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1 Karyotype dispersal of the common lizard *Zootoca vivipara* (Lichtenstein,  
2 1823) in eastern and northeastern Fennoscandia

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## 10 **Abstract**

11 The wide-ranging Eurasian common lizard *Zootoca vivipara* (Lichtenstein, 1823) is  
12 remarkably uniform morphologically but highly varied in its karyotype. Previous studies have  
13 revealed two distinctly different chromosomal forms of *Z. v. vivipara* in the Baltic basin.  
14 Moreover, a zone of secondary contact between these forms has been localized on the  
15 southern Baltic Sea seashore. Intraspecific karyotype diversity for *Z. vivipara* and new zones  
16 of secondary contact have recently been suggested for other parts of the Baltic Sea seashore.  
17 We studied the karyotype of *Z. vivipara* in central, western and northern parts of Finland. All  
18 the individuals karyotyped represented the Russian form of *Z. v. vivipara* that differs from the  
19 western form of the subspecies located at the southern and western Baltic Sea seashore.  
20 Together with previous data sets, our results suggest intraspecific karyotype diversity in the  
21 northern and northwestern parts of Fennoscandia. The results give support to the hypothesis  
22 of *Z. vivipara*'s re-colonization of the Baltic Sea basin. Moreover, the results support the  
23 previous observations of Voipio (1961, 1968 and 1969) who has reported variability in the  
24 shield pattern of *Z. vivipara* in the same region.

## 25 **Introduction**

26 The widely-ranged Eurasian common lizard *Zootoca vivipara* (Lichtenstein 1823) (family  
27 Lacertidae) is a squamate species with a huge distribution range from western Europe (the  
28 Pyrenees) throughout central, eastern and northern Europe up to eastern Asia (the Russian

1 Far East, islands Sakhalin and Kunashir and northern Japan). The species is characterized by  
2 viviparous and oviparous reproduction in different populations (Brana & Bea 1987) and  
3 substantial geographic variation in body size and reproductive output (Horváthová *et al.*  
4 2013). Despite such special characteristics *Z. vivipara* is remarkably uniform  
5 morphologically but polymorphic in its haplotype and karyotype. The species has 1) different  
6 diploid numbers:  $2n = 36/36$  in both sexes or  $2n = 35$  in female and  $2n = 36$  in male; 2)  
7 different size of female sex chromosomes:  $w$  microchromosome ( $m$ ) or  $W$   
8 macrochromosome ( $M$ ); 3) different systems of sex chromosomes:  $Zw$  in female and  $ZZ$  in  
9 male or  $Z_1Z_2W$  in female and  $Z_1Z_1Z_2Z_2$  in male; 4) different morphology of  $w$  and  $W$  sex  
10 chromosomes: acrocentric ( $a, A$ ), subtelocentric ( $ST$ ) or submetacentric ( $SV$ ) and 5) some  
11 differences in cytogenetic and molecular structure of  $w$  and  $W$  sex chromosomes:  
12 heterochromatic amount and some other features (Table 1.).

13 From all these karyotype characteristics six/seven separate chromosomal forms have been  
14 recognized among oviparous and viviparous females from different populations in Europe  
15 and in Asia. Among them, two new oviparous subspecies; *Z. v. carniolica* (Mayer, Böhme,  
16 Tiedemann & Bischoff 2000) and *Z. v. louislantzi* (Arribas 2009). Two recent studies even  
17 suggest that *Z. v. carniolica* may be approaching species status (Lindtke *et al.* 2010, Cornetti  
18 *et al.* 2014). Subspecies *Z. v. vivipara* may be subdivided into four viviparous chromosomal  
19 forms, three of which are closely related, although the taxonomy of the latter is still  
20 questionable, they can be easily recognized by their  $2n$  and some other karyotype  
21 characteristics (Table 1.).

22 In addition, several molecular and chromosomal studies have discussed the geographical  
23 distribution of different haplogroups (Heulin *et al.* 1999, 2011, Surget-Groba *et al.* 2001,  
24 2006, Velekei *et al.* 2014) and chromosomal forms (Kupriyanova 1990, Kupriyanova &  
25 Böhme 1997, Kupriyanova *et al.* 2005, 2006, 2007, Odierna *et al.* 2001, Puky *et al.* 2004) of  
26 *Z. vivipara*. Chromosomal studies have shown that described subspecies and separate  
27 chromosomal forms of *Z. v. vivipara* have their distinct distribution ranges in Europe and in  
28 Asia (Table 1). In central Europe, subspecies and forms occur in allopatric, parapatric and  
29 sometimes mosaic populations. Some of them appear to inhabit small areas while others are  
30 relict and rare within one country. However, the western form of *Z. v. vivipara* and the  
31 Russian form of *Z. v. vivipara* occupy a vast territory in Europe and Asia. It has been  
32 indicated that the Russian form has in its female karyotype 34 acrocentric ( $A$ ) chromosomes

1 and 1 acrocentric (A) W sex chromosome (35 chromosomes in total). The latter has short  
 2 arms at some metaphase plates and it is close to subtelocentric (ST). Therefore, it is  
 3 sometimes indicated as A/ST. Chromosomal formula is: ♀2n = 35: 34A + 1A, where W is A  
 4 (or A/ST). The western form has in its female karyotype 34 acrocentric (A) chromosomes  
 5 and 1 submetacentric (SV) W sex chromosome (the same 35 chromosomes in total).  
 6 Chromosomal formula is: ♀2n = 35: 34A + 1SV, where W is SV. The males of all forms of  
 7 *Z. v. vivipara* and of subspecies *Z. v. louislantzi* have in their karyotype 36 acrocentric  
 8 chromosomes: ♂ 2n = 36A with 4 acrocentric Z<sub>1</sub>Z<sub>1</sub>Z<sub>2</sub>Z<sub>2</sub> sex chromosomes (Table 1.).

9 Based on the karyotype (Kupriyanova 1990, 2004, Kupriyanova & Rudi 1990, Kupriyanova  
 10 & Böhme 1997, Kupriyanova *et al.* 2007, Odierna *et al.* 1998, 2001) and the Mt DNA data  
 11 (Surget-Groba *et al.* 2006, Velekei *et al.* 2014), the Russian form has been discovered from  
 12 the eastern Carpathian throughout Russia up to Sakhalin island and northern Japan, whereas  
 13 the western form has also been found in the populations in the eastern and western  
 14 Carpathians as well as in central and in western Europe up to the Pyrenees. So far, the highest  
 15 karyotype diversity has been discovered among the populations in the Carpathian Basin, due  
 16 to which a centre of evolution of different chromosomal forms of *Z. vivipara* has been  
 17 assumed to occur there (Kupriyanova & Böhme 1997, Odierna *et al.* 1998). Additionally,  
 18 from all the available biogeographical and chromosomal data, it can be concluded that the  
 19 Russian form is the most primitive one, whereas the western form has been derived from it  
 20 (Kupriyanova 1990, Kupriyanova & Rudi 1990, Odierna *et al.* 1998).

21 Based on the karyotype markers, many specimens of western form *Z. v. vivipara* have been  
 22 further identified in the western part of the Baltic region (Denmark, north of Germany, south  
 23 of Sweden), while specimens of Russian form could be found in its eastern part (Estonia,  
 24 north-west of Russia, south-east of Finland). Therefore, it was predicted that the southern  
 25 Baltic Sea is a zone of secondary contact between these two chromosomal forms  
 26 (Kupriyanova 1997).

27 In previous chromosomal studies, both the western and the Russian form of *Z. vivipara* have  
 28 been identified on the limited territory of this seashore, namely the Kaliningrad oblast  
 29 [Königsberger Gebiet] in western Russia (Kupriyanova *et al.* 2007, Kupriyanova &  
 30 Melashchenko 2011). Therefore, intraspecific karyotype diversity in northeastern Europe has  
 31 been confirmed and a zone of secondary contact between these forms localized. In addition,  
 32 the previous data by Kupriyanova & Melashchenko (2011) predicts more intraspecific

1 karyotype diversity and several new zones of secondary contact in Kaliningrad oblast and in  
2 other parts of the southern Baltic Sea seashore. This has been confirmed by a karyological  
3 study of *Z. vivipara* where both forms were discovered in Poland for the first time in 2012 by  
4 Kupriyanova and Böhme (2012) (see Fig. 1). Moreover, two new zones of their secondary  
5 contact with allopatric and in one case with a parapatric distribution in Kaliningrad oblast  
6 were found in 2014 by Kupriyanova and Melashchenko (2015).

7 Thus, the geographically distinct distribution of both forms has been demonstrated and the  
8 border of their distribution area in this part of northeastern Europe has been verified. The  
9 previous data sets suggest that during the postglacial time, populations of *Z. vivipara*  
10 belonging to the western form of *Z. v. vivipara* have been re-colonizing the Kaliningrad  
11 region from the west and south-west, and those belonging to the Russian form of *Z. v.*  
12 *vivipara* from the east and south-east. Moreover, the previous data enables us to predict  
13 karyotype diversity of *Z. vivipara* and some new zone(s) of secondary contact between the  
14 two forms in other parts of the Baltic basin as well as a trend of re-colonization of the Baltic  
15 region by *Z. vivipara* during the post-glacial period (Kupriynova & Melashchenko 2011,  
16 2014, Kupriyanova & Böhme 2012).

17 To test these predictions, we focused on the diagnostics of morphologically uniform  
18 specimens of *Z. vivipara* from populations along the eastern and northern sides of Gulf of  
19 Bothnia. We collected 33 specimens of *Z. vivipara*, obtained chromosomes and studied  
20 several previously listed karyotype markers to evaluate the karyotype diversity of *Z. vivipara*  
21 on the eastern and northern coasts of the Baltic Sea. The data allowed us to 1) define the  
22 karyotype of *Z. vivipara* from central and western parts of Finland as well as from a southern  
23 part of northern Finland; 2) identify the specimens; 3) verify a border of distribution of  
24 different forms of *Z. vivipara* on studied regions and 4) test a hypothesis of re-colonization by  
25 specimens of *Z. vivipara* of this part of Fennoscandia.

## 26 **Materials and Methods**

27 25 specimens from nine geographically distinct localities from central, western and northern  
28 Finland were collected and analyzed in May – June 2011 and 2012 (Table 2; Fig. 1). In  
29 addition, we analyzed eight individuals (6 females and 2 males) from an enclosure population  
30 originating from several natural populations in central Finland and located at Konnevesi  
31 Research Station of the University of Jyväskylä (locality 1 in Table 2).

1 The chromosomes were obtained according to the scraping and air-drying method from  
2 intestinal epithelial and lung cells as well as from the germinal lamina (i.e. the ovarian area  
3 where the earliest stages of oogenesis occur) with using 0.05 % colchicines (Odierna *et al.*,  
4 1993). In a subset of the samples, we used a different method where metaphase chromosomes  
5 were prepared from whole blood and a short term leucocyte culture in Kreavital Lymphocyte  
6 Karyotyping Medium with an addition of 0.1 ml 0.1 % phytohaemagglutinin M (Sigma-  
7 Aldrich) per 3.5 ml culture for 24 – 48 h and of 0.1 ml 0.002 % colchicine for 30 min  
8 (modification of the method of Moritz 1984, 1987). The slides were stained for 10 min with a  
9 5 % Giemsa solution in pH 7 phosphate buffer. Metaphase plates suitable for chromosome  
10 analysis were obtained from all samples studied.

## 11 **Results and discussion**

12 Chromosomal analysis showed that females of *Z. vivipara* from geographically separate  
13 localities 1-10 (Table 2; Fig. 1) have  $2n = 35:34$  acrocentric (A) macrochromosomes and one  
14 acrocentric macrochromosome (A), with short arms at rare metaphase plates, sometimes  
15 close to subtelocentric (A/ST). Acrocentric macrochromosome (A) is well known as W sex  
16 chromosome to *Z. vivipara* (Fig. 2 a – h). Therefore, chromosomal analysis identified these  
17 specimens as the Russian form of *Z. v. vivipara*.

18 A limited number of specimens (2 - 4 from each locality) does not allow us to assess inter-  
19 population or intra-population chromosomal variability (mosaics, polymorphism etc.).  
20 Nevertheless, we found for the first time that specimens of the Russian form inhabit the  
21 eastern and northern coast of the Baltic Sea (Fig. 1). The data indicates that the Russian form  
22 lives in many regions of Finland and in the southern part of northern Sweden. However, an  
23 earlier study has identified a western form in the southern and eastern parts of Sweden  
24 (Göteborg and Uppsala regions) (Kupriyanova *et al.* 1995). Moreover, according to  
25 molecular (Mt haplotype) data by Surget-Groba *et al.* (2006) two specimens of a western  
26 haplotype (VB haplogroup) have been identified from the south and the central-eastern  
27 Sweden (Runsten and Umeå localities) and positioned to western viviparous clade (clade E).  
28 At the same time, a specimen west to the border between Sweden and Finland (Kiruna  
29 locality) was identified as an eastern haplotype (VU haplogroup) and positioned to an eastern  
30 viviparous clade (clade D).

1 Comparison of molecular phylogenetic trees with karyotype characteristics of different  
2 chromosomal forms has demonstrated a good correlation between molecular and  
3 chromosomal data (see Kupriyanova 2004, 2013, Kupriyanova *et al.* 2006). Therefore, it is  
4 clear that chromosomal data supports the presence of main branches of the molecular trees of  
5 *Z. vivipara* and shows that their appearance is marked by the chromosomal rearrangements  
6 with the forming of several subspecies and separate chromosomal forms. From all these data,  
7 we may with confidence say that the specimens belonging to western viviparous clade (clade  
8 E, VB haplogroup) should be identified as western chromosomal form of *Z. v. vivipara*  
9 whereas those belonging to eastern viviparous clade (clade D, VU haplogroup) should be  
10 identifies as Russian chromosomal form. Thus all these data points to the direction that the  
11 Russian chromosomal form of the subspecies *Z. v. vivipara* inhabits north-eastern part of  
12 Sweden.

13 To conclude, our chromosomal data demonstrates that 1) the Russian form of *Z. v. vivipara*  
14 inhabits the central, western and southern parts of northern Finland; 2) these regions are not  
15 characterized by karyotype diversity of *Z. vivipara*; 3) diversity and a zone of secondary  
16 contact between two chromosomal forms of *Z. v. vivipara* may be predicted for other  
17 northern parts of Finland as well as for those of Sweden and Norway; 4) the border of the  
18 distribution area of the Russian form is located in the north-western and northern Baltic Sea  
19 seashore (in the northern parts of Sweden, Finland and/or Norway) and 5) the hypothesis of  
20 re-colonization of the area of the Baltic Sea by *Z. vivipara* is supported.

21 Regarding the re-colonization hypothesis, our results suggest that during the postglacial time,  
22 populations of *Z. vivipara* belonging to the western form of *Z. v. vivipara* came to the area  
23 from south and south-west whereas those belonging to the Russian form of *Z. v. vivipara*  
24 moved into Fennoscandia from east and south-east. Additionally, the present data is  
25 consistent with the previous chromosomal results on the presence of the Russian form both in  
26 the southern and eastern parts of Finland and in a neighbouring Karelian Russia territory,  
27 near the border between Russia and Finland (Kupriyanova *et al.* 2005).

28 *Z. vivipara* is distributed all over Finland (e.g. Terhivuo 1993) and it should be stressed that  
29 our results regarding the chromosomal characteristics correlate with the data of Voipio (1961,  
30 1968, 1969, 1992) reporting variability in the shield pattern of the *Z. vivipara* in  
31 Fennoscandia. Based on these patters, Voipio pointed out that *Z. vivipara* populations in  
32 southern and central Sweden include specimens with western and central European type of

1 shields patterns whereas specimens in northern Sweden, Finland and Russia (north of the 62°  
2 N) show patterns of the eastern type.

3 To summarize, we emphasize that the identification of specimens of *Z. vivipara* based on  
4 their morphology is very difficult and misidentifications may occur. Our results demonstrate  
5 the value of chromosome diagnostic of *Z. vivipara* from the geographically distant localities  
6 of Fennoscandia. Furthermore, intensive chromosomal studies of specimens from the areas of  
7 Finland, in particular those from its northern part, could show a presence of both  
8 chromosomal forms of the subspecies *Z. v. vivipara*. New chromosomal data could also give  
9 additional information about the karyotype diversity of *Z. vivipara* throughout the area of  
10 Fennoscandia and clarify the border of the distribution of the two forms in the region.

11 From the literature and the data obtained in this study, we predict that the western and  
12 Russian chromosomal forms of *Z. v. vivipara* occupy the northern and northwestern regions  
13 of Fennoscandia. However, more data from a wider set of localities, in particular from the  
14 northern part of Finland, Sweden and Norway, is needed to confirm this prediction as any  
15 chromosomal data for these territories is still missing. We would also like to stress that a  
16 more detailed study of a secondary contact zone and its characteristic (allopatry, sympatry,  
17 parapatry and/or hybrid zone) is needed as *Z. vivipara* may represent a group of cryptic taxa.  
18 The taxonomic status of the chromosomal forms *Z. v. vivipara* is still unclear and under  
19 discussion in the literature (Kupriyanova 2004, Kupriyanova & Melashchenko 2011). A  
20 combination of different types of approaches (chromosomal, molecular, morphometric, life-  
21 history and behavioral) would be helpful in evaluating the biodiversity and conservation  
22 issues of these unique populations of *Z. vivipara*.

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28

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1 **Table 1.** Karyotype characteristics of subspecies and different forms of *Zootoca vivipara*  
 2 (Lichtenstein 1823) and their distribution in Europe (Size of sex chromosomes: m =  
 3 microchromosome, M = macrochromosome; Morphology of sex chromosomes a/A =  
 4 acrocentric, ST = subtelocentric, SV = submetacentric; Mode of reproduction: O = oviparous,  
 5 V = viviparous). A modification of Table 1. in Kupriyanova (2013).

N/N	2n ♂/♀	Sex chromosomes Size/System/Morphology			Mode of reproduction	Localities	Species, subspecies, chromosomal forms
<b>The first group of karyotype</b>							
1.	36A/36: 35A + 1a	m	Zw	a	O	Central, South- western Europe	<i>Z. vivipara</i> , now <i>Z. v. carniolica</i>
2.	36A/36: 35A + 1a	m	Zw	a	V	Central Europe	<i>Z. vivipara</i> , now <i>Z. v. vivipara</i> Hungarian form
<b>The second group of karyotype</b>							
3.	36A/35: 34A + 1A (A/ST)	M	Z <sub>1</sub> Z <sub>2</sub> W	A, A/ST	O	Western Europe, the Pyrenees	<i>Z. v. vivipara</i> Pyrenean form, now <i>Z. v.</i> <i>louislantzi</i>
4.	36A/35: 34A + 1ST (A/ST)	M	Z <sub>1</sub> Z <sub>2</sub> W	ST, A/ST	V	Central Europe	<i>Z. vivipara</i> , now <i>Z. v. vivipara</i> Austrian form; <i>Z. v. pannonica?</i>
5.	36A/35: 34A + 1A	M	Z <sub>1</sub> Z <sub>2</sub> W	A	V	Asia, Eastern Europe, Baltic region	<i>Z. vivipara</i> , now <i>Z. v. vivipara</i> Russian form
6.	36A/35: 34A + 1SV	M	Z <sub>1</sub> Z <sub>2</sub> W	SV	V	Western, Central Europe, Baltic region	<i>Z. vivipara</i> , now <i>Z. v. vivipara</i> Western form

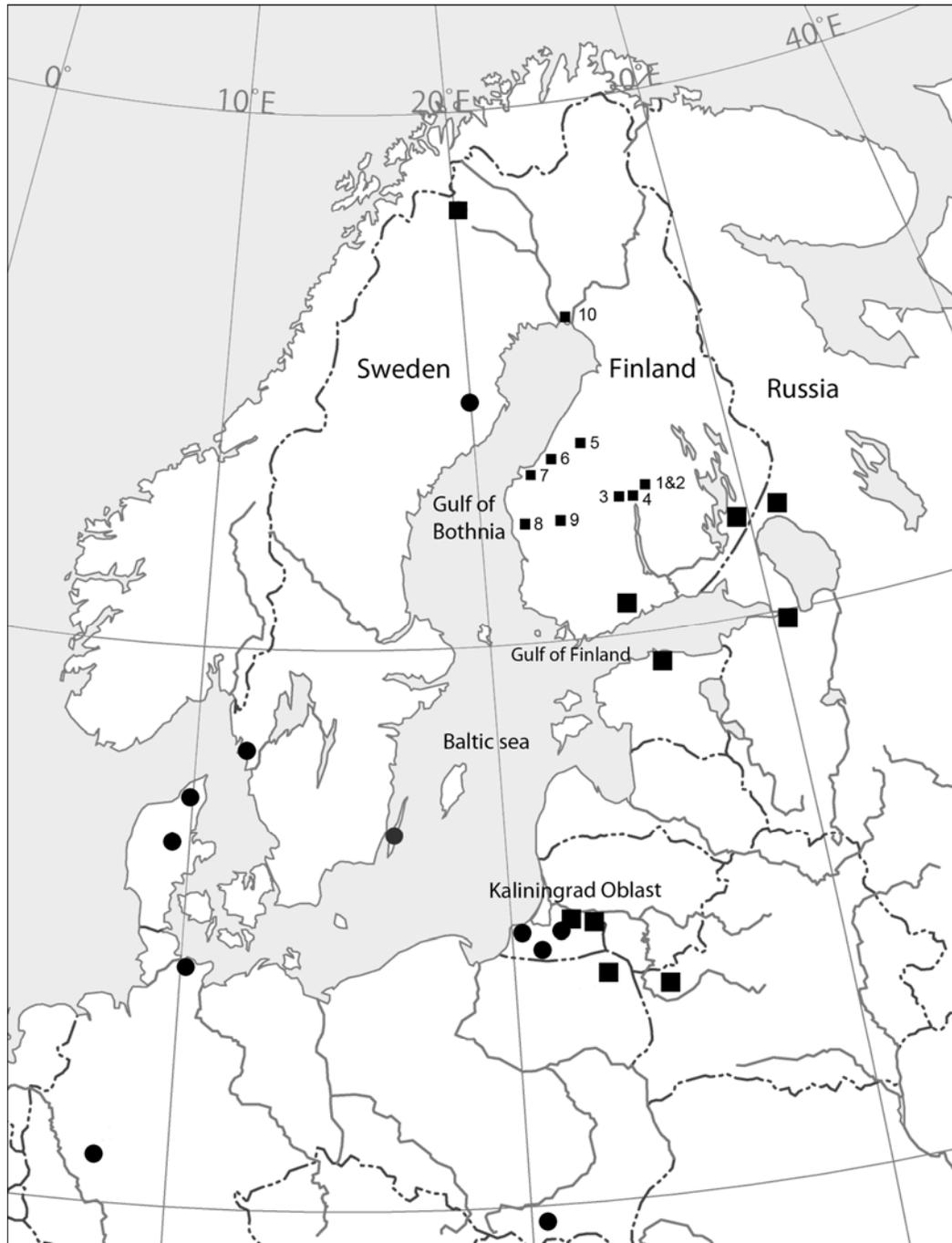
6

1 **Table 2.** Number and origin of specimens of *Zootoca vivipara* analyzed in this study.

Locality number	Number of female specimens	Number of male specimens	Locality	
			<b>Central Finland</b>	
1	6	2	Central Finland (enclosure population)	-
2	2	0	Konnevesi	62°36.964'N 26°20.746'E
3	5	0	Vesanka	62°30.277'N 25°558'E
4	4	0	Muurame	62°05.159'N 25°36.353'E
			<b>Western Finland</b>	
5	2	0	Alaveteli	63°42.351'N 23°17.784'E
6	4	0	Kortesjärvi	63°18.836'N 23°14.682'E
7	2	0	Vaasa	63°6.943'N 22°2.803'E
8	2	0	Närpiö	62°31.892'N 21°15.404'E
9	2	0	Kauhajoki	62°18.166'N 22°33.502'E
			<b>Northern Finland (north of the Baltic coast)</b>	
10	2	0	Tornio	65°54.093'N 24°27.957'E

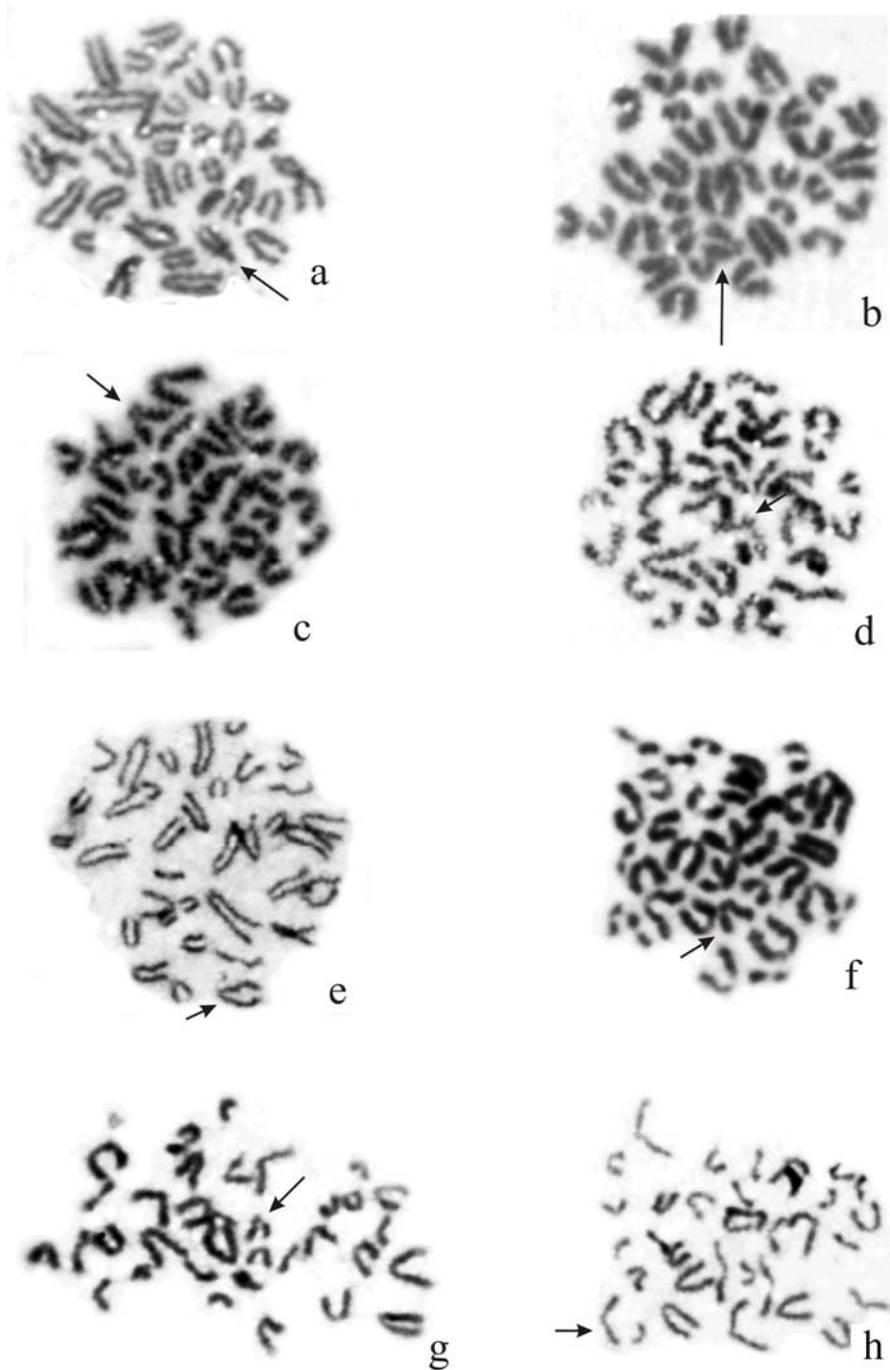
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- 1 **Figure 1.** The locations sampled in this study presented as the numbered squares that  
 2 match the coordinates in table 2. The map also shows wider distributions of Russian (■) and  
 3 western (●) forms.



4

1 **Figure 2.** Giemsa stained metaphase plates of females of *Zootoca vivipara* from: 1. Central  
 2 Finland (localities 1, 2, 4), 2. Western Finland (localities 5, 6, 7, 9) and 3. Northern Finland  
 3 (locality 10). Localities refer to table 2.  $2n = 34A + 1A$  (A/ST). Arrows point to acrocentric-  
 4 (A) (e, f, h) and acro-/subtelocentric (A/ST) (a, b, c, d, g) W sex chromosomes. According to  
 5 karyotype markers these females belong to the Russian form of *Z. v. vivipara*.



6