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PHYLOGEOGRAPHY AND SYSTEMATICS OF *Lacerta agilis* BASED ON MITOCHONDRIAL CYTOCHROME *B* GENE SEQUENCES: FIRST RESULTS

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The phylogeny and phylogeography of *Lacerta agilis* was inferred from the nucleotide sequences of the mitochondrial cytochrome *b* gene. *Lacerta agilis* (Sauria: Lacertidae) is a widespread species composed of several described subspecies. Fifty specimens of *Lacerta agilis* were studied from different locations throughout the distribution range. Two species, *Lacerta media* and *Lacerta praticola* were used as outgroup taxa. Within *Lacerta agilis* three genetically distinct groups were found. The first monophyletic group (morphologically treated as *L. a. brevicaudata* and *L. a. exigua* subspecies) includes specimens from a large part of European Russia, Caucasus, and Kazakhstan. The second monophyletic group comprises two subgroups of *L. a. agilis* specimens from Denmark, Germany, Czech Republic and *L. a. chersonensis* from Northwest Russia. Specimens of *L. a. boemica* from Northern Caucasus form the separate group, which appears to be genetically distinct from other groups of *Lacerta agilis.* Some hypotheses on the history of the distribution of *Lacerta agilis* are proposed considering the molecular data.

Key Words: Lizards, Lacertidae, *Lacerta agilis*, phylogeography, systematics, mitochondrial DNA, cytochrome *b.*

INTRODUCTION

For a long time all the taxa, including subspecies, have been described based on certain morphological and anatomical characters. Since molecular methods were available for studies on systematics and phylogeny of different groups of organisms, it has been possible to refine former subspecies divisions. This is especially interesting for studying species that have wide distribution ranges and thus present a high level of phenotypic variations, which sometimes lead to the multiplication of artificial descriptions of many different forms/morphotypes treated as subspecies.

The sand lizard, *Lacerta agilis*, is a widespread palearctic species. Its distribution range extends from the British islands in the west to Northwest China and the Lake Baykal in the east (Fig. 1). This eurytopic species occupies a variety of different habitats from open steppe to fields, hedgerows and woodland (Tertyshnikov et al., 1976). In a wood zone *Lacerta agilis* extends from subtropical forests in the south to middle taiga in the north. Western populations of this species prefer moister habitats than eastern ones, and eastern and southern populations occur at high elevations up to 2200 m (Szczerbak et al., 1976). *Lacerta agilis* displays a high level of variability. More than twenty subspecies and varieties of *Lacerta agilis* were described but most of them have been synonymized (Darevsky et al., 1976).

At present *Lacerta agilis* comprises 9 subspecies (Bischoff, 1988) based on color pattern and scalation. Two main groups of subspecies are considered within *Lacerta agilis* — western and eastern. The western group is represented by three subspecies, *L. a. agilis*, *L. a. argus*, *L. a. bosnica*, and *L. a. chersonensis*. The nominotypical subspecies, *L. a. agilis* occupies Western Europe and has a large intergradation zone with *L. a. argus* inhabiting Central Europe. *L. a. bosnica* occurs in the Balkans. *L. a. chersonensis* inhabits re-

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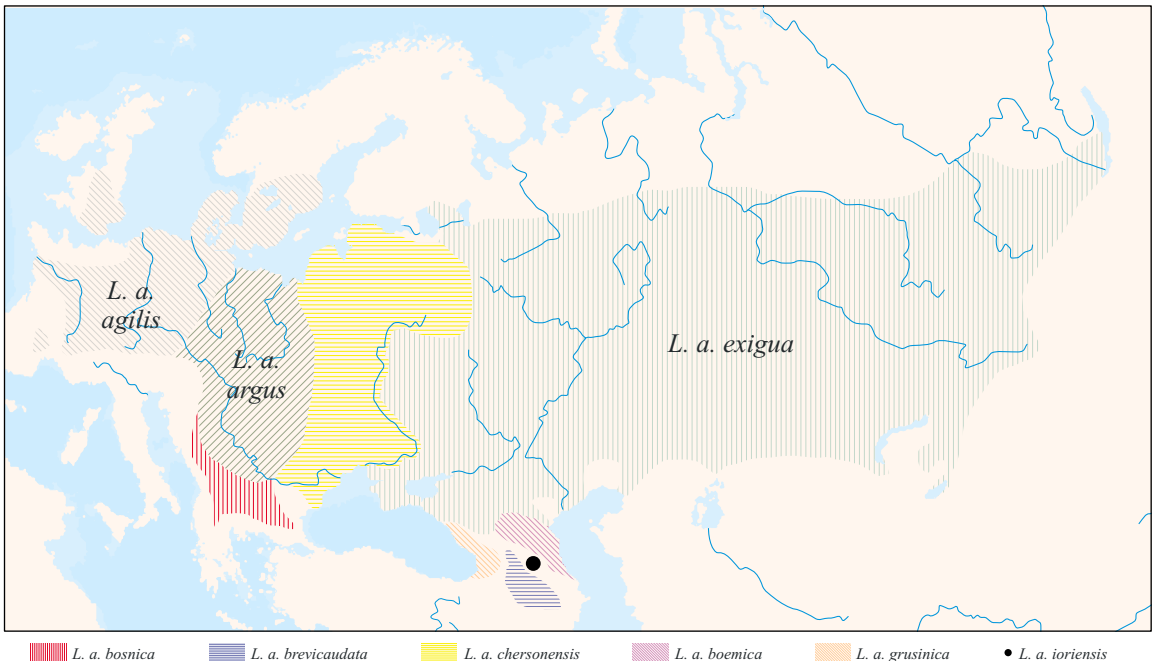

Fig. 1. Distribution of the sand lizard, *Lacerta agilis*, and ranges of subspecies.

gions in Europe from the Carpathian Mountains eastward and has a zone of intergradation with *L. a. exigua*, which together with four Caucasian subspecies (*L. a. grusinica*, *L. a. boemica*, *L. a. brevicaudata*, *L. a. ioriensis*) forms the eastern group (Fig. 1). Sukhov (1948) suggested that the eastern and western groups should be considered as separate species — *L. agilis* and *L. exigua*, which would not have an intergradation zone. The wide zone of intergradation has since been discovered and species status of the western and eastern forms has been refuted. Morphological differences between these two general groups are probably caused by geographic separation due to range restrictions in climatically unfavorable periods. Populations of *Lacerta agilis* from Altay and Eastern Kazakhstan and populations from Mongol Altay and Semipalatinsk region were described as separate subspecies, *L. a. altaica* (Kastschenko, 1898) and *L. a. kurtuana* (Kastschenko, 1909), respectively. The validity of these subspecies is still under discussion (Ananjeva et al., 1997), although some authors have confirmed the subspecies status of these forms (Čugunov, 1911; Yablokov, 1981a; Munkhbayar et al., 1998) based on morphological characters and coloration. Two Caucasian subspecies (*L. a. boemica* and *L. a. grusinica*) display some archaic characters and thus appear to be close to the hypothetical ancestral form (Darevsky at al., 1976; Roytberg, 1982, 1986). These subspecies have relict distribution ranges in Western Transcaucasia and Northeastern Caucasus (Darevsky et al., 1976).

The goals of this study are:

— to clarify the status of most subspecies;

— to infer general phylogeographical hypotheses and especially confirm the Caucasian origin of *Lacerta agilis*.

In this attempt to improve our knowledge about relationships among *Lacerta agilis* species a phylogenetic analysis was conducted using DNA sequences derived from a section of the mitochondrial protein encoding gene — cytochrome *b*.

MATERIAL AND METHODS

Sample Information

DNA was extracted from tissue samples deposited in the following collections: ZISP (Zoological Institute, St. Petersburg, Russia) and HLMD (Hessisches Landesmuseum, Darmstadt, Germany) (see *Appendix*).

Blood, liver, and muscle tissue were obtained from 50 samples of *Lacerta agilis* (five from nine

currently recognized subspecies: *L. a. agilis*, *L. a. boemica*, *L. a. brevicaudata*, *L. a. chersonensis*, and *L. a. exigua*) from localities spread over the species distribution area (Fig. 2). Samples of two species of the genus *Lacerta* were used as outgroup taxa, *Lacerta media* and *Lacerta praticola.*

Laboratory Protocols

DNA was extracted from liver and muscle tissue as well as from blood following standard proteinase *k* and phenol chlorophorm protocols (Sambrook et al., 1989). 1 – 6 µl Aliquots of isolated DNA were subjected to Polymerase Chain Reaction (PCR) using a primer pair which amplified an appoximately 1140 bp fragment of mtDNA containing a part of the cytochrome *b* gene and $tRNA^{threonine}$. The light and heavy strand primers used were modified versions of those given by Kocher (Kocher et al., 1989): mtA-new (L 14995) — 5′-TCCCAGCCCCATCCAACATCTCAGCATGATGAAACTTCG-3′ amd mtF-new (H 16060) — 5′-AGGGTGGAGTCTTCAGTTTTTGGTTTACAAGACCAATG-3′. Amplification conditions were as follows: after an initial denaturation step of 94°C for 300 sec, 31 cycles followed with a denaturation at 94°C for 45 sec, annealing at 40 – 47°C for 45 sec, and extension at 70°C for 120 sec. Cycle sequencing reactions were run with a two step program, 15 cycles followed with denaturation at 94°C for 45 sec, annealing at 47 – 53°C for 45 sec, extension at 70°C for 60 sec and 15 cycles of denaturation at 94°C for 45 sec, and extension at 60°C for 60 sec. Three sequencing primers were used (the light strand primer smtA (L 14995) — 5′-CAACATCTCAGCATGATGAAACTTCG-3′ and two heavy strand primers: smt-F (H 16060) — 5′-TCAGTTTTTGGTTTACAAGACCAATG-3′ and mt-B2 (H 15298) — 5′-GCCCAGAAkGATATTTGTCCTCA-3′ to obtain sequences of both strands using an automatic sequenser (ALF Express). Sequencing was performed for 5 – 11 h depending on the length of the sequenced fragment. Each sequence was verified by sequencing from heavy and light strand primers with large overlapping of the segments and from different PCR amplification products.

Phylogenetic Analysis

The sequences were aligned manually by pairwise comparison of each pair of taxa.

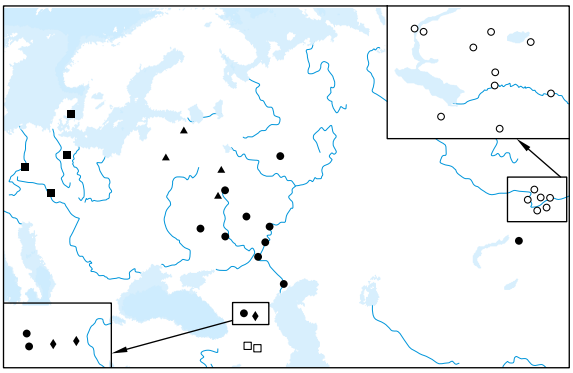

Fig. 2. Localities of analyzed samples of *Lacerta agilis*.

We prefer to compare different methods of analysis to reconstruct phylogenetic relationships, such as MP (Maximum Parsimony) and ML (Maximum Likelihood). In our experience, data sets based on a balanced sampling usually produce trees of almost congruent topology. If this topology is supported by these independent methods which employ different mathematical algorithms, we are confident in the results; ambiguous bifurcation rather indicate that the topology can not be resolved with the present data set.

All analyses were performed on a PC Pentium III 500 MHz with PAUP* software (version 4b2a), written by David L. Swofford (2000). All heuristic searches for optimal trees were carried out by TBR (Tree-bisection-reconnection) branch swapping with option MULPARS in effect.

Parsimony-based analyses. Starting trees were obtained by stepwise addition due to random addition of taxa does not lead to alternative, more parsimonious trees. For each bootstrap replicate, 10 heuristic searches were performed with random addition of taxa.

Distance-(minimum evolution) based analyses. A log-determinant (LogDet) distance measure was chosen since this transformation is robust and involves no assumptions on rates of substitution. General time-reversible (GTR) algorithm leads to exactly the same topology (this method involves assumptions on rates of substitution). Use of starting trees obtained by either neighbor-joining or random addition resulted in identical final topologies.

Analyses based on Maximum Likelihood. Starting trees were obtained by parsimony (see above), parameters were estimated, a new optimal tree was sought and the process was repeated until a stable topology was achieved (different starting trees led to the same final topology). Rates for variable sites were always assumed to follow a Gamma distribution, and both the shape of this distribution and the fraction of invariable sites were estimated. These settings correspond to GTR model with a rate of heterogeneity (Yang, 1994).

RESULTS

A 897 bp fragment of cytochrome *b* from 50 samples of five subspecies of *Lacerta agilis* from different localities and one sample each of *Lacerta media* and *Lacerta praticola* were obtained and used for analysis.

Mitochondrial Sequence Divergence

None of the sequences contained premature stop codons, and therefore do not appear to be nuclear copy pseudogenes (Zang and Hewitt, 1996). The strong bias against guanine on the light strand found in all analyzed sequences (A = 26.5 – 27.8%, C = 28.3 – 29.4%, T = 30.8 – 31.9%, G = 12.0 – 13.4%) is characteristic of the mitochondrial genome but not the nuclear genome. This bias against G provides a justification for using the GTR model of substitutions in our ML calculation.

Among the 897 bp of cytochrome *b* gene sequenced, 132 positions were variable and 98 were phylogenetically informative. As expected, the most variable sites occur in the third codon position ($n = 98$). Less variation occurs at the first position ($n = 21$) and a little variation was observed at the second position ($n = 11$). Transitions exceeded transversions at low levels of sequence divergence, which has been shown in previous studies on animal mitochondrial DNA (Brown et al., 1982; Hedges et al., 1991; Fuller et al., 1998). Our results indicate that transitions are three times more common than transversions in all analyzed sequences, 21 and 7.18 in average, respectively. The transition/transversion ratio among the samples of *Lacerta agilis* varies from 13 to 1, although the mean is 2.8.

Pairwise genetic distances (Logdet and GTR) among samples of *Lacerta agilis* range from zero to 8% for nucleotide sequences of the cytochrome *b* (in average 3.5%). When tested, genetic distances within different individuals of the same populations were very small varying from zero to 0.5%. Geographically close populations had little or no divergences. Genetic differences between *Lacerta agilis* samples and species used as outgroups average 13.5% and 26.4% with *Lacerta media* and *Lacerta praticola*, respectively.

Phylogenetic Relationships

As outgroup taxa we chose one species of *Lacerta*, *Lacerta media*, which is considered as relatively close based on morphological characters (Arnold, 1973; Schmidtler, 1986) and another species of the genus *Lacerta*, *Lacerta praticola*, which is considered to be more distant.

In all applied methods of phylogenetic reconstruction *Lacerta agilis* appears to be clearly monophyletic assemblage (bootstrap value of 100%).

Maximum parsimony and maximum likelihood analysis produced largely similar trees (Figs. 3, 4). Note that distance trees, independently of the algorithms used (Logdet, GTR), are fully identical with MP ones (data not shown).

Within the *Lacerta agilis* clade three major groups were clearly defined by all methods (Figs. 3, 4). The first one, eastern group (EG), comprises most of the analyzed samples and includes specimens from a large part of European Russia, Caucasus and Kazakhstan, represented by *L. a. brevicaudata* and *L. a. exigua* subspecies. The second, western group (WG) links the sand lizards from Europe and Northwestern Russia and is represented by *L. a. agilis* and *L. a. chersonensis* subspecies. The final group, "*boemica*" group (BG), is represented by three specimens from Central Caucasus, morphologically treated as *Lacerta agilis boemica* subspecies.

Monophyly of the three detected groups is well supported. However, only weak cladogenetic resolution was obtained within these groups as indicated by short internal branches and differing root position. In all the reconstructions used, there is only one ambiguity of topology. The BG tended to take up a basal position within *Lacerta agilis* in maximum parsimony reconstruction (Fig. 4), but in maximum likelihood tree it forms a clade together with the EG, while the WG turns to be the basal group (Fig. 3). However, by removing putative homoplasic characters (variable third codon position) and analyzing our data set (data not shown), it is clear that the BG group is dis-

tinct from the main lineage of *Lacerta agilis*. Six variable positions on first and second codon positions may be considered as "signatures" of the BG group and separate this group from other analyzed specimens of *Lacerta agilis* (positions: 253 Tv, 298 Tr, 589 Tr, 623 Tr, 649 Tr, and 784 Tr; Tv, transversion, Tr, transition).

According to our molecular data the EG shows a high genetic similarity with genetic divergence between populations less than 0.1%. WG divided into two subgroups that are genetically and geographically distinct. One subgroup is represented by specimens of *Lacerta a. agilis* from Denmark and Western Germany and specimens of *Lacerta agilis* ssp. from Eastern Germany and Check Republic, another is formed by specimens of *Lacerta a. chersonensis*, from Western Russia (Figs. 3, 4). The genetic distances between these two subgroups average 3.5%.

DISCUSSION

Phylogenetic Relationships between Subspecies of *Lacerta agilis* and Taxonomic Implications

According to our molecular analysis the *Lacerta agilis* clade is clearly distinct from two outgroups used and formed the separate clade in all applied analysis with bootstrap value of 100%. Therefore *Lacerta agilis* should be considered as a monophyletic species.

The results indicate that among analyzed populations and subspecies of *Lacerta agilis* three groups are clearly recognized (eastern, western and "*boemica*").

L. a. boemica takes a genetically distinct position among *Lacerta agilis* subspecies. Genetic distances of *L. a. boemica* to other groups average 7.65%. In the MP tree this group turned to be basal for other *Lacerta agilis* groups (Fig. 4), although in ML analysis it formed a cluster together with the EG (Fig. 3). According to the treatment of our data set two of three approaches show that this BG is placed in a basal position (MP and distance method). It is clear that the average of differences (transitions plus transversions) between EG and WG is less than that between BG and both, WG and EG. But checking the number of transitions and transversions, we realized that the differences between BG and EG are caused mostly by transitions and that the fewer differences between WG and EG are caused mostly by transversions. The GTR model in ML reconstruction that we selected takes into account the bias against guanine, but on the other hand it heavily overweights transversions. This is why we assume that BG is the basal group for all analyzed samples. Sukhov (1929) even referred to this subspecies as a separate species based on morphology. According to Yablokov (1981a), eastern populations of *Lacerta agilis* (*L. a. boemica*) of the northern slope of the Caucasus are clearly distinguished by morphological characters and coloration, which also confirmed a distinct position of this subspecies. Summarizing the data we suggest that *L. a. boemica* should be considered as a subspecies of *Lacerta agilis*.

Samples of *Lacerta agilis* from the eastern part of the distribution range (Central and South Russia, Central Caucasus, Armenia, Eastern Kazakhstan) (Fig. 2) form a separate genetically homogeneous group (EG) mostly represented by *L. a. exigua* (Fig. 4). Lizards from Altay and Eastern Kazakhstan and from Mongol Altay and Semipalatinsk region described as separate subspecies, *L. a. altaica* (Kastschenko, 1898) and *L. a. kurtuana* (Kastschenko, 1909) have slight morphological differences. 11 analyzed samples from Zaysan basin, which could be inhabited either by *L. a. altaica* or *L. a. kurtuana* or *L. a. exigua* subspecies, are genetically identical or show minor differences from analyzed samples of the EG (Fig. 4). Thus according to our molecular data the Zaysan region is inhabited by *Lacerta agilis exigua* subspecies. Specimens from Armenia are included in the EG of *Lacerta agilis* (Figs. 3, 4). Since 1958 the sand lizards from Armenia have been considered as a separate subspecies, *L. a. brevicaudata* (Peters, 1958). This Caucasian subspecies originates in the Armenian upland. At present it is represented only by relict populations. Despite a large morphological difference (Muskhelishvili, 1967) *Lacerta a. brevicaudata* appears to be genetically very close to its relative form, *L. a. exigua*. Our molecular results are in an agreement with Sukhov (1948), who also assigned the sand lizards from Armenia to *L. a. exigua*. Long isolation, relict range and some adaptive characters to specific conditions of the high mountain area could have caused some phenotypic differences between *L. a. brevicaudata* and *L. a. exigua* forms. Further studies are needed to clarify the taxonomical status of *L. a. brevicaudata* subspecies.

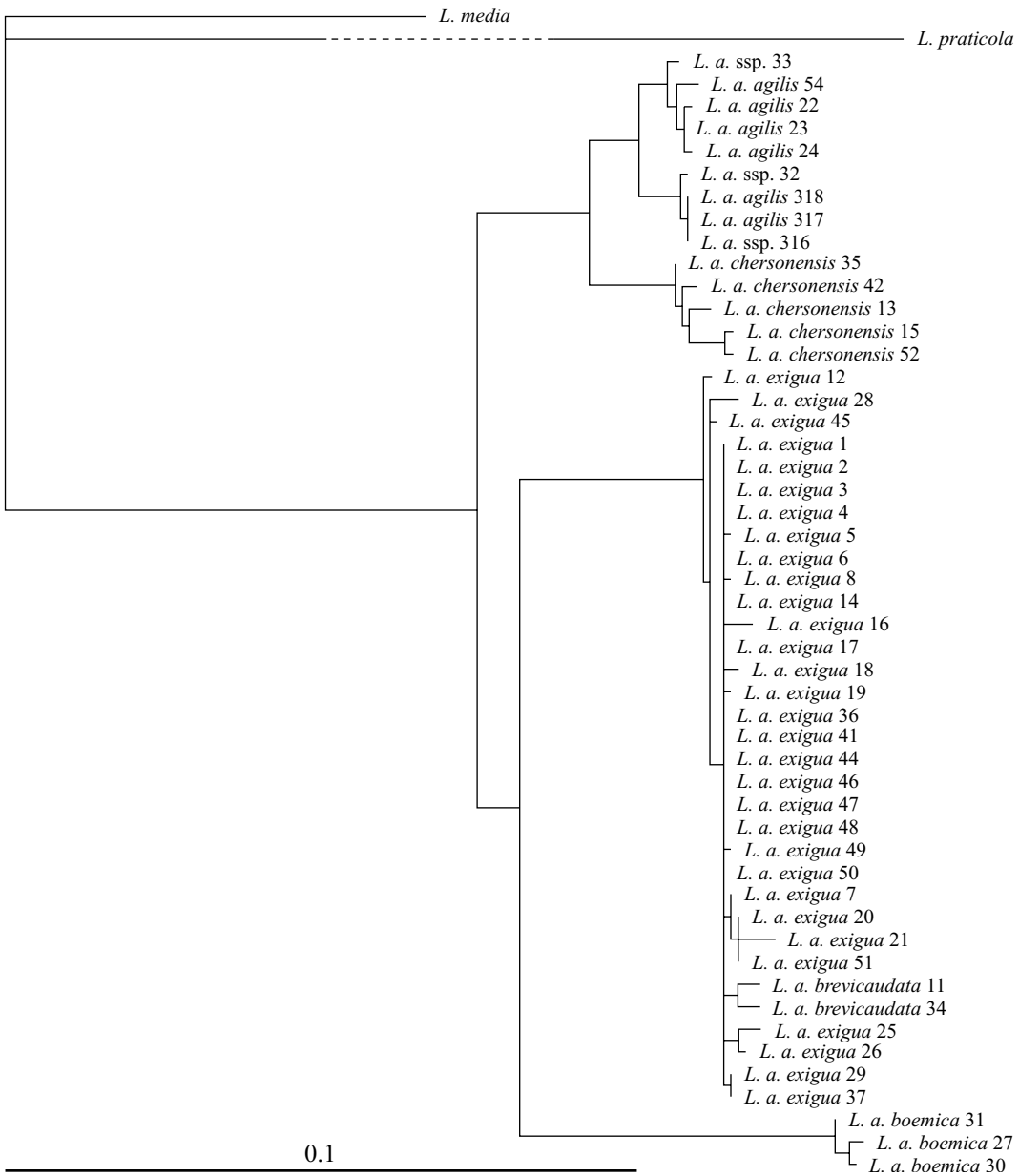

Fig. 3. Maximum likelihood (ML) tree for *Lacerta agilis* based on the analysis of mtDNA cytochrome *b* sequence data. Estimated fraction of invariable sites is 0.327, which rates of evolution were assumed to follow a gamma distribution with shape parameter 0.76 (4 rate categories represented by mean), rates of substitution were assumed to obey a six parameter, General Time-Reversible model, with rAC = 2.94; rAG = 13.12; rAT = 1.71; rCG = 2.04; rCT = 18.18; rGT = 1.

The WG comprises two subgroups (Figs. 3, 4). The first includes lizards from Western Germany and Denmark treated as a nomenotypic subspecies, *L. a. agilis* and *L. a.* ssp. from Eastern Germany and Czech Republic. The territory including the localities of our samples from Eastern Germany and Czech Republic are considered by Bischoff (1988) as a border between the distribution ranges of *L. a. agilis* and *L. a. argus*. These two forms are intermixed in the phylogenetic trees and show no or little genetic differences (Figs. 3, 4). Thus according to our molecular data, the sand lizards from Eastern Germany and

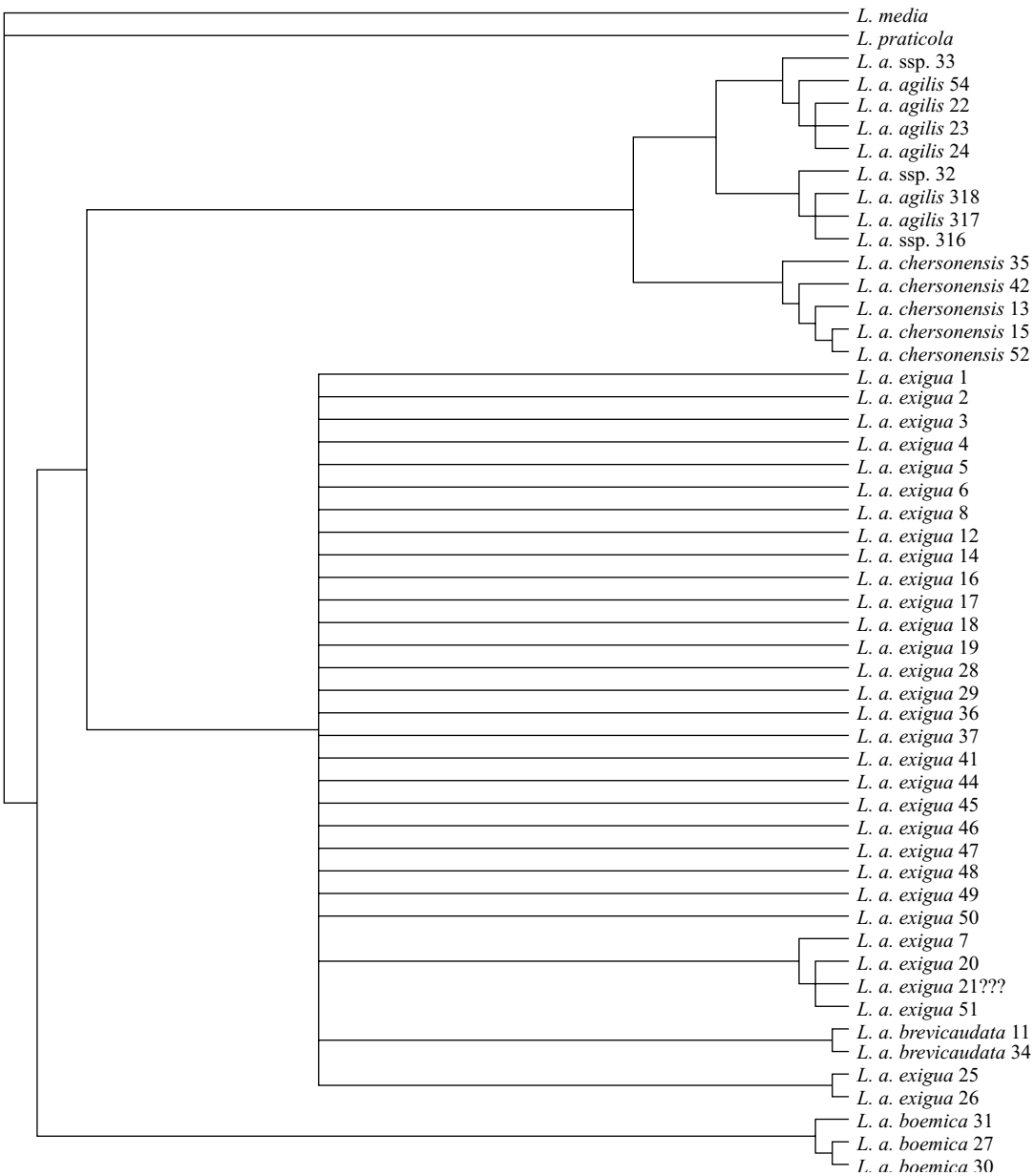

Fig. 4. A strict-rule consensus tree of 40 maximum parsimony trees depicting relationships among populations of lizards of *Lacerta agilis* derived from the analysis of cytochrome *b* sequence data. Number of parsimony-informative characters is 154; addition sequence is closest; length is 472 steps; scores of trees 1 – 40: CI = 0.75; RI = 0.88; RC = 0.66; HI = 0.37.

Czech Republic belong to *L. a. agilis* subspecies. The second subgroup of the WG is formed by specimens from Western Russia (Leningrad, Pskov, Moscow, and Tula regions). It is widely accepted that this area is occupied by *L. a. chersonensis* (Peters, 1958; Darevsky et al., 1976). Morphologically analyzed specimens combine the pholidosis characters (number of preanal rows and postnasal scale pattern) and coloration of three subspecies, *L. a. agilis*, *L. a. chersonensis*, and *L. a. exigua.* We assume that this clearly defined genetic subgroup should be considered as *L. a. chersonensis*, which occurs in the intermediate

part of the distribution range between the areas occupied by *L. a. agilis* and *L. a. exigua.* It is noteworthy that the Tula region is inhabited by two subspecies, *L. a. chersonensis* (La 52) and *L. a. exigua* (La 17) and appears to be a contact zone between them based on our molecular data (Fig. 2).

Phylogeography of *Lacerta agilis*

According to Yablokov (1981b) *Lacerta agilis* arose in the Late Miocene – Early Pliocene in the Caucasian region, supposedly an area of origin for many of present species of the genus *Lacerta*. Two presently recognized subspecies of *Lacerta agilis*, *L. a. boemica* and *L. a. grusinica*, represented by only relict populations in the Caucasian region, have some archaic morphological characters and may be closest to an ancestral form of the species. With the exception given by ML reconstruction (discussed above) *L. a. boemica* appears to be the basal group for *Lacerta agilis* species. This is also confirmed by the level of genetic divergences between BG and two other groups defined (7.6% in average).

The Caucasus was connected to the Russian platform in the Late Pliocene, which allowed the sand lizards to disperse eastward and westward throughout the Caspian lowland. Dispersing to the southwest along the Black Sea and southeast through the Iranian Plateau, *Lacerta agilis* reached Balkans in the west and the foothills of Pamiro-Altay and Tien Shan Mountains in the east, respectively. According to this scenario, the Balkan-Carpathian and Middle Asian distribution centers of *Lacerta agilis* species were formed, with the Caucasus-Middle East as primary radiation center. These areas were glacial refugia during cold period in Europe; later they became centers of postglacial recolonization. The range of *Lacerta agilis* became recurrently fragmented, probably as a result of numerous climatic oscillations and transgressions of the Caspian Sea. Colonization of the East European plain took place not less than 30 – 50 thousand years ago, while the Baltic region was occupied by *Lacerta agilis* only 10 – 12 thousand years ago (Yablokov et al., 1981b; Baranov, 1982; Gullberg et al., 1998). The Central European part of the range is more ancient and was formed not less than 100 thousand years ago (however, a north-south movement has to be assumed for Central European populations during the last glaciation). Concerning the Asian part of the range, there are two hypotheses: (1) the Southern radiation hypothesis suggests that the Asian part of the distribution was formed between two and three hundred thousand years ago (Balkhash-Zaysan refugia); (2) the North Caspian radiation hypothesis assumes that the Asian part of the distribution range is more recent; about several thousands years (Yablokov et al., 1981b; Baranov, 1982). The recent origin of the eastern part of the range of *Lacerta agilis* is prefered due to the high genetic similarity of the specimens from Eastern Kazakhstan (Balkhash-Zaysan region) to other samples of the EG (see above). This low level of divergence also leads us to support the hypothesis of the North Caspian radiation of *Lacerta agilis* (Yablokov, 1981b; Baranov, 1982). Thus *Lacerta agilis* could not remain in Balkhash-Zaisan refugia and therefore deviated forms, *L. a. altaica* and *L. a. kurtuana* probably could not evolve into subspecies status because of an insufficient time period. This is also proved by our molecular data.

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APPENDIX
Specimens Used in This Study

***Lacerta agilis agilis*:** HLMD — La 22, La 23, La 24, La 54: Germany, nearby Schriesheim, 49°29′ N 8°40′ E; HLMD — La 317, La 318: Denmark, Samsoe Island, 55°50′ N 10°36′ E.

***Lacerta agilis boemica*:** ZISP 70/20929 — La 27, ZISP 71/20929 — La 30: Russia, Caucasus, Cherek Kanjon, Blue Lakes, 43°31′ N 43°55′ E; ZISP 21034 — La 31: Russia, Caucasus, nearby Nalchik, 43°29′ N 43°36′ E.

***Lacerta agilis brevicaudata*:** ZISP 20683 — La 11: Armenia, Kotajsk distr., v. Adis, 40°17′ N 44°38′ E; ZISP 21158 — La 34: Armenia, v. Ankavan, 40°38′ N 44°29′ E.

***Lacerta agilis chersonensis*:** ZISP 20709 — La13: Russia, Pskov region, Sebezhskii district, 56°06′ N 2816 E; ZISP 21032 — La 15: Russia, 40 km N of Moscow, 56°09′ N 37°37′ E; ZISP

21102 — La 52: Russia, Tula region, v. Barsuki, 54°16′ N 37°28′ E; ZISP 20874 — La 35, La 42: Russia, Leningrad region, Luzhskii distr., 58°54′ N 29°46′ E.

***Lacerta agilis exigua*:** ZISP TS. 7 — La 12, ZISP 20924 — La 36: Russia, nearby Volgograd, 48°44′ N 44°27′ E; ZISP 21029 — La 17: Russia, 30 km NE of Tula, 54°27′ N 38°04′ E; ZISP 21031 — La 16: Russia, Tambov region, Khoper river, 52°14′ N 42°26′ E; ZISP 21033 — La 18: Russia, Volgograd region, nearby Kamyshin, 50°6′ N 45°25′ E; ZISP 21030 — La 19: Russia, Saratov region, nearby Engels, 51°12′ N 46°09′ E; HLMD — La 25: Russia, nearby Saratov, 51°34′ N 45°59′ E; ZISP 20873 — La 20, La 21, ZISP 21407 — La51: Russia, Belgorod region, v. Borisovka, 50°37′ N 36°00′ E; ZISP 21243 — La 37, ZISP 21117 — La 46: Russia, Voronezh region, 50°13′ N 39°36′ E; ZISP 21267 — La 44, ZISP 21266 — La 45: Russia, Astrakhan' region, 46°21′ N 48°04′ E; ZISP 21268 — La 50: Russia, Nizhny Novgorod region, Tonashevskii distr., 12 km NW of Pigma station, 57°57′ N 47°00′ E; ZISP 60/20928 — La 26, ZISP 61/20928 — La 29: Russia, Central Caucasus, Chegem Canyon, v. Khushto-Syrt, 43°26′ N 43°15″ E; ZISP TS. 73 — La 28: Russia, Central Caucasus, Baksan Canyon, nearby v. Jankhoteko, 43°34′ N 43°13″ E; ZISP 21572 — La 47: Kazakhstan, Dzhungar Alatau; 45°51′ N 79°59′ E; ZISP 21519 — La 1: Kazakhstan, Mramornyi pass, Markakol lake, 48°38′ N 85°58′ E; ZISP 21514 — La 2: Kazakhstan, Northern coast of Zaysan lake, Matveev Log; 48°48′ N 83°35″ E; ZISP 21522 — La3: Kazakhstan, Eastern coast of Markakol river, 48°49′ N 86°05″ E; ZISP 21520 — La4: Kazakhstan, right coast of Black Irtysh river, 48°00′ N 85°10′ E; ZISP 21524 — La 5: Kazakhstan, Northern coast of Zaysan lake, Kaznakovskaya crossing, 48°50′ N 83°25′ E; ZISP 21511 — La 6: Kazakhstan, Northern coast of Zaysan lake; 25 km NE v. Kara-Togai, 48°34′ N 84°43″ E; ZISP 21507 — La 7: Kazakhstan, Zaysan depression, 20 km SW v. Priozernyi, 47°34′ N 83°58′ E; ZISP 21509 — La 8: Kazakhstan, Zaysan depression, Bazaizhirkum sands, 47°53′ N 85°25″ E; ZISP 21513 — La 14: Kazakhstan, Zaysan depression; Southern Altay foothills, Koldgar river, 48°11′ N 85°11′ E; ZISP 21569 — La 48, La 49: Kazakhstan, Zaisanskii distr., Saur, 47°20′ N 85°17′ E.

***Lacerta agilis* ssp.:** HMLD — La 32, La 33: Germany, nearby Berlin, 52°31′ N 13°23′ E; HLMD — La 316: Czech Republic, Narodni Park, 48°48′ N 13°55′ E.