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Phylogenetic relationships between Afrotropical and Palaearctic *Crocidura* species inferred from Inter-SINE-PCR

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Abstract

The relationships between Afrotropical and Palaearctic Crocidurinae species have been evaluated on the basis of inter-SINE-PCR (IS-PCR). Two SINE families have been studied: mammalian interspersed repeats (MIR) and a short retroposon SOR, specific to the family Soricidae. Use of neighbor-joining and maximum parsimony analyses allowed us to recognize three major groupings: (i) 50-chromosome African species (*C. parvipes* and *C. olivieri*) and 28-chromosome European *C. leucodon*; (ii) species endemic to Ethiopia (36-chromosome *C. thalia*, *C. glassi*, *Crocidura* sp. B, united with *C. macmillani* and *C. baileyi*); (iii) Palaearctic 40-chromosome species group. Therefore, the major groupings of *Crocidura* shrews in the phylogenetic analysis of IS-PCR results cannot be distinguished as Palaearctic and Afrotropical groups of species: European *C. leucodon* unites with African 50-chromosome species with a high bootstrap support. The affiliation of *Suncus murinus* with Eurasian grouping is only supported by low bootstrap values.
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Keywords: SINEs; Inter-SINE-PCR; Shrews; *Crocidura*; Phylogeny

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A widely accepted view on the relationships between African, Asian and European species of Crocidurinae has been predominantly inferred from the chromosomal (Maddalena and Ruedi, 1994), allozyme (Maddalena, 1990) and mtDNA (Motokawa et al., 2000; Querouil et al., 2001) data. The allozyme approach has shown that, on the one hand, *Crocidura* genus includes several independent lineages (Ruedi, 1998), but on the other hand, all its species can be restricted to two major groups, formed by the Palaearctic and Afrotropical species, respectively (Maddalena, 1990). Analysis of nucleotide sequence of the cyt b gene suggested that *Crocidura* is paraphyletic (Motokawa et al., 2000), whereas the 16S rRNA gene data did not support this hypothesis (Querouil et al., 2001). Karyotype investigation revealed a bimodal distribution in the diploid numbers of *Crocidura* shrews with two peaks at around 40 chromosomes in the Palaearctic species and 50 in the African species (Maddalena and Ruedi, 1994). In order to assess the relationship among *Crocidura* shrews from different continents, the above two karyotypical groups, as well as *Crocidura* species with other tendencies in their chromosomal evolution, have been compared. *Suncus murinus* was also studied because of its unclear position within Crocidurinae. Another purpose of this study was to examine relationships among *Crocidura* of Ethiopia, an area with flora and fauna, known for high diversity and endemism. Earlier we carried out a comparative analysis of tandem DNA repeats in six *Crocidura* shrews from this country (Bannikova et al., 2001). In the present work we continue examination of their relationships and taxonomy on the basis of short dispersal DNA elements and compare the results with those of the Palaearctic species.

1. Material and methods

1.1. Specimens

Tissue samples have been taken from 38 shrew specimens (13 *Crocidura* species, *Suncus murinus* and *Neomys fodiens*) from Eurasia and Africa. Water shrew, *Neomys fodiens*, was used in the phylogenetic analysis as an outgroup. African shrews were collected by L.A. Lavrenchenko after extensive trapping during the Joint Ethio-Russian Biological Expedition (JERBE) held from 1995 through 2001. All specimens are deposited in the Zoological Museum of Moscow (Lomonosov) State University (ZMMU). The rest of the material is deposited in the Department of Vertebrate Zoology of Moscow (Lomonosov) State University. The list of species, collecting sites, number of specimens, and museum catalogue numbers are shown in Table 1.

Karyotypes of all species, except *C. baileyi*, were sourced in literature. *C. thalia*, *C. glassi* and *Crocidura* sp. B have 36 chromosomes (Lavrenchenko et al., 1997; Aniskin et al., 1998). *C. olivieri* and *C. parvipes* possess a 50-chromosome karyotype (De Hondt, 1972); the diploid number in *C. macmillani* is 28 chromosomes (Lavrenchenko, unpublished data). The karyotypes of *C. leucodon* ($2n = 28$, $NF = 56$) and species of *suaveolens* group ($2n = 40$, $NF = 50–56$) are described in

Table 1
List of specimens analysed

Species	Collection code	Field code	n	Collecting site
C. suaveolens	su3, su4, su6, su7	su3, su4, su6, su7	3	Dagestan, Madgalis
	su8	su8	1	Moscow
	su9, su10	su9, su10	2	Kalmykia
C. sibirica	sib195, sib624, sib1650, sib1651, sib1688	sib195, sib624, sib1650, sib1651, sib1688	5	Siberia, Kemerovo
C. gueldenstaedtii	gu11, gu12, gu15	gu11, gu12, gu15	3	North Caucasus, Tuapse
C. leucodon	le1, le2, le3	le1, le2, le3	3	Dagestan, Madgalis
C. olivieri	S-166027	733	2	Western Plateau, Middle Godjeb Valley (1220 m ASL, 07°15′N 36°47′E)
	S-172693, S-172813	1125, 1126	2	Vanzaye, shore of the Gumara River (1750 m ASL, 11°47′N 37°43′E)
C. parvipes	S-166033, S-166034	757, 758	2	Western Plateau, Middle Godjeb Valley (1220 m ASL, 07°15′N 36°47′E)
	S-167212, S-167213, S-167292	898, 899, 895	3	Koi River, 37 km SW of the Bebeka Coffee Farm (1130 m ASL, 06°39′N 35°14′E)
C. macmillani	S-166029	734	1	Western Plateau, Middle Godjeb Valley (1220 m ASL, 07°15′N 36°47′E)
	S-167293	933	1	Sheko Forest (1930 m ASL, 07°04′N 35°30′E)
C. thalia	S-165165, S-165166	468, 473	2	Eastern Plateau, Bale Mountains, Shawe River, Harenna Forest (1935 m ASL, 06°38′N 39°44′E)
C. glassi	S-164853, S-164856	373, 378	2	Eastern Plateau, Bale Mountains, Dinsho area (3170 m ASL, 7°06′N 39°47′E)
C. baileyi	S-172690	1089	1	Mount Guna near Yitba (3800 m ASL, 11°43′N 38°15′E)
Crocidura sp. B	S-165342	30	1	Western Plateau, Beletta Forest (1900 m ASL, 07°34′N 36°31′E)
Suncus murinus	Sm19, Sm20	Sm19, Sm20	2	Cambodia
Neomys fodiens	Nf2.92	Nf2.92	2	Siberia, Yenisey river

n—number of specimens.

a number of publications (Catzeflis et al., 1985; Grafodatsky et al., 1988; Sokolov and Tembotov, 1989).

1.2. DNA isolation

Genomic DNA was isolated from ethanol-fixed liver, kidney or muscles by pronase digestion, phenol–chloroform deproteinization and isopropanol precipitation (Sambrook et al., 1989).

1.3. PCR using SINE specific primers (IS-PCR)

The method of inter-SINE-PCR (IS-PCR) is based on amplification of the SINE-flanked DNA-fragments. Copies of SINE-elements (short interspersed elements, short retroposons) are located 100–1000 bp apart, and represent 80–400 bp DNA sequences, that are dispersed throughout the genome in hundreds of thousands. Our attention was drawn to a SINE family named the mammalian interspersed repeat (MIR) which numbers 10^5 copies in all mammalian genomes (Smit and Riggs, 1995; Jurka et al., 1995; Gilbert and Labuda, 2000). Full-sized MIR copies (260 bp) occur relatively seldom because of their ancient origin. In addition to the MIR elements, we studied a short retroposon SOR of 179 bp in length, which is specific to the family Soricidae. First it was isolated from the genome of *S. araneus* (Borodulina and Kramerov, 2001). The mutations in the above SINEs (MIR and SOR) per se, as well as deletions and insertions in the DNA regions that situate between their copies result in differences between IS-PCR products.

Inter-MIR-PCR was carried out with primers complementary to the most conserved regions of the central core sequence of the MIR element (Jurka et al., 1995). Four primers have been used in the following combinations: MIR17/MIL17 and OMIL17/OMIR17. In SOR-PCR, we used primer complementary to the beginning of the SOR element, and in that way initiated DNA synthesis in the upstream direction of the SINE. The sequences of the above primers are as the following:

MIR17, 5′-AGTGACTTGCTCAAGGT-3′;
MIL17, 5′-GCCTCAGTTTCCTCATC-3′;
OMIR17, 5′-ACCTTGAGCAAGTCACT-3′;
OMIL17, 5′-GATGAGGAAACTGAGGC-3′;
SOR, 5′-TGCTATC(G/A)CTCCAGCCC-3′.

Primers (100 pmole each) were labeled with [γ-^{32}P]-ATP (1 MBq) by polynucleotidekinase (Sambrook et al., 1989). PCR was carried out in 20 μl of a reaction mixture containing 10 mM of Tris–HCl buffer, pH 8.3; 50 mM KCl; 2.5 mM $MgCl_2$; 0.001% gelatin; dNTPs, 0.2 mM each; 4 pmole of each primer; 1 U of Taq-polymerase; and 25 ng of DNA template. Conditions of the MIR-specific PCR corresponded to the procedure by Jurka et al. (1995): denaturation, 30 s at 94 °C; annealing, 45 s at 56 °C; and elongation, 2 min at 72 °C, in a total of 27 cycles. The

initial denaturation and final synthesis lasted 3 min at 94 °C and 5 min at 72 °C, respectively. In the SOR-experiments, a consistently reproducible pattern was secured by increasing the annealing temperature, as against the calculated melting point. As a result, annealing was conducted for 45 s at 65 °C. PCR was performed in an MJ Research (USA) thermal cycler. PCR-products were denaturated and separated by the electrophoresis in a 6% polyacrylamide gel, containing Tris–borate buffer and 8 M urea. The gel was 50 cm long and 0.4 mm thick. Electrophoresis was run for 7 h at a constant power of 75 W. Dried gels were autoradiographed by gel exposure with X-ray films for 48 h.

1.4. Phylogenetic analysis

IS-PCR and restriction fragments (fingerprint bands) were scored for their presence/absence. To infer molecular phylogenetic relationships among the 39 Crocidurinae species of our data set, the binary matrices were analysed by neighbor-joining (NJ; Saitou and Nei, 1987) and maximum parsimony (MP; Swofford, 1998) analyses using TREECON (Van de Peer and de Wachter, 1994) and PAUP, version 4.0b4a (Swofford, 1998) software packages accordingly. The statistical confidence of groupings in the NJ and MP trees was evaluated by the bootstrap test based on 1000 replications. To estimate the dissimilarity among individual patterns Link et al. (1995) genetic distances were calculated using the formula $D_L = 1-J$, where J is the Jacquard's similarity index.

2. Results

2.1. Intraspecific variability

Ranges of individual and geographical variability of shrews are presented in Table 2.

The estimation of individual molecular polymorphism was done for the samples from the same locality. The level of individual variability, revealed by MIR-PCR, that was inferred from the D_L genetic distances, appeared to be minimum in *C. gueldenstaedtii* and *C. sibirica*, and maximum in *C. parvipes* and *C. olivieri*. The same tendency was observed in SOR-PCR with the D_L index ranging from 0.00 in *C. gueldenstaedtii* to 0.41 in *C. olivieri*. On the whole, the 40-chromosome Palaearctic species were less heterogeneous genetically, than the 50-chromosome Afrotropical ones. Among the former, *C. suaveolens* demonstrated the most remarkable level of individual variability (D_L = 0.16–0.32 in MIR-PCR and 0.12–0.26 in SOR-PCR), whereas *C. gueldenstaedtii* was genetically the most homogeneous (D_L = 0.03–0.09 in MIR-PCR and 0.00 in SOR-PCR).

Genetic distance between the samples from geographically distant localities was estimated as a level of geographical variability. The clustering of individuals (Figs. 1–3) and the D_L values did not necessarily reflect the geographical proximity of their capture locations (Table 2), such as in *C. olivieri*, *C. parvipes* and

Table 2
The intraspecific genetic variability of the *Crocidura* shrews inferred from inter-MIR- and inter-SOR-PCR data

PCR	Group of species	D_L—genetic distances of Link et al. (1995)	
		Individual variability, min–max	Geographical variability, min–max
MIR	50-Chromosome species	*C. parvipes*: 0.20–0.41, *C. olivieri*: 0.33	*C. parvipes*: 0.50, *C. olivieri*: 0.24–0.40
	40-Chromosome species	*C. gueldenstaedtii*: 0.03–0.09, *C. sibirica*: 0.03–0.10, *C. suaveolens*: 0.16–0.32	*C. suaveolens*: 0.13–0.36
SOR	50-Chromosome species	*C. parvipes*: 0.18–0.37, *C. olivieri*: 0.41	*C. parvipes*: 0.30–0.37, *C. olivieri*: 0.30
	40-Chromosome species	*C. gueldenstaedtii*: 0.00, *C. sibirica*: 0.02–0.15, *C. suaveolens*: 0.12–0.26	*C. suaveolens*: 0.08–0.28

C. suaveolens, which demonstrate approximately equal level of geographical variation. However, these data require further careful examination on larger geographical sample.

2.2. Interspecific variability

The MIR-PCR interspecific genetic distances ranged from 0.38 (between *C. suaveolens* and *C. gueldenstaedtii*) to 0.9 (between *C. suaveolens* and *C. parvipes*), with minimum values received for three species of the 40-chromosome group; in the pair *C. suaveolens*/*C. gueldenstaedtii* this index overlapped with the intraspecific D_L of the 50-chromosome species group. However, for the pairs *C. sibirica*/*C. suaveolens* or *C. sibirica*/*C. gueldenstaedtii* it was even higher than between *C. thalia* and *C. glassi* (0.57 and 0.42, respectively). *C. lasiura* appeared to be more distant from the other 40-chromosome species with D_L, the same as *C. leucodon* is distant from the rest of the 50-chromosome representatives ($D_L = 0.81$).

The general ratio of interspecific distances in SOR-PCR is similar to those in MIR-PCR; the minimum $D_L = 0.4$ was observed between *C. sibirica* and *C. suaveolens*, and maximum $D_L = 0.92$—between the Palaearctic *suaveolens* species group and Afrotropical 50-chromosome species *C. leucodon*. The D_L ratio was comparatively low among *C. suaveolens*, *C. gueldenstaedtii* and *C. sibirica* (0.4–0.51) and between *C. thalia* and *C. glassi* (0.33). The distance between the 50-chromosome species and *C. leucodon* was about the same as in MIR-PCR (0.85). The only significant difference between the D_L values, that were calculated from the MIR- and SOR-PCR data, concerns the distance between *C. lasiura* and other 40-chromosome shrews. In the latter, the distance values are the same as between *Crocidura* sp. B and other 36-chromosome species (0.66). On the whole, there was no overlap between the intra- and inter-specific genetic distances, despite the fact that the genetic diversity within the species is high and variable.

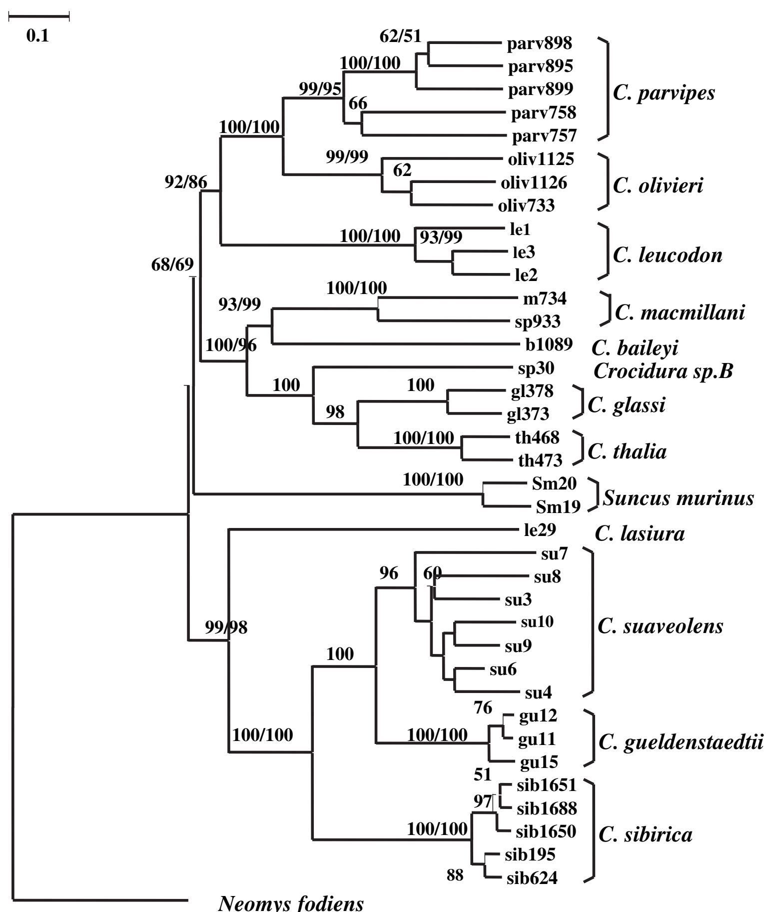

Fig. 1. The NJ tree of the relationships of 13 *Crocidura* species and *Suncus murinus* based on the Link et al. (1995) genetic distances by the results of inter-MIR-PCR. The bootstrap values (bv $\geq$ 50%) obtained from 1000 replics in NJ and MP analysis are given at the nodes over the slash (NJ/MP). When the branching patterns on NJ tree are not supported by MP, then only NJ bv are given. *Neomys fodiens* is used as an outgroup.

2.3. Phylogenetic analysis

Reasonably congruent phylogenetic trees have been generated employing NJ and MP algorithms, of which only the NJ ones are presented here, bearing the notations of the bootstrap values (bv) for both NJ- and MP-type analyses (Figs. 1–3).

2.4. Inter-MIR-PCR

The whole number of characters examined with inter-MIR-PCR of *Crocidura* and *Suncus* was very similar for the two combinations of primers: 341 for the experiments

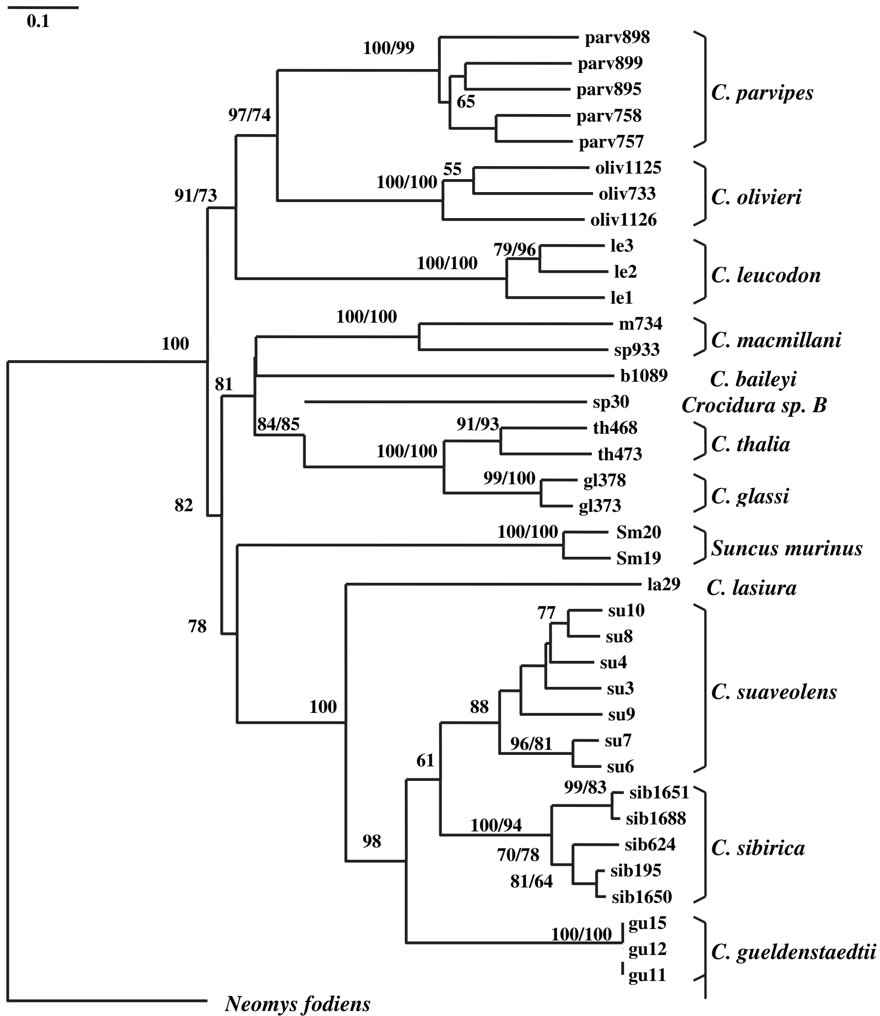

Fig. 2. The NJ tree of the relationships of 13 *Crocidura* species and *Suncus murinus* based on the Link et al. (1995) genetic distances by the results of inter-SOR-PCR. The designations are as in Fig. 1.

with mir17/mil17 and 329 in the case of omir17/omil17. The resulting topologies of inter-MIR-PCR trees, for different primer combinations, differed in the position of *C. leucodon* within the African species and the grouping of *C. thalia*, *C. glassi* and *Crocidura* sp. B. On the whole, the topology of the tree of IS-PCR conducted with omir17/omil17 primer combination appeared to be more similar to the one of inter-MIR-PCR combined data tree (Fig. 1).

The number of characters examined in the combined phylogenetic analysis of MIR-PCR-derived data with both systems of primers comprised 670. The number of parsimony-informative characters is 425. The topology of both NJ and MP trees was

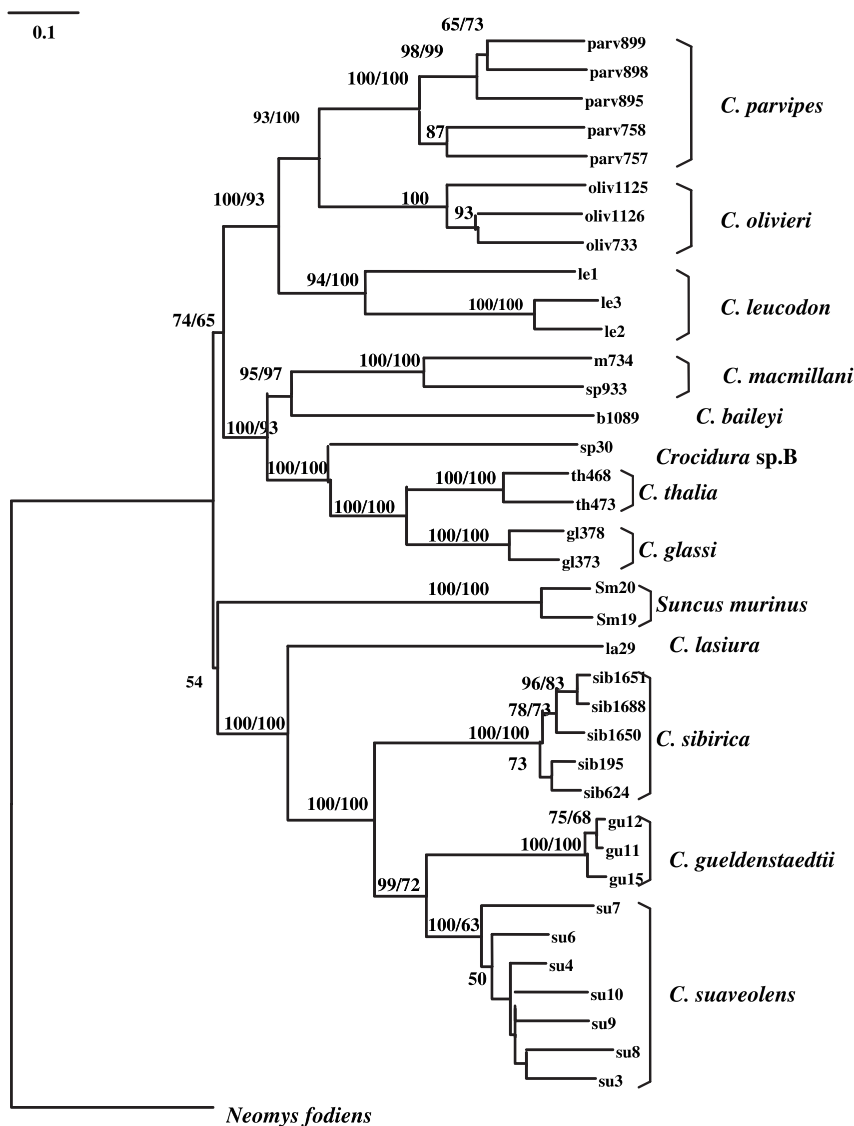

Fig. 3. The NJ tree of the relationships of 13 *Crocidura* species and *Suncus murinus* based on the Link et al. (1995) genetic distances by the combined results of inter-MIR- and inter-SOR-PCR. The designations are as in Figs. 1 and 2.

basically the same (Fig. 1) except for interspecific relationships among 36-chromosome species (*C. glassi*, *C. thalia* and *Crocidura* sp. B) and within the 40-chromosome species group (*C. suaveolens*, *C. sibirica*, *C. gueldenstaedtii* and *C. lasiura*). Both groups form strong supported clades but the NJ-supported topology of different species was not supported by the MP analysis. For example,

C. suaveolens represents a monophyletic taxon in the NJ tree but it is characterized by a polytomy in the MP tree. The combined data tree by the results of inter-MIR-PCR analysis moderately supported association of all the Ethiopian species presented in our research in the same cluster with European *C. leucodon* (bvNJ/bvMP = 68/69). Such a topology, according to which *C. leucodon* within the African clade appeared to be a sister branch of the 50-chromosome species (*C. parvipes* and *C. olivieri*) had a high bootstrap support in both NJ and MP analyses (92/86%). The 36-chromosome species and *C. macmillani* + *C. baileyi* formed a strong supported clade (100/96%). The position of *Suncus murinus* was unresolved.

2.5. Inter-SOR-PCR

Among 389 characters considered in the parsimony and NJ analysis of SOR-PCR results, 240 were parsimony informative. Contrary to the inter-MIR-PCR results, African species (+ *C. leucodon*) did not represent a united assemblage (Fig. 2). The cluster of endemic Ethiopian 36-chromosome species, *C. macmillani* and *C. baileyi* formed a sister group to the 40-chromosome Palaearctic species group + *Suncus murinus* (82%) in NJ analysis. However, this grouping was absent in the MP tree because the relationships of 36-chromosome species, 40-chromosome species, *C. macmillani*, *C. baileyi* and *Suncus murinus* were unresolved in MP analysis. Relatively low support was demonstrated for each of the species cluster in the case of the 40-chromosome Palaearctic species group. In the MP analysis, *C. gueldenstaedtii* appeared within the *C. suaveolens* clade which had the low bootstrap support. The relationships of *C. sibirica* and *C. lasiura* remain unresolved. The clade *C. leucodon*/(*C. parvipes* + *C. olivieri*) had good support both in NJ and MP analyses (bv = 91/73%). Affiliation of *Suncus murinus* with Eurasian grouping is supported by moderate bootstrap values (78%) in NJ and unresolved in MP trees.

2.6. Combined results of inter-MIR- and inter-SOR-PCR

If compared, inter-MIR- and inter-SOR-PCR trees differ in terms of branching and clustering of some taxa and specimens. However, these differences concerned only those clades that were not strongly supported by the NJ analysis and as a rule were unresolved on the MP trees. So we suggest that there are not very essential differences between them. Hence, we tend to treat these differences between the inter-MIR- and inter-SOR-PCR results as of little significance. Therefore we found it possible to join binary matrices for all results and discuss the combined inter-SINE-PCR tree.

The topology of the combined NJ and MP trees (660 parsimony-informative characters out of the total number 1059) is similar to the inter-MIR-PCR tree, concerning the relationships between the Palaearctic and Afrotropical species (Fig. 3). Integrated results of inter-MIR- and inter-SOR-PCR, indicate that the African species taken into analysis and European *C. leucodon* form a common cluster (bv = 74/65%). The 36-chromosome species along with *C. macmillani* + *C. baileyi* formed a sister clade to *C. parvipes* + *C. olivieri* together with *C. leucodon*.

Palaearctic 40-chromosome species tended to be a constant clade, in which *C. sibirica* appeared to be a sister group to *C. suaveolens*/*C. gueldenstaedtii* lineage, and *C. lasiura* is the most basal branch of the 40-chromosome species. *C. gueldenstaedtii* and *C. sibirica* formed a separate clade each, with a high bootstrap values in both NJ and MP analysis. As for *C. suaveolens* the bootstrap support of this clade in MP analysis was low (63%), confirming the tendency to the polytomy which had been shown for it in the MP analysis of both inter-MIR- and inter-SOR-PCR data independently.

The position of *Suncus murinus* remains unresolved.

3. Discussion

Our results suggest that 14 species of Crocidurinae can be assigned to four main lineages: (i) 50-chromosome African species (*C. parvipes* and *C. olivieri*) together with 28-chromosome European *C. leucodon*; (ii) endemic Ethiopian 36-chromosome *C. thalia*, *C. glassi* and *Crocidura* sp. B together with *C. macmillani* ($2n = 28$) and *C. baileyi*; (iii) Palaearctic 40-chromosome species group; (iv) *Suncus murinus* ($2n = 40$). The major groupings of *Crocidura* shrews in our phylogenetic analysis of the results of IS-PCR are not distinguished as Palaearctic and Afrotropical groups of species for European *C. leucodon* joined the African 50-chromosome representatives. Therefore we conclude that Eurasian and African shrews appear to be the non-monophyletic assemblages. These data do not support the outcome of the allozyme survey (Maddalena, 1990) but are congruent with the 16S rRNA nucleotide sequence data (Querouil et al., 2001).

The grouping of all examined African species in one cluster was not supported by inter-SOR-PCR and only moderate bootstrap support was found for this branching pattern when the data of inter-MIR- and inter-SOR-PCR were combined. The 50-chromosome African species and 40-chromosome Palaearctic ones appeared to be the most stable, strongly supported grouping around which other species clustered in a different manner depending on the kind of IS-PCR and chosen primers. The 36-chromosome species endemic to Ethiopian mountain forests and Afroalpine moorlands also represent a strongly supported and well resolved cluster, and cluster with a few more Ethiopian endemics, namely *C. macmillani* + *C. baileyi*.

It is essential to note that except for *C. macmillani*, all cytogenetically studied endemic Ethiopian *Crocidura* species (including *C. bottegoides* Hutterer et Yalden, 1990; *C. harenna* Hutterer et Yalden, 1990; *C. lucina* Dippenaar, 1980 and undiscribed *Crocidura* sp. B) possess a 36-chromosome karyotype (Lavrenchenko et al., 1997; our unpublished data) while only two with the diploid number of 36 have hitherto been found in the rest of Subsaharan Africa (*C. obscurior* from Ivory Coast and *C. luna* from Burundi) (Schlitter et al., 1999). Dippenaar and Meester (1989) believed that the five Ethiopian taxa (*C. macmillani*, *C. baileyi*, *C. glassi*, *C. lucina* and *C. thalia*) form a distinct group of closely related endemic species. Our data confirm this conclusion: *C. macmillani* and *C. baileyi* form a cluster which is

phyletically close to the group comprising the rest of the Ethiopian endemics. Taking the above into consideration, we may suppose that all the 36-chromosome species, endemic to Ethiopia, form a monophyletic group of a relatively recent origin.

By the results of combined phylogenetic analysis of inter-MIR-PCR and inter-SOR-PCR, *C. glassi* and *C. thalia* form a clade against *Crocidura* sp. B. Earlier the restriction DNA analysis (taxonprint) suggested the closer genetic affinity of *Crocidura* sp. B with *C. thalia* than with *C. glassi* (Bannikova et al., 2001). This inconsistency suggests that the question of the relationships of 36-chromosome species needs further investigation. It is noteworthy that in the IS-PCR with OMIL17/OMIR17 primers, *Crocidura* sp. B formed a cluster with *C. glassi* against *C. thalia* (bv = 91%). In the combined phylogenetic analysis of MIR-PCR data, NJ and MP trees were different in the part of *Crocidura* sp. B/*C. glassi*/*C. thalia* relationships. In the NJ tree *C. glassi* and *C. thalia* formed a well supported cluster (bv = 98%) distinct from *Crocidura* sp. B. In the MP tree *Crocidura* sp. B appeared to be closer to *C. thalia* rather than to *C. glassi* likewise in the taxonprint NJ and MP trees (Bannikova et al., 2001), although the bootstrap support of this branch pattern was low (54%). So the constant position for 36-chromosome species in relation to each other was not found in the experiments with different primers. At the same time we rely more on the IS-PCR data than on the restriction DNA analysis. The reason for probable bias between the results obtained by taxonprint and IS-PCR and the less reliability of the data of restriction DNA analysis are likely caused by such source of convergence as the independent explosive amplification of the same monomer of satellite DNA in different non-sister species lineages. On the whole the difficulties in the resolving of the relationships among *Crocidura* sp. B, *C. glassi* and *C. thalia* may be associated with their recent origin as well as in the case of *suaveolens* species group.

The estimation of the level of divergence between "good" Ethiopian species, possessing the identical karyotypes, enabled us to evaluate the taxonomic significance of the genetic distances among disputed 40-chromosome Palaearctic species (*C. suaveolens*, *C. gueldenstaedtii* and *C. sibirica*). The results demonstrate that the interspecific genetic divergence between different species is similar in the cases of the 40- and 36-chromosome species groups (except for *C. lasiura* in the former), and is more than 1.5 times lower than that of the 50-chromosome species. Thus, if the species validity of *C. thalia* and *C. glassi* is doubtless, *C. suaveolens*, *C. gueldenstaedti* and *C. sibirica* must be treated as full species as well. Contrary to the results of comparison of West European populations of *C. suaveolens* with *C. gueldenstaedtii* from Georgia (Vogel et al., 2003), our data demonstrate that *C. suaveolens* from Eastern Europe (Moscow, Kalmykia and Dagestan) and population of *C. gueldenstaedtii* from the North Caucasus (Tuapse) represent separate units.

Our results revealed a surprisingly strong relationship between European *C. leucodon* and African *C. olivieri* and *C. parvipes*. It contradicts allozyme electrophoresis data according to which *C. olivieri* and *C. leucodon* belong to different lineages (Maddalena, 1990). On the other hand, a close relationship has been shown for *C. leucodon* and West African *C. obscurior* on account of comparison between the sequences of their 16S rRNA genes (Querouil et al., 2001). It seems possible that some recent African species are the descendants of the same with

C. leucodon ancestor which migrated to Europe at some early stage of their radiation. If so, *C. leucodon* may be suggested as one of the most ancient species among Palaearctic *Crocidura* sharing the plesiomorphic features with their African 50-chromosome relatives.

After comparing the data concerning clustering and chromosome numbers of the studied species, a clear correlation can be traced in all cases with the exception of the 28-chromosomal *C. macmillani* and *C. leucodon*. These species cluster with two distinct clades formed by the species with dissimilar chromosome numbers ($2n = 36$ and $2n = 50$, respectively). Their identical chromosome number is obviously the result of independent convergent evolution that may be revealed by morphology and different banding patterns.

The position of *Suncus murinus* with respect to the genus *Crocidura* is lacking a sufficient support.

Most authors currently accept *Suncus* as a full genus (Hutterer, 1993; Wolsan and Hutterer, 1998). According to recent analysis of 16S rRNA, five *Suncus* species examined were positioned aside from representatives of *Crocidura* and *Sylvisorex* genera and none of them clustered among the *Crocidura* species (Querouil et al., 2001). Contrary to this findings, in biochemical allozyme examination (Jenkins et al., 1998) and in molecular study based on cyt b sequences (Motokawa et al., 2000) the clustering of *Suncus* among *Crocidura* species was observed. Nucleotide sequence data of the cyt b gene suggested that Oriental species *C. watasei* was more closely related to *Suncus murinus*, rather than to other studied East Asian *Crocidura* (Motokawa et al., 2000). Electrophoretic data, obtained from 25 protein loci in *Suncus etruscus* (Ruedi, 1998), indicated a close relationship of this species to *C. suaveolens* and both *Suncus etruscus* and *C. suaveolens* appeared to be fairly distant from *C. leucodon*. It is known that the 40-chromosome race of *S. murinus* is distributed in Nepal, Bangladesh, Vietnam, Malaysia, Indonesia, Taiwan and Japan (Yosida, 1982). The karyotype comparisons revealed a surprisingly high degree of chromosome homology between this 40-chromosome race of *S. murinus* and Oriental *C. dsinezumi* ($2n = 40$, $NF = 56$) which possess the karyotype close in its characteristics to that of the species of *suaveolens* group. The authors of the latter work conclude that *S. murinus* and species of *suaveolens* group retained more ancestral traits than the more advanced species, *C. leucodon* (Biltueva et al., 2001). All these facts are in a good agreement with our inter-SOR-PCR findings when *S. murinus* demonstrates a tendency to cluster with 40-chromosome Palaearctic species.

Additional molecular surveys involving more African species are required to clarify phylogenetic relationships between *Suncus* and *Crocidura* as well as groupings between Afrotropical and Eurasian species.

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