Comparison of skeletal muscle protein bands and trunk vertebrae count between *Ommatotriton ophryticus nesterovi* and *O. o. ophryticus* populations in Turkey

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Abstract: Skeletal muscle was investigated by SDS-PAGE protein electrophoresis for western versus eastern Turkish populations of the newt *Ommatotriton ophryticus*. The number of protein bands resolved was 25 in the west and 26 in the east. The modal number of trunk vertebrae was also compared among 10 populations of the eastern group (Artvin, Trabzon, Giresun, and Ordu) and 3 populations of the western group (Zonguldak, Bartın, and Karabük). The eastern group had 13 vertebrae except Şinik (Trabzon) specimens, which had 14 vertebrae, whereas the western group had 12 vertebrae. These data supported the recognition of 2 taxa that would correspond to *O. o. nesterovi* in the west *O. o. ophryticus* in the east.

Key words: Skeletal muscle, SDS-PAGE, *Ommatotriton ophryticus*, protein band

Introduction

The banded newt, *Triturus vittatus* (Jenyns, 1835), is distributed throughout the western Caucasus, Turkey, Lebanon, Syria, Israel, Iraq, and perhaps, Jordan (Borkin et al., 2003; Litvinchuk et al., 2005b; Pearlson and Degani, 2007). For the taxonomical status of banded newt, *Triturus vittatus* (Jenyns, 1835), it was suggested before that 3 subspecies, *T. v.*

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*vittatus* (Jenyns, 1835), *T. v. ciliensis* (Wolterstorff, 1906), and *T. v. ophryticus* (Berthold, 1846), are distributed in Turkey (Schmidtler and Schmidtler, 1967; Atatür, 1974; Özet and Yılmaz, 1994; Arntzen and Olgun, 2000; Borkin et al., 2003). In contrast, Steinitz (1965) reported that only *T. v. vittatus* subspecies is distributed in Southern Anatolia and there are 2 subspecies of *T. vittatus* (*T. v. vittatus* and *T. v. ophryticus*) in Turkey. The species of the genus *Triturus* are divided into 2 groups: the large-bodied newts and the small bodied newts. Although the banded newt, *T. vittatus*, is intermediate in size, it is considered to be more closely related to the large-bodied group. Recently, Litvinchuk et al. (2005a) proposed the *Ommatotriton* genus name for the banded newt. On the other hand, Litvinchuk et al. (2005a) also reported that *T. vittatus* is proposed to have 2 species (*O. ophryticus* and *O. vittatus*) in Turkey in terms of their trunk vertebrae count, genome size, and allozyme data. According to their findings, the northern taxon, *O. ophryticus*, is subdivided into 2 geographic fragments: the western group is allocated to the *O. ophryticus nesterovi* subspecies while the eastern group is named *O. ophryticus ophryticus*. Smith et al. (2008) studied CO1 DNA barcoding amphibians and they stated that the 2 species were shown to be deeply divergent. *Ommatotriton vittatus* was considered to represent 2 allopatric species – *O. vittatus* in the southern and *O. ophryticus* in the northern part of the range (Litvinchuk et al., 2005a) – with 4 subspecies of variable morphology. Similarly, Arntzen and Olgun (2000), Litvinchuk et al. (2001), and Litvinchuk et al. (2005b) mentioned that trunk vertebrae count and genome sizes were different in the western and eastern populations of *O. ophryticus* in Turkey.

To test the suggestion made by Litvinchuk et al. (2005a) that *O. ophryticus* could be represented by 2 different subspecies in northern Turkey, the present study aimed i) to compare skeletal muscle proteins of the *O. o. nesterovi* from one western Black Sea and *O. o. ophryticus* from 9 eastern Black Sea populations in Turkey for the first time by SDS-PAGE, and ii) to compare trunk vertebrae count in 3 *O. o. nesterovi* and 10 *O. o. ophryticus* populations in Turkey.

**Materials and methods**

**Collecting samples**

Totally 203 adult (106 females and 97 males) *O. ophryticus* specimens (24 specimens from the western Black Sea and 179 specimens from the eastern Black Sea) were collected from 21 different populations. From the collected specimens, 107 individuals were used to determine trunk vertebrae count and 96 individuals were used for SDS-PAGE. *O. ophryticus* specimens were collected from April to June in 2005. The specimens were caught by netting from their breeding ponds.

**Trunk vertebrae count**

The number of trunk vertebrae was determined for 93 specimens from 10 populations belonging to the eastern group (Artvin, Trabzon, Giresun, and Ordu) and for 14 specimens from 3 populations belonging to the western group (Zonguldak, Karabük, and Bartın) (Figure 1) by dissecting of dorsal muscle according to Litvinchuk et al. (2005b).

**SDS-PAGE**

In SDS-PAGE experiments, totally 96 adult (46 females and 50 males) *O. ophryticus* specimens (12 specimens from the western Black Sea and 84 specimens from the eastern Black Sea) were used from 10 populations, (6 ♀♀ and 6 ♂♂ from Zonguldak (Kilimli), 4 ♀♀ and 6 ♂♂ from Giresun (Tamdere), 5 ♀♀ and 4 ♂♂ from Rize (Gündoğdu), 31 ♀♀ and 34 ♂♂ from Trabzon (Akçaabat 3 ♀♀ and 4 ♂♂; Çamburnu 5 ♀♀ and 4 ♂♂; Hidrînebi 4 ♀♀ and 4 ♂♂; Kirazlık 3 ♀♀ and 6 ♂♂; Maçka 5 ♀♀ and 6 ♂♂; Uzungöl 7 ♀♀ and 6 ♂♂; Gürbülak 4 ♀♀ and 4 ♂♂) in Turkey (Figure 1). In each SDS-PAGE experiment 1 specimen was used for each population and the experiments were repeated for all specimens in each population. Skeletal muscle protein samples of the newts were obtained by grinding 0.1 g of skeletal muscles of each specimen in liquid nitrogen and by adding 0.1 mL of double distilled water (DDW) and 0.2 mL of 2 × 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). Samples were boiled for 2 min in the 2 × SDS gel-loading buffer to denature the proteins prior to gel-loading (Lutz et al., 2001). The SDS-PAGE 99 program was used for boiling in a thermal block. The
size of the minigels was 8.3 × 7.3 cm and the resolving gels were 12% (w/v) gradient. The 12% gradient gels were prepared by putting 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 into a beaker. The 5% stacking gels were prepared by putting 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 into a beaker (Sambrook et al., 1989). For SDS-PAGE experiments, 1.85 μg of protein of each 1 μL sample was applied to the wells. 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 (1 h), which was 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 into washing solution 2 (1 h), prepared with 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 are shown in Figure 2. Appearances of the protein bands on the gels were clear and the bands were counted by 2 different researchers. Although the bands were clearly seen on the gels, when the gels were scanned the appearances of the protein bands were not so clear and some of vague bands appeared in Figure 2. An arrow was added to indicate the lacking protein band that belonged to O. o. nesterovi specimen in Figure 2.

Results

Figure 2 shows SDS-PAGE patterns of the skeletal muscle proteins in O. ophryticus. Total number of skeletal muscle protein bands was determined as 25 for the western Black Sea (Zonguldak) population while there were 26 bands in the eastern Black Sea populations. The lacking protein band for Zonguldak specimens is shown in Figure 2. After analyzing the
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data obtained from all specimens used in the SDS-PAGE experiments, no difference was found between males and females. The number of trunk vertebrae in *O. ophryticus* was 13–14 in the eastern group (Artvin, Trabzon, Giresun, and Ordu) while it was 12 in the western group (Zonguldak, Karabük, and Bartın). The Table shows the trunk vertebrae count results.

**Discussion**

In the present study, skeletal muscle protein bands of the *O. ophryticus* samples from 1 western and 9 eastern Black Sea populations in Turkey were compared by using SDS-PAGE.

SDS-PAGE results showed that total number of skeletal muscle protein bands was 25 in the western Black Sea population while there were 26 bands in the 9 eastern Black Sea populations. Using the total number skeletal muscle protein bands to compare different species or subspecies was also performed by Bülbül and Kutrup (2007b). The results on the total number of protein bands of the green toad (*Bufo viridis*) showed that Hatay specimens were similar to Kayseri, Rize, and Tekirdağ specimens, while Mersin specimens had 2 different protein bands compared to other specimens in their study. Similarly Hasnain et al. (2005) studied the soluble muscle proteins in 4 fish species. The authors found that 16 protein bands were diagnostic for *Channa gachua* and *Channa striatus* while 10 and 15 bands were diagnostic for *C. marulius* and *C. punctatus* by SDS-PAGE. Bülbül and Kutrup (2007a) also stated that molecular weights of the skeletal myosin, actin, troponin C, and tropomyosin proteins differed between *Xenopus tropicalis* and *Hyla japonica*. In addition to these data, a detailed and systematic study of skeletal fiber types in limb muscle of 2 frog species, *Rana* and *Xenopus*, by using myosin isofrom-based criteria was performed by Rowlerson and Spurway (1988). They found different myosin isoforms in 2 different frog species. This result provided strong evidence that the different fiber types in *Rana* and *Xenopus* limb muscle contained different myosin heavy chains (MHC) isoforms. These data revealed that total number of skeletal muscle protein bands and biochemical characteristics of the skeletal muscle proteins could be different among species or subspecies.

In the present study, trunk vertebrae counts of the western and eastern Black Sea specimens of *O. ophryticus* were also compared. The number of trunk vertebrae in *O. o. ophryticus* specimens was 13–14 in the eastern group while it was 12 in the *O. o. nesterovi* specimens in the western group. This result is consistent with the result reported by Litvinchuk

**Table. Trunk vertebrae counts of Ommatotriton ophryticus nesterovi and O. o. ophryticus specimens from Turkey.**

<table>
<thead>
<tr>
<th>Subspecies Name</th>
<th>Locality</th>
<th>n</th>
<th>Voucher Number</th>
<th>Trunk vertebrae count</th>
</tr>
</thead>
<tbody>
<tr>
<td>O. o. nesterovi</td>
<td>Karabük (Yenice)</td>
<td>2 (1♂, 1♀)</td>
<td>TrUB-01-02</td>
<td>12</td>
</tr>
<tr>
<td>O. o. nesterovi</td>
<td>Bartın (Kozcaguz)</td>
<td>2 (1♂, 1♀)</td>
<td>TrUB-03-04</td>
<td>12</td>
</tr>
<tr>
<td>O. o. nesterovi</td>
<td>Zonguldak (Ereğli)</td>
<td>10 (5♂, 5♀)</td>
<td>TrUB-05-14</td>
<td>12</td>
</tr>
<tr>
<td>O. o. ophryticus</td>
<td>Trabzon (Gürbulak)</td>
<td>23 (5♂, 18♀)</td>
<td>TrUB-15-37</td>
<td>13</td>
</tr>
<tr>
<td>O. o. ophryticus</td>
<td>Trabzon (Hıdırnebi)</td>
<td>15 (7♂, 8♀)</td>
<td>TrUB-38-52</td>
<td>13</td>
</tr>
<tr>
<td>O. o. ophryticus</td>
<td>Trabzon (Beşirli)</td>
<td>12 (6♂, 6♀)</td>
<td>TrUB-53-64</td>
<td>13</td>
</tr>
<tr>
<td>O. o. ophryticus</td>
<td>Trabzon (Şinik)</td>
<td>9 (7♂, 2♀)</td>
<td>TrUB-65-73</td>
<td>14</td>
</tr>
<tr>
<td>O. o. ophryticus</td>
<td>Trabzon (Of)</td>
<td>8 (3♂, 5♀)</td>
<td>TrUB-74-81</td>
<td>13</td>
</tr>
<tr>
<td>O. o. ophryticus</td>
<td>Trabzon (Yalncak)</td>
<td>2 (2♂)</td>
<td>TrUB-82-83</td>
<td>13</td>
</tr>
<tr>
<td>O. o. ophryticus</td>
<td>Trabzon (Sürmene)</td>
<td>2 (1♂, 1♀)</td>
<td>TrUB-84-85</td>
<td>13</td>
</tr>
<tr>
<td>O. o. ophryticus</td>
<td>Artvin (Erenköy)</td>
<td>11 (4♂, 7♀)</td>
<td>TrUB-86-96</td>
<td>13</td>
</tr>
<tr>
<td>O. o. ophryticus</td>
<td>Ordu (Uluşey)</td>
<td>4 (2♂, 2♀)</td>
<td>TrUB-97-100</td>
<td>13</td>
</tr>
<tr>
<td>O. o. ophryticus</td>
<td>Giresun (Kümbet)</td>
<td>7 (3♂, 4♀)</td>
<td>TrUB-101-107</td>
<td>13</td>
</tr>
</tbody>
</table>
et al. (2005b). The modal number trunk vertebrae count was 12-13 in the western group and 13-14 in the eastern group in their study. Similarly, Arntzen and Olgun (2000) also found that modal count was 13 in the western Black Sea samples while it was 14 in the eastern Black Sea samples. For other Urodelas, Litvinchuk and Borkin (2003) reported that the number of trunk vertebrae varied between 14 and 21 in the family Hynobiidae. The lowest number was recorded in Ranodon sibiricus, whereas the highest count was in Onychodactylus fischeri. The modal number of trunk vertebrae was 15 in Ranodon sibiricus (and, probably, Hynobius nigrescens); 16 in Hynobius leechii (and, probably, Hynobius nebulosus); 17 in Salamandrella keyserlingii, Hynobius naevis, and Batrachuperus mustersi; and 18 in Onychodactylus japonicus. O. fischeri was characterized by the higher modal number, which was equal to 20. On the other hand, number of trunk vertebrae varies from 16 to 23 in slender salamanders, Batrachoseps, and variation was found within populations, among populations, and among species (Anderson, 1960; Wake, 1966; Brame and Murrey, 1968; Yanev, 1978). Chan et al. (2001) stated that except for 1 individual with 14 trunk vertebrae all Cynops pyrrhogaster and Cynops cyanurus specimens had 13 trunk vertebrae.

The results in the present study for O. ophryticus species showed that western Black Sea specimens were different from eastern Black Sea ones according to the total number of skeletal muscle protein bands and trunk vertebrae counts contrary to the former common suggestion that 3 subspecies, T. v. vittatus, T. v. cilicensis, and T. v. ophryticus, are distributed in Turkey (Schmidtler and Schmidtler, 1967; Atatürk, 1974; Özet and Yılmaz, 1994; Arntzen and Olgun, 2000; Borkin et al., 2003). In agreement with our results, Litvinchuk et al. (2005a) reported that the banded newt, T. vittatus, is represented by 2 species (O. ophryticus and O. vittatus) in Turkey according to their trunk vertebrae count, genome size, and allozyme data. They proposed that the northern taxon, O. ophryticus, is subdivided into 2 geographic fragments: the western group is allocated to the O. o. nesterovi subspecies while the eastern group is named O. o. ophryticus. Smith et al. (2008) studied CO1 DNA barcoding amphibians and they stated that the 2 species were shown to be deeply divergent. O. vittatus was considered to represent 2 allopatric species – O. vittatus in the southern and O. ophryticus in the northern part of the range (Litvinchuk et al., 2005a) – with 4 subspecies of variable morphology. Similar to their proposal, we recommend that O. ophryticus be subdivided into 2 geographic fragments as O. o. nesterovi in western Black Sea and O. o. ophryticus in eastern Black Sea regions.

The skeletal muscle differences revealed by the present study for the O. ophryticus specimens needs to be supported by more comprehensive populations, especially for the western Black Sea specimens.

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References


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