* is present on Turin Hills (Piedmont, NW Italy)

Davide Marino[1], Angelica Crottini[2], Franco Andreone[3,*]

[1] *Associazione Natura Invisibile, Via Avigliana, 24, I-10138 Torino, Italy*
[2] *CIBIO, Research Centre in Biodiversity and Genetic Resources, InBIO, Universidade do Porto, Campus Agrário de Vairão, Rua Padre Armando Quintas, No. 7, 4485-661 Vairão, Portugal*
[3] *Museo Regionale di Scienze Naturali, Via G. Giolitti, 36, I-10123 Torino, Italy*
**Corresponding author.* E-mail: franco.andreone@gmail.com

Submitted on: 2020, 1st July; revised on: 2020, 13th July; accepted on: 2020, 30th July
Editor: Stefano Scali

Abstract. The presence of *Rana temporaria* on Turin Hills (Piedmont NW Italy) has been confirmed through morphological and molecular analyses. Breeding individuals of this species were found at two sites and assessed by either morpho-chromatic and genetics. This new finding represents an interesting confirmation of ancient record reported in 1877 by the renowned naturalist Michele Lessona, and highlights that the species is likely quite cryptical and secretive and has a distribution wider than formerly presumed.

Keywords. Common frog, *Rana temporaria*, new findings, Turin Hills, NW Italy.

The common frog *Rana temporaria* shows a conspicuous wide geographical and elevational distribution, being present in most of Europe and in the northern and central regions of western Asia, from northern Spain, France, United Kingdom and the Scandinavian Peninsula and Russia (Urals and adjacent western Siberia) to northern Kazakhstan (Lanza, 1983; Kuzmin, 1999; Lanza et al., 2009; Sillero et al., 2014), from sea level to around 2846 m a.s.l. (Vences et al., 2003, 2013; Maurino and Doglio, 2010; Tiberti and von Hardenberg, 2012; Di Nicola et al., 2019). In southern Europe this species is usually associated to montane habitats, being absent from southern and central Iberia, most of southern Italy and Caucasus, and shows a patchy distribution in the Balkans and in the Mediterranean islands. In Italy it is found on the Alpine and northern Apennine reliefs, with a fragmented and irregular distribution in Arezzo, Florence and Forlí-Cesena provinces, and a relict population on the Monti della Laga (Rieti Province, NE Latium) (Capula and Bagnoli, 1983; Razzetti et al., 2007).

In Piedmont (NW Italy) *R. temporaria* is quite common on the Alps, on northern Apennines and on southern hilly reliefs, known as "Langhe", with scattered, findings at low altitudes (Andreone et al, 1988; Andreone and Sindaco, 1989, 1999). In a pioneer contribution on anuran distribution, the renowned naturalist, writer, lecturer, minister and Darwin translator Michele Lessona published a colour map (realised by his son-in-law L. Camerano) with the inferred distribution of *R. temporaria* in Piedmont and Aosta Valley known at that time. In this map the species was also reported on the hill system bordering the town of Turin, known as "Collina di Torino" (Lessona, 1877). No precise localities or toponyms were provided together with this map, although Lessona provided considerations on abundances, human uses and life history traits on this species in Piedmont. Although Lessona was Turin Zoological Museum's director (Camerano,

ISSN 1827-9635 (print)
ISSN 1827-9643 (online)

Fig. 1. *Rana temporaria*, male from Turin Hills, Piedmont (NW Italy) (A: dorsolateral view; B: ventral view) (photographs by F. Andreone).

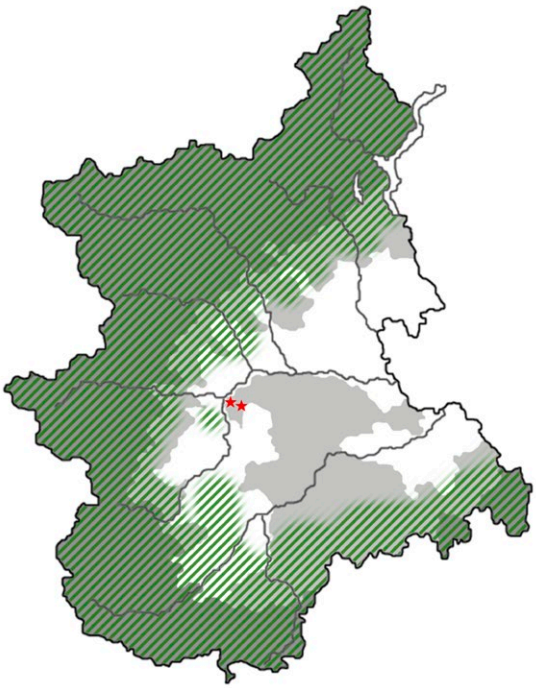

Fig. 2. The distribution of *Rana temporaria* in Piedmont and Aosta Valley (NW Italy) based upon current data. Dark and light gray show Alps, Prealps and inner hills. The transverse (green) bars represent the known distribution. The (red) stars mark the confirmed sites recently found on the hill system near Turin.

1894), no preserved specimen of this species from Turin Hills is currently present in the historical herpetological collection of Turin University (now hosted by the Museo Regionale di Scienze Naturali: Gavetti and Andreone, 1993), and we did not find any record of *R. temporaria* from Turin Hills on the historical catalogues or quoted by previous authors, such as Camerano (1884) and Tortonese (1953). Until now *R. temporaria* was not reported for Turin Hills, neither during the realization of the herpetological distribution atlas of Piedmont and Aosta Valley (Andreone and Sindaco, 1999), nor in the Italian atlas (Sindaco et al., 2006), and this record was considered anecdotal. This assumption was contradicted by recent observations, reported in this paper. During a survey carried out on the 8th March 2017 to confirm the presence of the Alpine newt (*Ichthyosaura alpestris apuana*) on Turin Hills (Marino, 2018), one of us (DM) reported a single brown frog at Valsalice (altitude: 315 m a.s.l.; coordinates: 45°03'04.5"N, 7°42'46.6"E). This individual corresponded in morphology and colouration to a typical *R. temporaria*. This record was promptly reported on iNaturalist, and further confirmed by the herpetological online community. This individual (a male) was quite large (around 60 mm snout-vent length), had a relatively short snout and hindlimbs, that, adpressed along body barely reached the eye (Fig. 1). The colouration of this individual was brownish, with sparse dark spots on the back, intense purple shading under the throat and missed the yellowish belly and groin shadings typical of the agile frog *R. dalmatina*¸ the other brown frog confirmed in his area. A few days later another female and some males were found around an artificial pond, where typical eggs-clumps were also found. On the 29th March this species was also found at at Reaglie (altitude: 380 m a.s.l.; coordinates: 45°02'44.9"N, 7°44'55.1"E), about 10 Km away from Valsalice (Fig. 2).

To further confirm the species identity we analysed the tissue samples of two adults, one tadpole and one egg. We also took the tissue samples of two adults *R. dalmatina* of Reaglie for comparison. Total genomic DNA was extracted from these samples using proteinase K digestion (10 mg/ml concentration) followed by a standard salt extraction protocol (Bruford et al., 1992). We sequenced a fragment of ca. 550 bp of the 3' terminus of

the mitochondrial rrnL gene. Polymerase chain reactions (PCR) were performed in a final volume of 25 µl using 0.75 µl each of 10 pmol primer, 0.4 µl of total dNTP 10 mM (Promega), 0.1 µl of 5 U/ml GoTaq (Promega), 5 µl 5X Green GoTaq Reaction Buffer (Promega) and 4 µl of MgCl2 25mM (Promega). For primers and cycling protocols, see Crottini et al. (2011). Successfully amplified PCR products were treated to inactivate remaining primers and dNTPs. Purified PCR templates were sequenced using dyelabelled dideoxy terminator cycle sequencing on an ABI3730xl at Macrogen Inc. Sequences were checked by eye, edited (when necessary), aligned using the BioEdit sequence alignment editor (version 7.0.5.3; Hall 1999) and compared to the GenBank dataset. All newly determined sequences have been deposited in GenBank (MT459788-MT459793).

All these observations support the presence of *R. temporaria* on the hill system around Turin and confirm old Lessona's ancient maps, providing a significant novelty in terms of species distribution in Piedmont. It remains somehow surprising that no records of *R. temporaria* were reported since Lessona's contribution (including the lack of museum specimens and photographs): we believe this absence might be due to the species overall similarity with the agile frog *R. dalmatina*, and to a deficit of field research during suitable periods and in suitable sites. In fact, *R. temporaria* apparently prefers stream systems (in particular in late winter), which were not so frequently surveyed on the Turin Hills.

Finally, we cannot exclude that in the 19th Century the species was more widespred and common than today, and since then this species has reduced its distribution, as it is already known for *Pelobates fuscus* and *Zootoca carniolica,* two species which survived with patchy populations at a few sites along the Po River (Andreone and Sindaco, 1999). The presence of *R. temporaria* in the hill system of Turin may represent a vestige of an ancient and more widespread distribution range for the species, as already supposed for the Alpine newt (Andreone and Sindaco, 1987; Marino 2018). Further research efforts should be devoted to better characterize the distribution of this species around the hill system of the city of Turin, and between the city and "Langhe", to valorise all remnant populations of this species.

ACKNOWLEDGEMENTS

We thanks the authorities and friends who accompanied us in the field: G. Tonelli, M. Dadda and the park guards of "Parco del Po e Collina Torinese". The Portuguese National Funds through FCT – Foundation for Science and Technology – supported the Investigador FCT (IF) grant to AC (IF/00209/2014). This work is also funded by National Funds through FCT within the IF/00209/2014/CP1256/CT0011 Exploratory Research Project. L. Cavigioli greatly helped with the realization of the distribution map. Permits to carry out research were issued by Regione Piemonte (det. n. 234 of 21/6/2018). Finally, we thank the two reviewers who provided useful comments to a previous version version of this contribution.

REFERENCES

Andreone, F., Delmastro, G.B., Boano, G. (1988): Distribuzione delle rane rosse nel Piemonte occidentale (Amphibia, Anura, Ranidae). Pianura **2**: 7-20.

Andreone, F., Sindaco, R. (1987): Sulla presenza e la neotenia di *Triturus alpestris apuanus* (Bonaparte 1839) nella Collina di Torino (Amphibia, Urodela, Salamandridae). Boll. Mus. Reg. Sci. Nat. Torino **5**: 103-112.

Andreone, F., Sindaco, R. (1989): Materiali per un'erpetologia del Piemonte e della Valle d'Aosta (Amphibia, Reptilia). Riv. Piem. St. Nat. **10**: 205-225.

Andreone, F., Sindaco, R. (1999): Erpetologia del Piemonte e della Valle d'Aosta. Atlante degli Anfibi e dei Rettili. Monografie **26** (1998). Museo Regionale di Scienze Naturali, Torino.

Bruford, M.W., Hanotte, O., Brookefield, J.F.Y., Burke, T. (1992): Single-locus and multilocus DNA fingerprint. In: Molecular genetic analysis of populations: a practical approach, pp. 225-270. Hoelzel, A.R., Ed., IRL Press, Oxford.

Camerano, L. (1884): Monografia degli Anfibi Anuri italiani. Mem. R. Acc. Sci. Torino, Sci. Fis. Mat. **35** (2): 187-284.

Camerano, L. (1894): Michele Lessona, notizie biografiche e bibliografiche. Boll. Musei Zool. Anat. Comp. R. Univ. Torino **188** (9): 1-72.

Capula, M., Bagnoli, C. (1983): Il *Triturus alpestris* (Laurenti) e la *Rana temporaria* Linnaeus nell' Appennino Centrale (Amphibia). Boll. Mus. Civ. St. Nat. Verona **9**: 333–344.

Crottini, A., Glaw, F., Casiraghi, M., Jenkins, R.K.B., Mercurio, V., Randrianantoandro, C., Randrianirina, J.E., Andreone, F. (2011) A new *Gephyromantis* (*Phylacomantis*) frog species from the pinnacle karst of Bemaraha, western Madagascar. Zookeys **81**: 51-71.

Di Nicola, M.R., Cavigioli, L., Luiselli, L., Andreone, F. (2019): Anfibi e Rettili d'Italia. Edizioni Belvedere, Latina.

Gavetti, E., Andreone, F. (1993): Revised catalogue of the herpetological collection in Turin University. I. Amphibia. Mus. Reg. Sci. Nat. Torino, Cataloghi X, Museo Regionale di Scienze Naturali, Torino.

Hall, T.A. (1999): BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series **41**: 95-98.

Kuzmin, S. (1999): The amphibians of the former Soviet Union. Sofia, Pensoft.

Lanza, B. (1983): Guide per il riconoscimento delle specie animali delle acque interne italiane. 27. Anfibi, Rettili (Amphibia, Reptilia). Collana del progetto finalizzato "Promozione della Qualità dell'Ambiente AQ/1/205. Consiglio Nazionale delle Ricerche, Roma.

Lanza, B., Nistri, A., Vanni, S. (2009): Anfibi d'Italia. Quaderni di Conservazione della Natura, numero 29. Ministero dell'Ambiente e della Tutela del Territorio e del Mare, I.S.P.R.A., Grandi & Grandi Editori, Rome.

Lessona, M. (1877): Studi sugli Anfibi Anuri del Piemonte. Atti Accad. naz. Lincei. Memorie (Classe Sci. fis., matem. e nat.) **274** (1876-1877): 1019-1098.

Marino, D. (2018). Il tritone apuano (*Ichthyosaura alpestris apuana* Bonaparte, 1839) sulla Collina di Torino: aggiornamento della distribuzione a 30 anni dalla sua riconferma. Riv. Piem. St. Nat. **39**: 347-358.

Maurino, L., Doglio, S. (2010): Vivere al limite nelle Alpi Piemontesi: la rana temporaria nel Parco Naturale Val Troncea. In: Atti VIII Congresso Nazionale Societas Herpetologica Italica (Chieti, 22-26 settembre 2010), pp. 331-334. Di Tizio, L., Di Cerbo, A. R., Di Francesco, N. & Cameli, A., Eds., Ianeri Edizioni, Pescara.

Razzetti, E., Zanghellini, S., Bernini, F. (2007): *Rana temporaria* Linnaeus, 1758. In: Amphibia. Fauna d'Italia, pp. 417-424. Lanza B., Andreone, F., Bologna, M.A., Corti, C., Razzetti, E., Eds, Calderini, Bologna.

Sillero, N., Campos, J., Bonardi, A., Corti, C., Creemers, R., Crochet, P.-A., Crnobrnja-Isailovic, J., Denoel, M., Ficetola, G.F., Gonçalves, J., Kuzmin, S., Lymberakis, P., de Pous, P., Rodríguez, A., Sindaco, R., Speybroeck, J., Toxopeus, B., Vieites, D., Vences, M. (2014): Updated distribution and biogeography of amphibians and reptiles of Europe. Amphibia-Reptilia **35**: 1-31.

Tiberti, R., von Hardenberg, A. (2012): Impact of introduced fish on common frog (*Rana temporaria*) close to its altitudinal limit in alpine lakes. Amphibia-Reptilia **33**: 303-307.

Tortonese, E. (1953): Spigolature di erpetologia pedemontana. Natura, Milano **44**: 24-34.

Vences, M., Grossenbacher, K., Puente, M., Palanca, A., Vieites, D.R. (2003): The Cambalès fairy tale: elevational limits of *Rana temporaria* (Amphibia: Ranidae) and other European amphibians revisited. Folia Zoologica **52**: 189-202.

Vences, M., Hauswaldt, J.S., Steinfartz, S., Rupp, O., Goesmann, A., Künzel, S., Orozco-terWengel, P., Vieites, D.R., Nieto-Roman, S., Haas, S., Laugsch, C., Gehara, M., Bruchmann, S., Pabijan, M., Ludewig, A-.K., Rudert, D., Angelini, C., Borkin, L.J., Crochet, P-.A., Crottini, A., Dubois, A., Ficetola, G.F., Galán, P., Geniez, P., Hachtel, M., Jovanovic, O., Litvinchuk, S.N., Lymberakis, P., Ohler, A., Smirnov, N.A. (2013): Radically different phylogeographies and patterns of genetic variation in two European brown frogs, genus *Rana*. Mol. Phylogenetics Evol. **68**: 657-670.