

On the Distribution and Subspecies Affiliation of *Triturus dobrogicus* (Amphibia: Salamandridae) in Bulgaria

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Abstract: Literature data on the distribution of *Triturus dobrogicus* in Bulgaria were summarized and 6 new locations were reported. An UTM map of the species distribution was presented. The known sites (historical and new) covered in total 1.36 % of the UTM squares (10x10 km) in Bulgaria. The major diagnostic traits of specimens from Bulgaria were compared with those from the type locations of *T. d. dobrogicus* and *T. d. macrosoma*. The assumption was made that the Bulgarian populations along the Danube River belong to *T. d. macrosoma*, and this at Durankulak Lake (the Northern Black Sea region) was supposedly relict and should be affiliated to *T. d. dobrogicus*.

Key words: *Triturus dobrogicus macrosoma*, distribution, taxonomy, Bulgaria, Danube River.

Introduction

The Danube crested newt *Triturus dobrogicus* (KIRITZESCU, 1903) inhabits the Vienna and Pannonia Plains, the valley of the Lower Danube, and the Dnieper delta (e.g. ARNTZEN 2003; LITVINCHUK, BORKIN 2009). The species was found in Bulgaria mainly in the immediate vicinity of the Danube, but one location in the Northern Black Sea region was also reported (GHERGHEL, IFTIME 2009). Maps of *T. dobrogicus* occurrence have been presented in several contemporary publications (e.g. BISERKOV 2007; BESHKOV 2011; STOJANOV *et al.* 2011) but no particular finds have been cited in them.

According to LITVINCHUK, BORKIN (2000), *T. dobrogicus* has two valid subspecies – *T. d. dobrogicus* (KIRITZESCU, 1903) in the Danube and the Dnieper deltas, and – *T. d. macrosoma* (BOULENGER, 1908) in the rest of the species areal. There is no holotype defined for *T. d. dobrogicus* but there are 9 syntypes from the Danube delta preserved in the Grigore Antipa Museum in Bucharest, Romania (FUHN, FREYTAG 1961). The same authors stated one of them (male) as a lectotype and presented a description

of certain external traits and indices for the specimens of the type series (see p. 172 in FUHN, FREYTAG 1961). According to LITVINCHUK, BORKIN (2000), the holotype (female) of *T. d. macrosoma* most probably had come from the vicinities of Vienna, Austria, and it was kept in the British Museum; the authors gave in the same paper a description of some traits and indices of the specimen in question. The two taxa are relatively well discernible morphologically, but their detachment has not been confirmed yet by using cytogenetic or molecular methods (e.g. LITVINCHUK *et al.* 1994, 1999). The main traits to distinguish the two subspecies (LITVINCHUK, BORKIN 2000, 2009) are, as follows: the so-called Wolterstorff index (WI, the percentage of the forelimb length from the body length between the anterior and posterior limbs), the Ltc/L index (the percentage of the head width from the body length), the belly and sides coloration, and the number of rib-bearing vertebrae (RBV). There was no material from Bulgaria studied in the cited papers, so the taxonomic affiliation of the Bulgarian populations remains unproven.

The aim of this work was to generalize and map the data on *T. dobrogicus* distribution in Bulgaria and to make an attempt to clarify the subspecies affiliations of its Bulgarian populations.

Material and methods

All available literature sources, as well as unpublished data from 2009–2012, were used in mapping the species occurrence. Locations were mapped according to the Universal Transverse Mercator (UTM) grid 10x10 km.

The morphological studies were carried out on 58 specimens (24 males and 34 females) from 6 sites in the Bulgarian Danube valley: Kudelin, Vidin, Orsoya, Batin, Nova Cherna and Srebarna (respectively FP39, FP57, FP65, LJ93, MJ57 and NJ08 in Fig. 1). Two specimens from the vicinities of Vienna, Austria (from the collection of the National Natural History Museum in Sofia, museum No. III-30-26) were used as comparative material, and they were regarded here as specimens representing the *T. d. macrosoma* type location. The diagnostic indices were calculated from the measurements of the following traits: body length from the tip of the snout to the anterior edge of the cloaca; head width between the corners of the mouth; length of the forelimb from the base to the end of the longest finger; body length between the anterior and posterior limbs. The measurements were done on living newts anaesthetized with 0.25 g/l water solution of benzocaine, using a caliper-gauge with 0.05 mm precision. The measurement data were described and analyzed using the following statistics: arithmetic mean (Mean), standard deviation (SD), minimum (Min) and maximum (Max), as well as Analysis of variance (ANOVA). All measurement data were normally distributed (Kolmogorov-Smirnov d test: $p > 0.05$ in both sexes). Statistical procedures were performed with STATISTICA 7.0 (StatSoft. Inc.1984-2004.).

Results and discussion

The reviewed literature presented 18 particular finding sites of *T. dobrogicus* in Bulgaria; we here additionally report on 6 new (Appendices I and II). All the 24 locations belonged to 17 UTM squares, which comprise 1.36% of the total count of squares in the country (Figure 1). Most of the literature data on the locations carried distinguishable geographic

landmarks (settlements). Some of the data given in ARNTZEN (2003), which were based on interpretation of other sources of information, were not enough convincing. The author cited three locations of *T. dobrogicus* in Bulgaria in the following manner: „Vidin, Arčar und zwischen Vidin und Arčar (Buresch und Zonkow 1941, Beshkov 1984)”. The first site was actually reported in the work of BURESCH, ZONKOV (1941), but the rest were not mentioned in that paper, or in the publication of BESHKOV (1984) (this discrepancy was also noted by TZANKOV, STOYANOV 2008). No locations were reported in the work of BESHKOV (1984), only a map was given with two symbols for the species in that part of the country, one of which could be assigned to the town of Vidin area, and the other – to the village of Archar area. The presence of the species in the region of Archar was confirmed in field investigations by B. Naumov in 2012. There was no data in the publications cited by ARNTZEN (2003) about the third location “between the town of Vidin and the village of Archar”, and its particular description did not allow mapping on the 10-km grid.

Almost all known sites of *T. dobrogicus* in Bulgaria are located in the immediate riverine area of the Danube, at no more than 3–4 km from the river. The site at the village of Katselovo (UTM: MJ21 in Fig. 1) is relatively outlying from the Danube bank. It was reported by UNDJIAN (2000) as „A micro reservoir near Katselovo village” and was presented as a location of *T. dobrogicus*, despite the author himself had not seen the newts (information on observations by the construction technician I. Kolev from May 1998). The distance from Katselovo to the Danube is 30 km as the crow flies, but it is about 100 km along the Lom River. It should be noted here that the whole area between Katselovo and the Danube is covered by arable land in monoculture tracts, and the only possible corridor for the newts is namely the Lom River valley. In our opinion, it is not quite proper to use inquiry data as a base to pronounce new sites of finding species of the genus *Triturus* because of the great morphological similarity among the species. In the discussed case, the specimens should be related most probably to *Triturus karelinii* (STRAUCH, 1870). This species is widely spread in Bulgaria and have been found near Katselovo: at the village of Nissovo in the Lom River valley (UTM: MJ23, TZANKOV, STOYANOV 2008) and at the village of Blagoevo (UTM: MJ51, N. Tzankov, unpublished

data). It is also possible that the newts have been carried there by man; such an opportunity was discussed by UNDIJAN (2000) as well. No symbol for the presence of the species in the vicinities of the village of Katselovo was placed in the map of the distribution of *T. dobrogicus* in the work of BESHKOV (2011) despite the fact that the author had cited the paper of UNDIJAN (2000). Supposedly, BESHKOV (2011) also doubted the reliability of that information, although not commenting it. For the same reason, this locality is not cited in the work of STOJANOV *et al.* (2011). If it be assumed that the species was really *T. dobrogicus*, then it should be assumed that the village of Katselovo is its location furthest from the Danube in the Danubian Plain in Bulgaria. Yet, this site of finding in all cases could not be taken as proven and therefore it was presented on the map here with a question mark.

The Durankulak Lake at the Northern Black Sea coast (UTM: PJ23 in Fig. 1) is of special interest as the only known location out of the Danubian Plain in Bulgaria. This site was reported by GHERGHEL, IFTIME (2009), and three specimens from it are known, which have been collected in the first half of the 20th century. Taking into account the total areal of the species (LITVINCHUK, BORKIN 2009), it is clear that this site is quite remote from the rest of the locations, and unlike them, it has no connection with the Danube. There are no other data on finding any newts in the most northern part of the Black Sea coast (Bulgaria) except for the publication of GHERGHEL, IFTIME (2009). *T. dobrogicus* is very rare in Northern Dobrudzha, and COVACIU-MARCOV *et al.* (2006) have reported only one location – at the town of Istria at about 100 km to the north of Durankulak. The rest of the known locations of *T. dobrogicus* in the north-eastern edge of the Balkans are in the Danube itself and in its delta, as well as in the nearby lake formation Razim–Sinoe (see FUHN 1960; ARNTZEN *et al.* 1997).

In order to elucidate the subspecies affiliation of the Bulgarian populations of *T. dobrogicus*, we compared according to the basic diagnostic traits specimens from Bulgaria with specimens from the type localities of the two taxa, as well as with the diagnoses given by LITVINCHUK, BORKIN (2000, 2009). The number of specimens we collected from every site of finding was small, that was why we combined them into two groups: (i) western (referred to as BgWest) which included 30 specimens from the locations at Kudelin, Vidin and Orsoya (respectively,

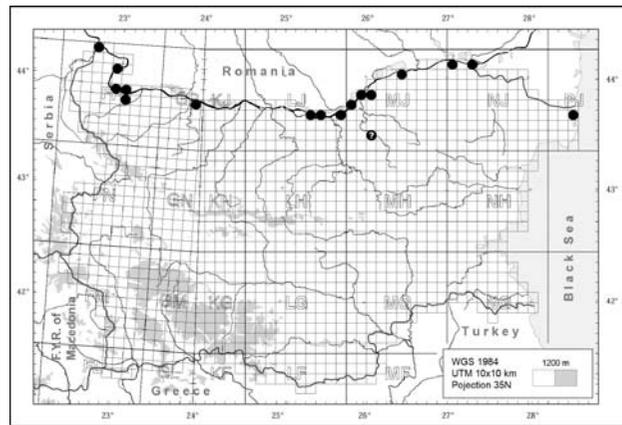


Fig. 1. Occurrence of *Triturus dobrogicus* in Bulgaria on an UTM grid

FP39, FP57 and FP65 in Fig. 1); (ii) eastern (referred to as BgEast) which included 28 specimens from the locations at Batin, Nova Cherna and Srebarna (respectively, LJ93, MJ57 and NJ08 in Fig. 1). The specimens from Durankulak (described in the work of GHERGHEL, IFTIME (2009), PJ23 in Fig. 1) could be regarded as a third group (referred to as BgBlsc), but their number was too small to be subjected to statistical analyses. ANOVA of the two diagnostic indices calculated for the studied specimens – WI and Ltc/L (Tables 1 and 2), did not reveal any statistically significant difference between the western and the eastern groups from the Bulgarian Danube river valley (Table 3).

According to the WI values, the studied males from Bulgaria of all the three groups fell into the “realm” of *T. d. macrosoma*, as it had been defined by the diagnoses – by the mean values, as well as by the variation range (Fig. 2). The mean values for the western and eastern groups of females from the Bulgarian Danube river valley were near to the threshold value for the differentiation of the two taxa. The maximum WI values in both groups were very near to the mean value for *T. d. dobrogicus*. One of the female specimens from Durankulak possessed a WI value typical for *T. d. macrosoma*, but the other had a much higher WI, almost equal to the arithmetical mean for the syntypes of *T. d. dobrogicus*. It should be noted that both of females from the type location of *T. d. macrosoma* (including the holotype) had WI higher than the threshold value for that taxon (43.5 and 42.5 for the females from Vienna, although the threshold value is 41).

The index Ltc/L was pointed out as the first trait in the differentiation between the two taxa in

the diagnoses given by LITVINCHUK, BORKIN (2000), but it was not mentioned at all in the work of LITVINCHUK, BORKIN (2009). In our study, the mean values of the index for both groups of specimens from the Bulgarian part of the Danube valley, of both sexes, fell into the “realm” of *T. d. macrosoma* (Fig. 3). In contrast to WI, Ltc/L better corresponded to *macrosoma* for the females than for the males. The index Ltc/L was not given for the specimens from Durankulak, so their taxonomic affiliation could not be verified by this index.

In addition to the different indices, the two subspecies differ also in some elements of their coloration. According to LITVINCHUK, BORKIN (2009), there are always numerous white specks on the lower part of the flanks of *T. d. macrosoma*, whereas for the nominal subspecies, there are always few or no specks. All specimens from the Bulgarian Danube examined by us, as well as those from Vienna, had numerous white specks on the flanks of the body. This trait was not described for the specimens from Durankulak, and it could not be adequately assessed on the submitted photographs because of the camera angle. The other distinction between the two subspecies refers the size and pattern of the dark spots on the belly. According to LITVINCHUK, BORKIN (2009), these spots of *T. d. dobrogicus* are usually large, and they are arranged (or merged) in such a way that one medial or two lateral bands are formed, while the

spots of *T. d. macrosoma* are typically of medium size and uniformly distributed over the surface of the belly. In our opinion, this trait is not sufficiently objective; still we could classify the studied specimens from the Bulgarian Danube valley in the following manner: spots of medium size, uniformly distributed, characteristic for *T. d. macrosoma*, possessed 38% of the specimens; large merged spots, characteristic for *T. d. dobrogicus*, possessed 41% of the specimens; other type of coloration was seen in 21% of the specimens. The newts from Durankulak had medium sized spots which were uniformly distributed in two of the specimens, and they were arranged in lateral bands in the third specimen. The spots of the specimens from Vienna were also of medium size, grouped medially in the male and uniformly distributed in the female.

The comparisons gave grounds to conclude that the studied specimens from the Bulgarian Danube River valley were morphologically much closer to *T. d. macrosoma* than to *T. d. dobrogicus*, and they should be affiliated to the first subspecies. In the work of VÖRÖS, ARNTZEN (2010), it is mentioned that the population from Svishtov area (LJ63 in Fig. 1) does not show significant genetic differentiation from the Pannonian samples, which corresponds to our conclusion. Most likely, this taxon occurs in the Bulgarian territory along the whole Danube valley (probably in all UTM squares the Danube crosses).

Table 1. Descriptive statistics of the diagnostic indices of the studied specimens of males

Males	WI				Ltc/L			
	Means	SD	Min	Max	Means	SD	Min	Max
BgWest (n = 10)	50,44	2,76	45,55	54,20	13,42	0,88	11,99	14,68
BgEast (n = 14)	48,56	2,05	45,36	51,88	13,64	0,45	13,03	14,64
All Groups (n = 24)	49,35	2,50	45,36	54,20	13,55	0,65	11,99	14,68

Table 2. Descriptive statistics of the diagnostic indices of the studied specimens of females

	WI				Ltc/L			
	Means	SD	Min	Max	Means	SD	Min	Max
BgWest (n = 20)	40,59	2,61	35,79	45,84	12,50	0,78	11,01	14,05
BgEast (n = 14)	42,75	4,11	36,89	48,70	12,48	1,29	9,48	13,97
All Groups (n = 34)	41,48	3,43	35,79	48,70	12,49	1,00	9,48	14,05

Table 3. Summarized results of ANOVA of BgWest and BgEast according to the diagnostic indices.

	Males			Females		
	F	df	p	F	Df	p
WI	3,6801	22	0,0681	3,5198	32	0,0698
Ltc/L ro	0,6446	22	0,4306	0,0033	32	0,9549

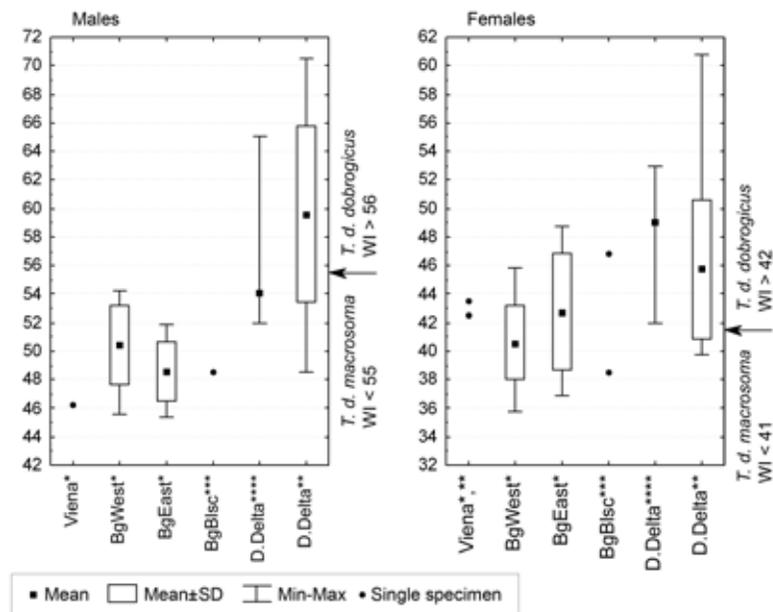


Fig. 2. Comparison of WI values (in %) of *T. dobrogicus* from Bulgaria with those from the type locations of the two taxa (* this study, ** LITVINCHUK, BORKIN (2000); *** GHERGHEL, IFIME (2009), **** FUHN, FREYTAG (1961)). The threshold values of the two taxa according to the diagnoses (after LITVINCHUK, BORKIN 2009) are given on the right axis

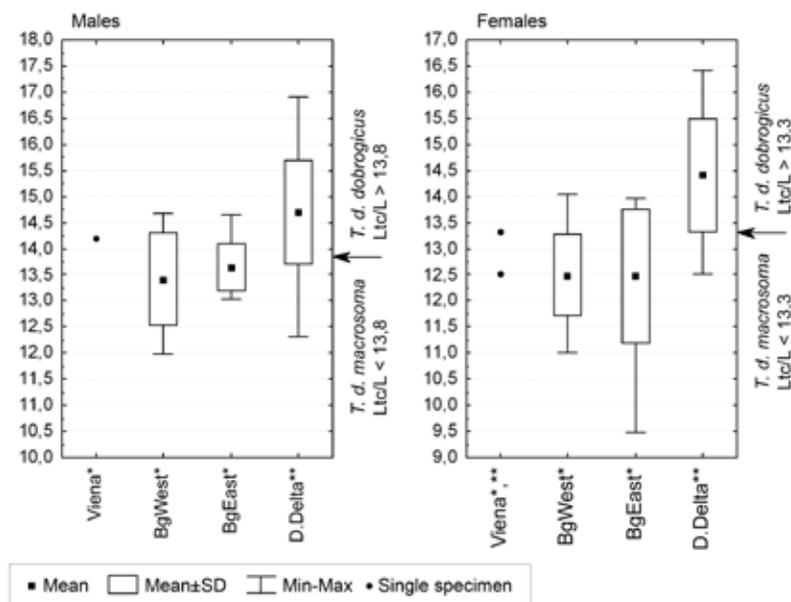


Fig. 3. Comparison of Ltc/L values (in %) of *T. dobrogicus* from Bulgaria with those from the type locations of the two taxa (* this study, ** LITVINCHUK, BORKIN (2000)). The threshold values of the two taxa according to the diagnoses (after LITVINCHUK, BORKIN 2000) are given on the right axis

As to the specimens from Durankulak, they were of very low number and the outer morphology description given by GHERGHEL, IFIME (2009) was not complete enough to draw an explicit conclusion about their subspecies affiliation. Yet, the number of rib-bearing vertebrae (a trait that was not studied by us) in these specimens was 15 in the male and 16 in both females, which corresponded to the diagnosis of *T. d. dobrogicus* (according to LITVINCHUK,

BORKIN, 2000, 2009 the number of RBV is usually 16 in *T. d. dobrogicus* and 17 in *T. d. macrosoma*). However, it should be noted that the holotype of *T. d. macrosoma* also has 16 RBV. According to certain hypotheses on the palaeogeography of the Black Sea region (e.g. VARUSHCHENKO 1982), the level of the Black Sea about 10,000 years ago was ca. 100 m lower than today. The Danube delta was much wider, located to the south-east from its today's position

and it formed a wetlands complex with the Dniester and the Dnieper deltas. LITVINCHUK (2005) supposed on that base that the locations of *T. dobrogicus* in the lower reaches of the Dnieper were remains of a much broader past areal. Such a palaeogeographic scenario could explain very well the species finds at Durankulak and aroused the assumption that the location was relict. In this context, it seems more probable that the Durankulak population belongs to the nominal subspecies, but at all events, it is necessary to study larger number specimens from that region.

In conclusion, it should be emphasized once more that the individualization of the taxon *macrosoma* is based only on morphologic differences. Moreover, none of the morphological traits itself does not clearly distinguish the two taxa (there is overlap of values), but this is also true for the species of *Triturus cristatus* group in general (see ARNTZEN, WALLIS 1994, 1999). Intraspecific taxonomy of *T. dobrogicus* has not been studied in detail by cytogenetic and molecular methods. In the work of LITVINCHUK *et al.* (1994), it is mentioned that the NEI's genetic distance between investigated specimens from Transcarpathian region (in the area of *T.*

macrosoma) and from the Danube delta is practically equal to zero (see Table 2 in LITVINCHUK *et al.* 1994). Moreover, some results of the study of LITVINCHUK *et al.* (1999) indicate that the genome size of the investigated specimens from the Danube delta is similar to this one of the specimens from the area of *T. d. macrosoma* (even the specimens from the delta occupy an intermediate position compared to these from Transcarpathian region and from Serbia; see Table 1 in LITVINCHUK *et al.* 1999). In view of the foregoing we believe that more detailed studies are needed to confirm (or reject) the status of *T. d. macrosoma* as a separate taxon. For all that, we are still standing by the assertion that the two separate taxa exist, because it has not been disproven either by any factual arguments in the referred literature.

Acknowledgements: We would like to express our gratitude to D. Chobanov, M. Ilieva and P. Zehindzhiev for the provided material and to A. Stojanov, D. Dobrev and E. Vacheva for their help in the field investigations. The field studies were funded by the Ministry of Education, Youth and Science (Bulgarian Science Fund grant No. DO 02-352/2009). Newts were captured under the permits of the Ministry of Environment and Water (Permits No. 182/09.03.2009, No. 258/09.04.2010 and No. 411/14.07.2011).

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Received: 16.10.2012

Accepted: 21.02.2013

Appendix I

(Published records of *T. dobrogicus* in Bulgaria in order of the UTM coordinates)

FP55: Archar (ARNTZEN, 2003 according to BESHKOV, 1984; confirmed by B. Naumov – 1 specimen, 01.04.2012); **FP57**: Vidin (BURESCH, ZONKOV, 1941); **GP34**: Oryahovo (KOVATSCHEFF, 1905; BURESCH, ZONKOV, 1941); **LJ63**: Svishtov (SCHUMANN, 1930; WALLIS, ARNTZEN, 1989), the Svishtov swamps (KOVATSCHEFF, 1903); **LJ73**: Vardim Island (given as „Beskut Island” by BURESCH, ZONKOV, 1941); **MJ04**: Mechka (UNDJIAN, 2000); **MJ15**: riverside of the Danube River at Ruse (KOVATSCHEFF, 1903; UNDJIAN, 2000), the Limana locality, Ruse (UNDJIAN, 2000), Sredna Kula quarter, Ruse (given as „Izvor Dere” by KOVACHEV, 1912); **MJ21**: a small reservoir near Katselovo (UNDJIAN, 2000) (in our opinion this locality is not certain – see Results and Discussion); **MJ25**: Obratzov Chiflik quarter, Ruse (KOVACHEV, 1912), Lipnik Park, Ruse (UNDJIAN, 2000); **MJ57**: Fish farm Nova Cherna (STOYNEVA, MICHEV, 2007; confirmed by M. Ilieva, P. Zehindzhiev – 15 specimens, October 2010); **NJ08**: Srebarna (KOVACHEV, 1912; BURESCH, ZONKOV, 1941; BESHKOV, 1998); **NJ28**: Silistra (KOVATSCHEFF, 1905; BURESCH, ZONKOV, 1941); **PJ23**: Durankulak Lake (GHERGHEL, IFTIME, 2009); **unknown UTM** (not shown in the map): between Vidin and Archar (ARNTZEN, 2003).

Appendix II

(New records of *T. dobrogicus* in Bulgaria in order of the UTM coordinates)

FP39: a drainage canal at Kudelin (6 specimens, 14.07.2009, B. Naumov, D. Dobrev, A. Stojanov); **FP57**: a flooded riverbank forest near Vidin (20 specimens, 14.07.2009, B. Naumov, D. Dobrev, A. Stojanov); **FP64**: a drainage canal near Orsoya (2 specimens, 18.03.2010, B. Naumov, E. Vacheva); **FP65**: the Orsoya swamp (2 specimens, 19.03.2010, B. Naumov, E. Vacheva); **LJ93**: a drainage canal northwest of Batin (5 specimens, May 2011, D. Chobanov); **NJ08**: the canal between Srebarna Lake and the Danube River (8 specimens, 16.05.2009, B. Naumov).

