

## Comparison of Skeletal Muscle Protein Bands among Five Populations of *Bufo viridis* in Turkey by SDS-PAGE

Ufuk BÜLBÜL\*, Bilal KUTRUP

Department of Biology, Faculty of Arts and Sciences, Karadeniz Technical University, 61080 Trabzon - TURKEY

Received: 16.01.2007

**Abstract:** Skeletal muscle protein bands of *Bufo viridis* samples from 5 different populations in Turkey were investigated by SDS-PAGE (Sodium Dodecyl Sulphate-Poly Acrylamide Gel Electrophoresis). The total number of skeletal muscle protein bands was 23 in Hatay, Kayseri, Rize, and Tekirdağ samples, and 25 in the Mersin sample. It can be ascertained from the present study that some populations of *B. viridis* in Turkey could be different according to skeletal muscle protein bands and SDS-PAGE results comparing different muscle protein bands could be helpful for taxonomical investigations.

**Key Words:** Skeletal muscle, SDS-PAGE, *Bufo viridis*, protein band

### ***Bufo viridis*'in Türkiye'deki 5 Populasyonunda İskelet Kası Proteinlerinin SDS-PAGE Yardımıyla Karşılaştırılması**

**Özet:** Bu çalışmada, *Bufo viridis*'in Türkiye'deki 5 farklı populasyonunda iskelet kası protein bantları SDS-PAGE vasıtasıyla incelenmiştir. Toplam iskelet kası protein bandı sayısı, Hatay, Kayseri, Rize ve Tekirdağ örneklerinde 23 olarak bulunurken, Mersin örneğinde 25 protein bandı bulunmaktadır. Bu çalışma ile, Türkiye'deki bazı *B. viridis* populasyonlarının iskelet kası protein bantlarının farklı olabileceği ve farklı iskelet kası proteinlerini karşılaştıran SDS-PAGE sonuçlarının taksonomik incelemelerde yardımcı olabileceği anlaşılmaktadır.

**Anahtar Sözcükler:** İskelet kası, SDS-PAGE, *Bufo viridis*, protein bandı

### **Introduction**

Although the taxonomic status of the green toad, *Bufo viridis*, was recently reported as *Bufo variabilis* (Stöck et al., 2006), its status is still not clear in Turkey. Commonly it is suggested that a single subspecies, *Bufo viridis viridis*, is distributed in lowland areas (Terentjev and Chernov, 1949; Mertens and Wermuth, 1960; Eiselt, 1965). In contrast, Flindt and Hemmer (1968) reported specimens from Adana as *B. viridis arabicus*. On the other hand, Fuhn (1960), Eiselt and Schmidtler (1973), and Esterbauer (1992) stated that *B. v. arabicus* is distributed in Iran, Iraq, Israel, and Syria. In addition, Kete (1991) reported *Bufo arabicus* specimens in southern Anatolia whereas Balletto et al. (1985) stated that *B. arabicus* is distributed in Saudi Arabia and it is sympatric with *B. viridis* in some localities of the Sinai Peninsula. In a study regarding the taxonomical status of

the green toad in Turkey, Tosunoğlu (1996) reported that Adana samples were different from İzmir samples in their albumin and some globulin (G3 and G5) fractions. Tosunoğlu (1999) also stated that the green toad specimens from southern Anatolia (Mersin, Adana, Hatay, and Şanlıurfa) were found to be relatively different from those of the other regions of Turkey in color, pattern, and serum globulin group. Furthermore, Tok (1999) stated that specimens from the Datça Peninsula showed similarity to those from Adana–Hatay because they both have a thicker first finger. The specimens from İzmir, Thrace, and the Black Sea were found to be different from Datça samples and Adana–Hatay samples of this study. In contrast, Kutrup et al. (2006) did not observe any trend suggesting an increase in the tibia length from north to south. The Mersin population in southern Anatolia showed such an increase; however, the Hatay

\* E-mail: ufukb@ktu.edu.tr

population in southern Anatolia did not show any differences in terms of the tibia length. In a different taxonomical approach, Borkin et al. (2000) studied the nuclear DNA content in some green toads from Turkey and Iran. They reported that specimens from Adapazarı and Ardahan were similar to the *B. v. viridis* specimens from Central Asia, Israel, and Europe. Finally Stöck et al. (2006) referred the *B. viridis* populations to *B. variabilis* in Turkey according to their mitochondrial DNA data.

To extend the taxonomical data for *B. viridis* in Turkey by a different perspective, the purposes of the present study were i) to compare skeletal muscle proteins of the green toad, *B. viridis*, by SDS-PAGE, in 5 different populations from Turkey for the first time; ii) to investigate whether the southern Anatolian specimens are different enough from other specimens to be regarded as distinct subspecies or species levels.

### Materials and Methods

Totally 49 adult green toad specimens (21 females and 28 males) were captured from 5 populations (3 ♀♀ and 6 ♂♂ from Hatay (Harbiye), 5 ♀♀ and 6 ♂♂ from Kayseri (Bünyan), 4 ♀♀ and 5 ♂♂ from Mersin (Mezitli), 5 ♀♀ and 7 ♂♂ from Rize (Gündoğdu), and 4 ♀♀ and 4 ♂♂ from Tekirdağ (Çorlu), in Turkey (Figure 1). In each SDS-PAGE experiment, one specimen was used for each population and the experiments were repeated for all specimens in each population. Skeletal muscle protein samples from 5 populations of *Bufo viridis* obtained by grinding 0.1 g skeletal muscles of each specimens in liquid

nitrogen and by adding 0.1 ml of double distilled water (DDW) and 0.2 ml of 2 x SDS gel-loading buffer (100 mM Tris-Base pH 6.8, 4% SDS electrophoresis grade, 0.2% bromophenol blue, and 20% glycerol) (Sambrook et al., 1989). The samples were boiled for 2 min in 2 x SDS gel-loading buffer to denature the proteins prior to loading the samples onto gels (Lutz et al., 2001). SDS-PAGE 99 program was used for boiling in a thermal block. The size of the minigels was 8.3 x 7.3 cm and the resolving gels were 12% (w/v). The 12% gels were prepared in a beaker by adding 3.3 ml of DDW, 4 ml of 30% acrylamide mix (29.2% acrylamide and 0.8% N,N'-methylene-bis-acrylamide), 2.5 ml of 1.5 M Tris pH 8.8, 0.1 ml of 10% SDS, 0.1 ml of 10% ammonium persulfate, and 0.004 ml of TEMED. The 5% stacking gels were prepared in a beaker by adding 2.7 ml of DDW, 0.67 ml of 30% acrylamide mix, 0.5 ml of 1.5 M Tris pH 6.8, 0.04 ml of 10% SDS, 0.04 ml of ammonium persulfate, and 0.004 ml of TEMED (Sambrook et al., 1989). For the SDS-PAGE experiments, 1.85 µg of protein of each 1 µl sample was applied to the wells. The gels were run at a constant current of 20 mA for 60 min (Lutz et al., 2001). Coomassie Brilliant Blue (CBB) R-250 was used to stain the gels. The gels were put into washing solution 1, prepared by mixing 50 ml of methanol, 10 ml of glacial acetic acid, and 40 ml of DDW for 60 min. Then the gels were put in washing solution 2, prepared by mixing 7 ml of glacial acetic acid, 5 ml of methanol, and 88 ml of DDW for 60 min (İnan, 2005). Finally the gels were scanned in a scanner and they are shown in Figure 2.

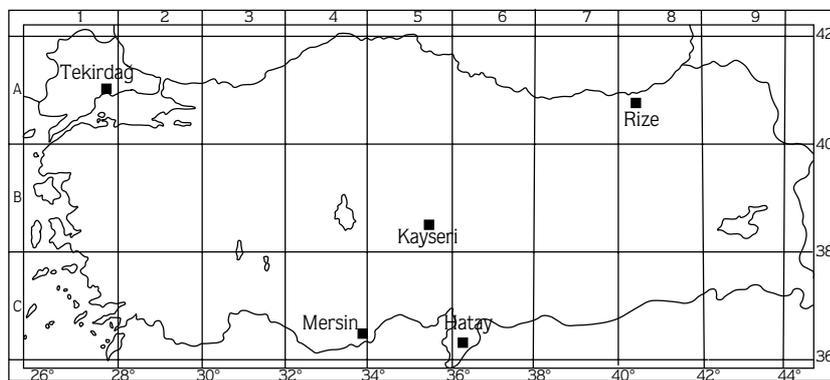


Figure 1. Localities of the studied samples.

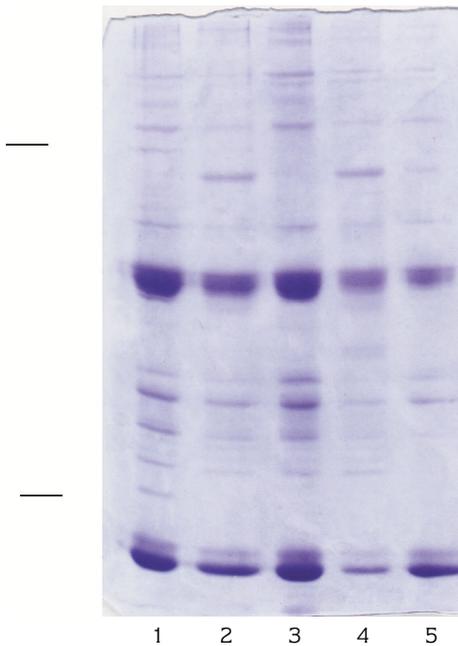


Figure 2. SDS-PAGE results of the 5 different *Bufo viridis* specimens. 100  $\mu$ l (185  $\mu$ g) of protein samples was applied to the wells. Numbers from 1 to 5 represent the names of the populations as following: 1. Mersin, 2. Hatay, 3. Rize, 4. Tekirdağ, 5. Kayseri.

## Results

Figure 2 shows SDS-PAGE patterns of the skeletal muscle proteins from *B. viridis*. Total number of skeletal muscle protein bands was 23 in Hatay, Kayseri, Rize, and Tekirdağ samples and there were 25 bands in the Mersin sample. The additional 2 protein bands are shown in Figure 2. All specimens were used in the SDS-PAGE experiments and we have not found any differences between males and females.

## Discussion and Conclusion

In the present study, we compared skeletal muscle protein bands of the *Bufo viridis* samples from 5 different populations in Turkey by SDS-PAGE.

SDS-PAGE results showed that the total number of skeletal muscle protein bands was 23 in Hatay, Kayseri, Rize, and Tekirdağ samples and 25 in the Mersin sample. Our SDS-PAGE characterization on the species or subspecies level for the green toad *B. viridis* was consistent with the study by Hasnain et al. (2005) for soluble muscle proteins in 4 fish species. The authors

found by SDS-PAGE that 16 protein bands were diagnostic for *Channa gachua* and *Channa striatus*, 10 bands for *C. marulus* and 15 bands for *C. punctatus*. These data showed that the total number of skeletal muscle protein bands could vary among classes, species, or subspecies.

The results on the total number of protein bands in our study showed that Hatay specimens were similar to Kayseri, Rize, and Tekirdağ specimens. On the other hand, the Mersin specimen had 2 different protein bands compared with other specimens in our study. Similar to the present study, Kutrup et al. (2006) found differences between Hatay and Mersin populations. They did not observe any trend suggesting an increase in the tibia length from north to south. The Mersin population in southern Anatolia showed such an increase, however. The Hatay population in southern Anatolia did not show any differences in terms of tibia length. On the other hand, Tosunoğlu (1999) stated that toads from a desert area, southern Anatolia (Adana, Mersin, Hatay, and Şanlıurfa), showed longer first fingers than those from a humid area (İzmir) and the southern Anatolia populations of the *B. viridis* were found to be relatively different than the other regions of Turkey in color, pattern, and serum globulin group. Similarly, we found that the skeletal muscle result of the Mersin specimens was not consistent with the Kayseri, Rize, and Tekirdağ specimens while the Hatay specimens were found to be similar with those specimens from these 3 populations.

The results of the present study revealed that the taxonomic status of *B. viridis* in Turkey, especially for the comparison of southern Anatolia populations with those in other regions of Turkey should be investigated further in detail. The unique approach of the present study showed that more populations from Adana, Hatay, and Mersin should be compared by SDS-PAGE among each other and also with those in other regions of Turkey. The skeletal muscle difference revealed by our study for Mersin and Hatay specimens needs to be supported with other taxonomical comparison methods, such as mitochondrial DNA sequences, morphological data and blood serum differences, to understand whether the difference between Mersin and Hatay populations is because of ecological variations or molecular diversity.

As can be seen in the present study, skeletal muscle protein bands could vary in the green toad, and the SDS-PAGE results for comparing different muscle proteins

could be helpful for taxonomical investigations. Similarly, Hasnain et al. (2005) used skeletal muscle differentiation in their study on 4 species of the genus *Channa* and they reported that different fish species had different numbers of skeletal muscle protein bands. Using myosin isoform-based criteria, a detailed and systematic study of skeletal fiber types in the limb muscles of 2 frog species, *Rana* and *Xenopus*, was performed (Rowlerson and Spurway, 1988). They found different myosin isoforms in the 2 different frog species. Furthermore, limb muscle differences were studied in *Xenopus laevis* (Lannergren, 1979). These data provided strong evidence that the

different fiber types in *Rana* and *Xenopus* limb muscle contained different myosin heavy chains isoforms.

### Acknowledgments

We thank Professor Dr. Hiroaki Kagawa and Masaaki Ouchi, graduate student, the Graduate School of Natural Science and Technology, Okayama University, Japan, for their laboratory help with the SDS-PAGE methods.

Corresponding author has been awarded an AIEJ Short-term Student Exchange Promotion Program Scholarship from Okayama University, Japan.

### References

- Balletto, E., Cherci, M.A. and Gasperetti, J. 1985. Amphibians of the Arabian Peninsula. Fauna Saud. Arab. 7: 318-392.
- Borkin, L.J., Rosanov, J. and Litvincuk, S.N. 2000. Nuclear DNA content in some green toads (*Bufo viridis* complex) of Turkey and Iran. Russ. J. Herpetol. 7: 171-180.
- Eiselt, J. 1965. Einige amphibien und reptilen aus der nordoslichen Türkei, gesammelt von Herrn H. Steiner. Ann. Naturhist. Mus. Wien. 67: 387-389.
- Eiselt, J. and Schmidler, J.F. 1973. Froschlurche aus dem Iran unter berücksichtigung auseriranischer populationsgruppen. Ann. Naturhist. Mus. Wien. 77: 181-243.
- Esterbauer, H. 1992. Die herpetofauna des östlichen Golan und Hermongebietes funde und Bernerkungen zur systematik und ökologie. Zoology in the Middle East. 7: 21-35.
- Flindt, R. and Hemmer, H. 1968. Über *Bufo viridis* im Vordereen Orient. Senckenbergiana Biol. 49: 99-106.
- Fuhn, I. 1960. Fauna Republicii Populare Romine - Amphibia. Editura Academiei Republicii Populare Romine, pp. 209-215.
- Hasnain, A., Hasan, A.S., Ahmad, R. and Pandey, R.B. 2005. Identification of species – marker bands in native and SDS-PAGE patterns of soluble muscle proteins of four species of Genus *Channa* (Channidae: Channiformes) with evidence of some intraspecies heterogeneity. Asian Fish. Sci. 18: 49-58.
- İnan, K. 2005. Türkiye'nin çeşitli kaplıcalarından *Anoxybacillus* türlerinin izolasyonu, karakterizasyonu ve tanımlanması. MSc thesis, KTU, Trabzon, 89 pp.
- Kete, R. 1991. İzmir ve Adana Bölgesi (Amphibia: Anura) populasyonlarının taksonomik yönden incelenmesi. Turk. J. Zool. 16: 60-70.
- Kutrup, B., Bülbül, U. and Yılmaz, N. 2006. Effects of the ecological conditions on morphological variations of the green toad, *Bufo viridis*, in Turkey. Ecol. Res. 21: 208-214.
- Lannergren, J. 1979. An intermediate type of muscle fibre in *Xenopus laevis*. Nature. 279: 254-256.
- Lutz, G.J., Brenner, S.N., Bade, M.J. and Lieber, R.L. 2001. Identification of myosin light chains in *Rana pipiens* skeletal muscle and their expression patterns along single fibres. J. Exper. Biol. 204: 4237-4248.
- Mertens, R. and Wermuth, H. 1960. Die Amphibien und Reptilien Europas. Verlag Waldemar Kramer., Frankfurt a., Main, pp. 264.
- Sambrook, J., Fritsch, E.F. and Maniatis, T. 1989. Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory, New York.
- Rowlerson, A.M. and Spurway, N.C. 1988. Histochemical and immunohistochemical properties of skeletal muscle fibers from *Rana* and *Xenopus*. Histochem. J. 20: 657-673.
- Stöck, M., Moritz, C., Hickerson, M., Frynta, D., Dujsebeyeva, T., Eremchenko, V., Macey, J.R., Papenfuss, T.J. and Wake, D.B. 2006. Evolution of mitochondrial relationships and biogeography of Palearctic green toads (*Bufo viridis* subgroup) with insights in their genomic plasticity. Mol. Phy. Evol. 41: 663-689.
- Terentjev, P.V. and Chernov, S.A. 1949. Key to amphibians and reptiles. Israel Program for Scientific Translation, Jerusalem, pp. 315.
- Tok, C.V. 1999. A morphological investigation on the Anuran species of the Reşadiye (Datça) Peninsula (Anura: Bufonidae, Hylidae, Ranidae). Turk. J. Zool. 23: 565-581.
- Tosunoğlu, M. 1996. Batı ve Güney Anadolu *Bufo viridis* (Anura: Bufonidae) populasyonlarında morfolojik ve serolojik araştırmalar. Turk. J. Zool. 20: 103-111.
- Tosunoğlu, M. 1999. Türkiye *Bufo viridis* (Anura: Bufonidae) populasyonları üzerine morfolojik, osteolojik ve serolojik araştırmalar. Turk. J. Zool. 3: 849-871.