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Revalidation of *Myotis petax* Hollister, 1912 and its new status in connection with *M. daubentonii* (Kuhl, 1817) (Vespertilionidae, Chiroptera)

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A combined approach based on the complex use of molecular, morphological and ecological data has shown that the 'eastern' group of forms of transpalearctic Daubenton's bat, *Myotis daubentonii* (Kuhl, 1817), deserves a status of distinct species, and conforms to *M. petax*, described by Hollister in 1912 from the Republic of Altai in the south of Western Siberia. In our genetic analysis we used SINEs (short interspersed elements) of nuclear DNA as genetic markers, and by means of Inter-SINE-PCR, have clearly demonstrated a species distinctiveness of *M. petax*. Our further analysis has also shown, that they considerably differ from *M. daubentonii* s.str. in skull proportions, dental features, as well as in bacular shape and size. Both species also differ in their ecology and general appearance, especially coloration.

Key words: *Myotis daubentonii*, *M. petax*, taxonomy, ranges, SINEs, morphology, ecology

INTRODUCTION

Daubenton's bat, *Myotis daubentonii* (Kuhl, 1817), has always been regarded as one of the most common Palearctic bat species, being widespread throughout most of Europe (including UK and Ireland) northwards to about 60 latitude and eastwards through the south of Siberia, Transbaikalia, northern Mongolia, north-eastern China and Korea, to the continental Far East, Kamchatka peninsula and the islands: Sakhalin, the Kuriles (Russia) and Hokkaido (Japan — e.g., Kuzyakin, 1950; Ellerman and Morrison-Scott, 1966; Krivosheev, 1984; Yoshiyuki, 1989). Up to 12 valid names were formerly accepted as synonyms of *M. daubentonii* (Bogdanowicz, 1994; Pavlinov *et al.*, 1995). Some of them were recognised as subspecies, though the views on their number and status have significantly evolved. While Ellerman and Morrison-Scott (1966) distinguished five subspecies, namely the nominate (northwestern Europe), *M. d. volgensis* (Eversmann, 1840) (Eastern Europe to central Siberia), *M. d. ussuriensis* Ognev, 1927 (Eastern Siberia from Baikal to the Far East, northeastern China to Japan and the Kuriles), *M. d. loukashkini* Shamel, 1942 (Manchuria) and

M. d. laniger (Peters, 1870) (southern China and northeastern India), further reduction of European *M. nathalinae* Tupinier, 1977 (southwestern Europe) to a subspecies of *M. daubentonii* (Hanák and Horáček, 1984; Koopman, 1994) increased their number to six. It is noteworthy, that in the view of the latest molecular analyses on bat taxonomy the subspecific status of *M. nathalinae* gained additional support as well (Ruedi and Mayer, 2001).

In 1912 a distinct species, *M. petax*, was described from Chuiskaya Steppe (Altai, south of Western Siberia) by Hollister. However, Ognev (1928) proposed it become a synonym of *M. d. daubentonii*, and since then only once '*petax*' appeared as a species in Findley's revision (1972), who even excluded it from '*daubentonii*' species group, pointing out its closer relationships with some American species, such as *M. volans* (Allen, 1866).

Bogdanowicz (1990, 1994), in his turn, reduced the number of subspecies to three, treating *M. d. loukashkini* from one side as a synonym of *M. d. ussuriensis*, and including all European and the rest of Siberian forms into *M. d. daubentonii*. The third subspecies he recognised, *M. d. laniger*, was later given a specific rank by Topál (1997).

Kruskop (2001) has shown the presence of two major groups of forms in the *M. daubentonii* complex: the 'western', or 'European' (excluding the Altai form *petax*) and the 'eastern', or 'Asian'. Later he designated their status as 'groups of subspecies': namely '*daubentonii*' and '*petax*', respectively, with three subspecies in the latter (Kruskop, 2004). Since it was demonstrated that '*petax*' had to be referred to as the eastern form, the use of this name (versus '*ussuriensis*') for the whole subspecies was justified, based on its seniority. Matveev (2004), who used SINEs of nuclear DNA as genetic markers, has also demonstrated the presence of the two major forms in the complex, but in his turn presented much greater level of divergence between them, justifying a species distinctiveness of the 'eastern' form, inhabiting most of Siberia, Transbaikalia, Far East, Manchuria, Japan and likely the Kuriles.

In the present paper we produce evidence supporting the specific status of the 'eastern' form of 'Daubenton's bat', derived from molecular, morphological, as well as ecological data, with *Myotis petax* applied as its legitimate name.

Materials and Methods

Inter-SINE(MIR)-PCR

Samples

The total of 13 specimens of both forms of '*M. daubentonii* complex' have been taken into analysis, with six of them representing the 'western' form, and seven — the 'eastern' one. The scope of biopsy sampling has covered most of the geographic ranges of both forms within Russia, excluding the Urals (Appendix I). In addition, *Eudiscopus denticulus* and *Myotis blythii* were taken as a complex outgroup. The tissue samples were preserved in 96% ethanol and stored at +4°C.

DNA Isolation

DNA was isolated from ethanol-preserved tissues (wing membrane, pectoral muscle and liver) by phenol–chloroform extraction, following the treatment of tissue homogenates with proteinase K.

Conditions of Inter-SINE(MIR)-PCR

Polymerase chain reaction (PCR) was conducted using one primer, complementary to the most conserved region of the central core sequence of the MIR element (Jurka *et al.*, 1995): mil17, 5'-CCTCAGTTTCCTCATC-3'. The primer (100 pmole) was labelled with [γ^{32}P]-ATP (1 MBq) by polynucleotidekinase (Maniatis *et al.*, 1982). The PCR was conducted in 20 µl of the reaction mixture containing 70 mM of Tris-HCl buffer (pH 8.6), 16.6 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 0.001% Triton X100, dNTPs (0.2 mM each), 4 pmole of the primer, 1 unit of Taq polymerase and 25 ng of genomic DNA as a template. The conditions of MIR-specific PCR corresponded to an earlier described scheme (Jurka *et al.*, 1995): denaturation, 30 s at 94°C; annealing, 45 s at 56°C; elongation, 2 min at 72°C — all in 27 cycles.

The initial denaturation and final synthesis lasted for 3 min at 94°C and 5 min at 72°C, respectively. The reaction was conducted in an MJ Research thermal cycler. The PCR products were denaturated and separated by electrophoresis in a 6% polyacrylamide gel containing a Tris-borate buffer and 8 M urea (composition similar to the one of a sequencing gel). Gel length and width were 50 cm and 0.4 mm, respectively. Electrophoresis was run for 7 h at a constant power capacity of 75 W. Dried gels were autoradiographed by their exposure against Retina X-ray film for 16 to 48 h.

Phylogenetic Analysis

The data from the Inter-MIR-PCR fingerprint were compiled into a binary matrix, with 1 or 0 marking presence or absence of the marker, respectively (Appendix II). The matrix was analysed by the Wagner maximum parsimony (MP) method in PAUP software package, version 4.0b4a (Swofford, 1998), and by the neighbour joining (NJ) method in TREECONW software package (Van de Peer and De Wachter, 1994). Genetic distances (DL) were calculated according to Link *et al*. (1995) and correlated with Jacquard's similarity index (J) by the following formula: DL= 1 - J. Thousand bootstrap iterations have been made.

Morphometry

About 190 specimens of *Myotis daubentonii* s.l. (skulls and dry or alcohol-preserved skins) from various parts of the distribution range have been investigated. For comparison, several specimens of similar species, *Myotis macrodactylus* and *M. capaccinii*, have been taken as well. Most of the specimens are deposed in the collections of Saint-Petersburg Zoological Institute (Russian Academy of Sciences) and Zoological Museum of Moscow State University (see Appendix). The number of adult males and females in relatively large samples were approximately equal.

The following cranial measurements have been taken (appropriate abbreviations are given in parentheses): condylobasal length (CBL), condylocanine length (CC^1L), skull width on the level of auditory bullae (W), braincase width (BCW); braincase height posteriorly to the auditory bullae (BCH), width of interorbital constriction (IOW), rostral width on the level of preorbital foramens (WR), rostral length from preorbital foramen to the alveole of I^1 (LR), $C–M^3$ length (C^1M^3), length of C^1 including cingulum (LC^1), length of the interval between cingulum of C^1 and P^4 ('pseudodiasteme', PD), molariform toothrow length (P^4M^3), width of M^3 (WM^3), length of M^3 (LM^3), distance between outer margins of C^1 (C^1C^1), distance between outer margins of M^3 (M^3M^3), lower jaw length from the alveole of I_1 to the articulated process (LMD), lower jaw height on the level of the tip of the coronoid process (HMD), $C–M_3$ length (C_1M_3). All measurements were taken with a digital caliper under a dissecting microscope to the nearest 0.01 mm. Some external measurements from 112 specimens of both 'forms' have been taken as well. The matrix of cranial measurements was processed statistically by the forward stepwise discriminant analysis (Statistica 5.0 software package for Windows).

External measurements were taken mainly from the alcohol-preserved specimens, to the nearest 0,1 mm: head and body length (L), tail length (C), ear length (A), tragus length (Tr), tibia length (Cr), length of hind foot without claws (Pl), forearm length (R), length of the first digit without claw (D1), and length of 3–5 metacarpals (Mc3, Mc4, Mc5).

Bacular Morphology

Seventeen penial bones from the representatives of both 'forms' of *M. daubentonii* s.l. (8 adults and 1 juvenile of *petax* from Altai Mts., Republic of Tuva, Transbaikalia, Russian Far East and Sakhalin Islands, and eight of *M. daubentonii* s.str.) and two from *M. macrodactylus* were extracted. All bones were cleaned with a standard method according to White (1951). The distal part of penis, taken from the ethanol-preserved specimen, was submerged into 6% solution of KOH, with addition of Alizarin red to dye the osseous tissues, for 8–16 hours. Cleaned bones were stored in glycerin. All prepared bacula were sketched and measured in camera lucida under the binocular microscope.

Field Equipment

For field observations and collection of the material we used the heterodyne bat detectors D-100 and D-200 (Pettersson Elektronik, Sweden), mist-nets, mobile traps (Borissenko, 1999), as well as biopsy punches and plastic tubes with 96%-ethanol for tissue sampling.

RESULTS

Inter-SINE(MIR)-PCR

The total of 163 characters have been picked up by the analysis. The topology of the MP- and NJ-trees was identical in their

major nodes: insignificant differences could only be observed in the bootstrap values (Fig. 1). On this reason, only NJ-dendrogramme, as the one reflecting the genetic distances, is shown (the MP-derived bootstrap values are indicated for the appropriate nodes as well).

Both *daubentonii* and *petax* clusters are supported by 100% bootstrap values (Fig. 1), while the position of *M. blythii* is uncertain: the bootstrap support is very low (67% and 66% for the MP and NJ, accordingly). Therefore, the major cluster, including *daubentonii*, *petax* and *M. blythii*, is in fact a polytomy. The reason is that the number of common characters, shared between all *petax* and *daubentonii* samples, is insignificant and comparable to the number of those, shared between each of them and *M. blythii*. The indices of the genetic distance (GD) between the two forms varied from 0.83 to 0.88 (Table 1) and were as high as between each of these forms and such distant representative of the same genus as *M. blythii*: namely 0.81–0.84 for *daubentonii*/*blythii* and 0.86–0.91 for *petax*/*blythii*. This is considerably higher than the internal values for both forms, i.e., 0.18–0.28 for *daubentonii*

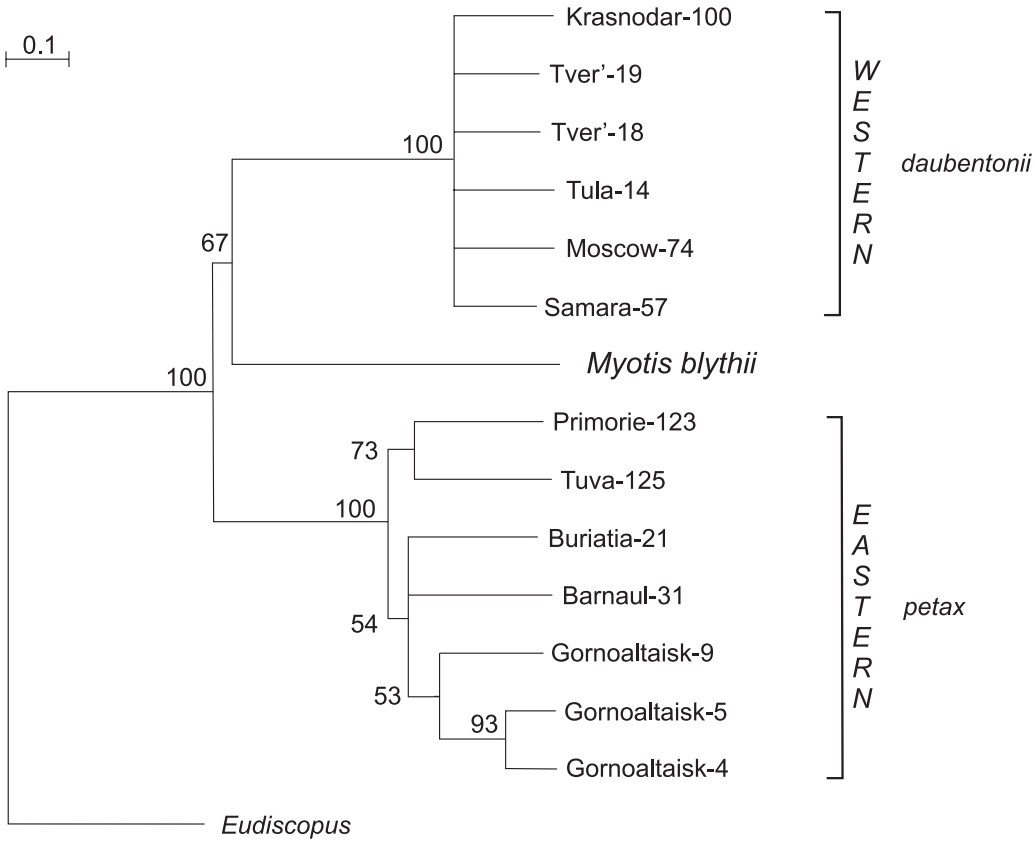

FIG. 1. Neighbour-joining (NJ) dendrogramme of the relationships between *Myotis daubentonii* s.str. and *M. petax* based on Inter-SINE-PCR results. NJ bootstrap values are shown above nodes and maximum parsimony (MP)-derived values — under the nodes. Clusters with bootstrap support less than 50% are shown as unresolved. Collector's numbers (acronym 'Rus' omitted) are given after appropriate names of geographic localities. *Eudiscopus denticulus* and *Myotis blythii* were chosen as a complex outgroup. Unexpected position of the latter on the tree is explained in the text

TABLE 1. Genetic distances between and within *M. daubentonii* s.str. and *M. petax*, as well as between each of them and two outgroups (*E. denticulus* and *M. blythii*). Distances calculated according to Link *et al*. (1995) on the basis of Inter-SINE(MIR)-PCR data

Taxon	*Eudiscopus denticulus* Vtm-11	*Myotis blythii* Rus-51	*daubentonii* Moscow Rus-74	*daubentonii* Tula Rus-14	*daubentonii* Tver' Rus-18	*daubentonii* Tver' Rus-19	*daubentonii* Krasnodar Rus-100	*daubentonii* Samara Rus-57	*petax* Barnaul Rus-31	*petax* Altai Rus-4	*petax* Altai Rus-5	*petax* Altai Rus-9	*petax* Buriatia Rus-21	*petax* Tuva Rus-125
Myotis blythi Rus-51	0.97													
daubentonii Moscow Rus-74	0.96	0.83												
daubentonii Tula Rus-14	0.96	0.84	0.22											
daubentonii Tver' Rus-18	0.96	0.82	0.21	0.25										
daubentonii Tver' Rus-19	0.96	0.83	0.26	0.20	0.18									
daubentonii Krasnodar Rus-100	0.96	0.82	0.28	0.28	0.20	0.19								
daubentonii Samara Rus-57	0.95	0.81	0.24	0.24	0.22	0.21	0.23							
petax Barnaul Rus-31	0.97	0.91	0.88	0.88	0.86	0.84	0.86	0.85						
petax Altai Rus-4	0.97	0.89	0.86	0.86	0.84	0.84	0.84	0.83	0.28					
petax Altai Rus-5	0.96	0.89	0.86	0.86	0.84	0.84	0.84	0.83	0.35	0.13				
petax Altai Rus-9	0.97	0.89	0.86	0.86	0.84	0.84	0.84	0.83	0.37	0.27	0.30			
petax Buriatia Rus-21	0.95	0.88	0.87	0.86	0.85	0.84	0.84	0.84	0.33	0.43	0.40	0.32		
petax Tuva Rus-125	0.96	0.88	0.88	0.88	0.86	0.84	0.86	0.86	0.44	0.49	0.44	0.40	0.37	
petax Primorie Rus-123	0.94	0.86	0.87	0.87	0.85	0.83	0.85	0.84	0.45	0.55	0.52	0.44	0.35	0.34

and 0.13–0.55 for *petax*. As for the intraspecific variation, the bootstrap values and GD-indices appear to be also higher in the *M. petax* cluster (see above). Interestingly, much higher maximum values of GD between different specimens of *M. petax* (0.13–0.55 vs. 0.18–0.28), though not reaching the interspecific parameters, simultaneously overlap with those, characterizing the divergence between different subspecies in other mouse-eared bats, such as *M. blythii* (0.39–0.61). These maximum values have separated the Far East specimen of *M. petax* (Rus-123) from the Altai ones (Rus-4, 5, 9, 31; Table 1).

Morphometry

Nineteen skull measurements have been processed with the forward stepwise discriminant analysis; all of them were standardized to the total dispersion to decrease the size influence. Four samples of each species were taken into analysis as identified: from Tobolsk, Bashkiria, the Moscow region and the Caucasus for *M. daubentonii*, and from the vicinities of Vladivostok, Khassan peninsula, Lake Baikal and the Republic of Tuva — for *M. petax*, while all others — as undetermined. The samples of each species, together with the appropriate 'undetermined' specimens, formed their special clusters with high overlap inside each of them. At the same time, no overlap occurred between the two clusters of the first discriminant function (Fig. 2). Moreover, the squared Mahalanobis (D^2) distances between the group centroids varied from 6.02 to 61.27 (Table 2); the distances between the groups of the same cluster were nearly twice smaller than those between the clusters, and the distances from each specimen to the group centroids varied more widely: from about 6.32 to 185.77. The mean values of Mahalanobis D^2 inside each cluster and between them were as follows: 28.37–33.08 and 56.16–67.15 for *petax*, 22.99–33.13

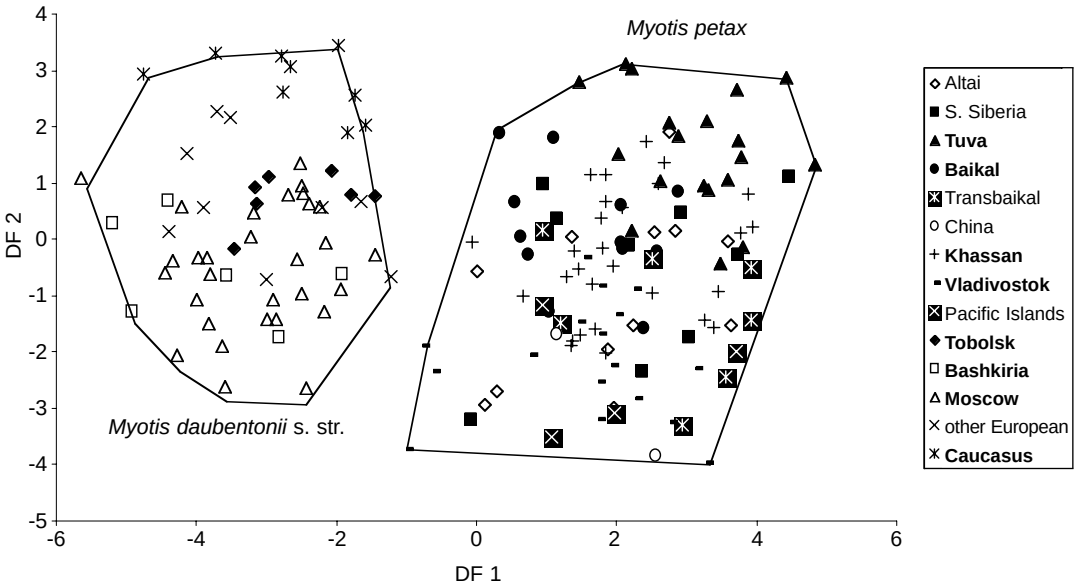

FIG. 2. Bivariate scatterplot of distribution of *Myotis daubentonii* s.str. and *M. petax* in the space of the first two discriminant functions. DF 1 has had greater correlations (all negative) with P^4M^3, BCH, HMD, LC^1, IOW, C^1M^3, C_1M_3, while DF 2 (positively) — with P^4M^3, C^1C^1, M^3M^3, CBL, C^1M^3, C_1M_3, CC^1L and LMD. 'Learning' samples in the legend are typed in bold

and 45.50–62.46 for *daubentonii*, respectively.

Apart from the above, *M. petax* differs from *M. daubentonii* in somewhat shorter upper and lower toothrows (5.12 ± 0.15 mm and 5.46 ± 0.15 mm vs. 5.36 ± 0.12 mm and 5.65 ± 0.13 mm, n = 132 and 59, respectively), and lower braincase (5.61 ± 0.14 mm vs. 5.93 ± 0.15 mm); interorbital width and width of the third upper molar overlap insignificantly. Despite slightly smaller skull in *M. petax*, the rostral and canine widths are somewhat smaller in the 'European' species. Among dental features, the paraconule and paraloph of the third upper molar are usually more or less definite in *M. petax*, while often missing in *M. daubentonii*.

Externally, *M. daubentonii* has slightly longer forearm (37.74 ± 1.02 mm, n = 50) and hind foot (9.63 ± 0.35 mm, n = 10), than *M. petax* (37.16 ± 1,1 mm and 8.24 ± 0.6 mm, respectively, n = 62). Besides, on the larger part of its distribution area *M. petax* differs from *M. daubentonii* in having shorter and more grayish dorsal pelage (which is more or less brown in the latter). However, this feature is rather variable in the bats from Altai and the Far East.

We have also studied photos of the skull and skin of the holotype of *M. petax* (NMNH 175189), kindly provided by Dr. R. W. Thorington (Smithsonian Institution, Washington D.C., USA). Unfortunately, their quality does not allow us to present them here. Despite the fact that the original description by Hollister may not allow the affiliation of the name *Myotis petax* with the concrete taxon, the skull proportions, fur texture and coloration in the holotype are typical of the Altai and Eastern Siberian populations of the discussed species.

Bacular Morphology

A penial bone of *M. petax* has a maximum length of 1.18–1.53 mm (nearly twice as large as in *M. daubentonii*, 0.46–0.79 mm), not distinctly narrowing to the tip (Fig. 3). All bacula of the adult specimens in our collection only slightly exceeded the measurements provided by Yoshiyuki (1989), who reported the mean length of about 1.04 mm.

Apart from the overall size, the bacula of *M. petax* are relatively narrower than in *M. daubentonii* (their width is 1.5–2 times less than the maximum length, while in the latter — 1.15–1.7 times; the mean values for this ratio in our sample — 1.8 and 1.4, respectively), with deep (up to 0.5 mm) and usually narrow basal concavity. Among the European populations of *M. daubentonii*, we have found somewhat similar proportions in the specimens from the Samara region only. The bacular urethral groove of *M. petax* is noteworthy too: it is deep and proportionally narrower (Fig. 4). As for *M. macrodactylus*, its baculum well differs from those of both species (see Fig. 3).

TABLE 2. Mahalanobis D^2 distances between the sample centroids (above the diagonal), and P-level of differences between the learning samples (below the diagonal)

Region	Khassan	Tuva	Selenga	Vladivostok	Tobolsk	Moscow	Bashkir	Caucasus
Khassan		10.23	6.02	10.31	30.95	32.80	47.03	42.05
Tuva	0.000		7.61	21.87	47.79	48.17	61.27	47.21
Selenga	0.031	0.013		12.96	32.26	26.20	41.24	37.66
Vladivostok	0.000	0.000	0.000		38.92	32.40	40.25	46.70
Tobolsk	0.000	0.000	0.000	0.000		14.43	15.89	20.08
Moscow	0.000	0.000	0.000	0.000	0.000		9.93	21.62
Bashkir	0.000	0.000	0.000	0.000	0.019	0.023		26.28
Caucasus	0.000	0.000	0.000	0.000	0.000	0.000	0.000	

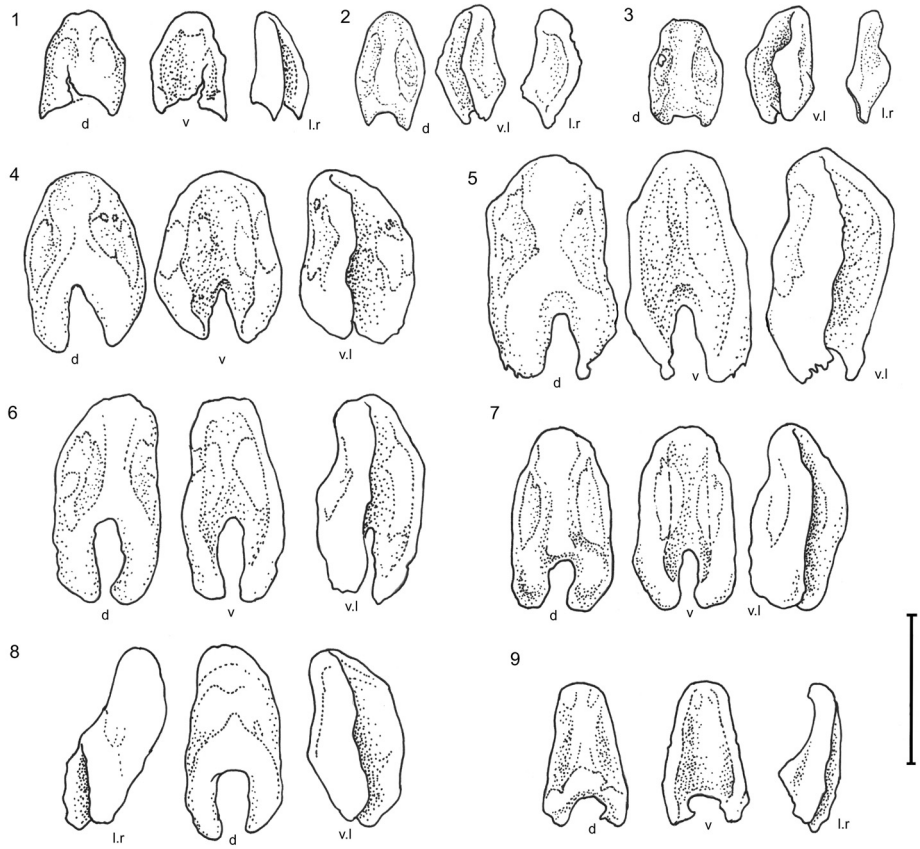

FIG. 3. Penial bones of *M. daubentonii* (1–3), *M. petax* (4–8), and *M. macrodactylus* (9): 1 — Moscow region; 2 — Samara region; 3 — Caucasus; 4 — Altai Mountains; 5 — Republic of Tuva; 6 — eastern Transbaikalia; 7, 9 — Russian Far East (lake Khassan); 8 — Sakhalin. d — dorsal view, v — ventral view, l.l. and l.r. — left and right lateral views, respectively; v.l. — ventro-lateral view. Scale bar = 1 mm

In addition, we have studied a penial bone of the juvenile specimen of *M. petax*: it was distinctly shorter (but not narrower), with relatively shallow basal concavity, like in *M. daubentonii* (Fig. 4).

DISCUSSION

The initial goal of our genetic analysis was, on the one hand, to test the conclusions of Kruskop (2001), who had demonstrated that 'the Altai form of *Myotis daubentonii*', namely *petax*, in fact belonged to the eastern complex of forms, and not to the western one, as it had been widely accepted before that. On the other hand, it was important to check its status genetically, as this form was treated as a distinct species by some specialists (Findley, 1972) from one side, and had never been included into any molecular surveys before, from another.

The results of Inter-SINE(MIR)-PCR have undoubtedly confirmed the above view on the 'original' *petax*, i.e. from the Republic of Altai (here — Rus-4, 5, 9 and 31) as a representative of the 'Asian', or 'eastern complex'. But what is even more important is that according to the chosen molecular marker *M. daubentonii* appeared to be diphyletic, and in fact includes two distinct, and perhaps even fairly distant

species, which conform to the 'European' and 'Asian' complexes outlined by Kruskop (2001, 2004).

The Inter-SINE-PCR indices of genetic distance, as well as the fact that *daubentonii* and *petax* have formed two strongly supported homogenous clusters, can only be considered as a validation of the species distinctiveness of *M. petax*. Moreover, the two forms are separated by the GD-values (0.83 to 0.88) comparable to those between each of them and *M. blythii* (see Table 1). These values are considerably higher than those within both forms, i.e., 0.18–0.28 for *daubentonii* and 0.13–0.55 for *petax*, totally conforming to the appropriate intraspecific parameters in the rest of Vespertilionidae studied earlier (Bannikova *et al.*, 2002; Matveev *et al.*, 2002; Matveev, 2004).

This indirectly proves that *M. daubentonii* and *M. petax* may in fact be not closely related at all, though the present research does not allow speculating about the relationships between *petax* and other *Myotis* species. Nonetheless, this issue undoubtedly represents a definite interest and demands a special investigation with the use of sufficient material, including Nearctic species, among which *M. petax* was placed by Findley (1972).

The intraspecific structure of *M. daubentonii* s.str. and *M. petax* has not been revealed by the molecular marker, as the sample size was not sufficient. Nevertheless, it appears that *M. daubentonii* is noticeably more uniform, at least within Russia's borders, than *M. petax*. The Far-Eastern specimen of *M. petax* appeared to be genetically the most distant from the Altai ones, in other words geographically the most remote populations. On the one hand, this may serve as evidence of the presence of several subspecies of *M. petax*, which seems to be rather probable, taking its wide range into consideration, as well as a great variety of habitats and ecological conditions through its range. On the other hand, the reason may be in a more compact area of sampling in the case of *M. daubentonii* (Fig. 5). However, additional studies

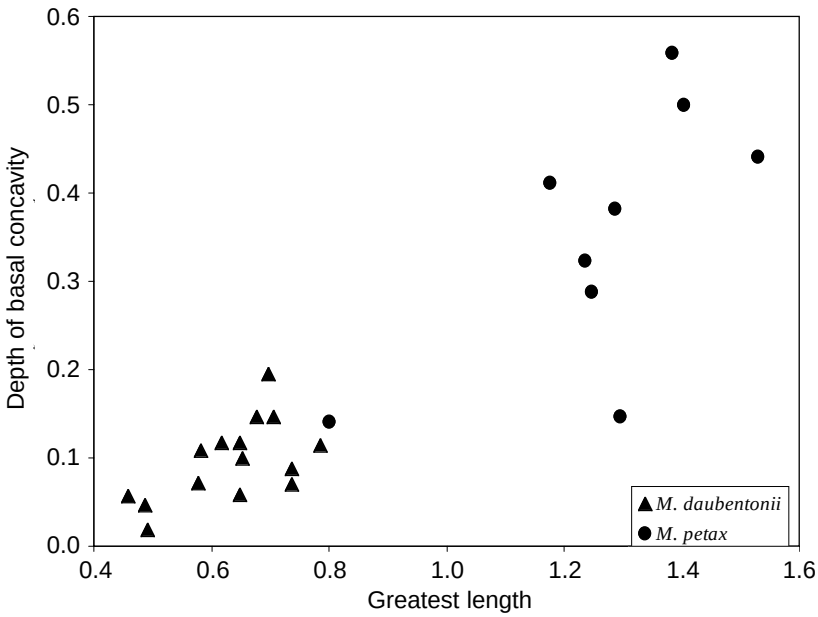

FIG. 4. Scatterplot of the greatest length and depth of the basal concavity of penial bones in *M. daubentonii* and *M. petax*. Marginal sample of *M. petax* is the juvenile specimen from Russian Far East

on sufficiently numerous samples are necessary to pick up the subspecific structure of both species with Inter-SINE-PCR.

The principal component analysis applied previously (Kruskop, 2001, 2004) did not separate *daubentonii* and *petax* in full. However, the level of difference between them appeared to be rather high, and could even be compared with that between some other species. The above molecular results induced us to re-evaluate our previous approach to the morphometric analysis of the two species in the way described above. This has yielded the cogent results, completely correlating with our molecular data, inferred from Inter-SINE(MIR)-PCR: the Mahalanobis D^2 between the groups of the same cluster of either *M. daubentonii* or *M. petax* were nearly twice smaller than those between the clusters, the same as the maximum internal GD-values for *M. daubentonii* and *M. petax* were in the similar ratio with those characterising the distance between them, and totally conforming to the interspecific indices received earlier for the rest of the studied Vespertilionidae species (Matveev, 2004).

The bacula morphology also confirms and illustrates our conclusions about the species distinctiveness of *M. petax*. At the end of 1980s Yoshiyuki (1989) described os penis of the 'eastern Daubenton's bat' (under the name *M. d. ussuriensis*) using the material from three specimens, and this has been the only remaining description of a baculum of *M. petax* to date. The difference in the bacular morphology between the two species is quite pronounced, as has been described above: in general aspect the whole bone of *M. petax* looks more similar to that of *M. dasycneme* (e.g., Smirnov, 2000), rather than that of *M. daubentonii*. Obviously, the bacular features on their own appear to be good

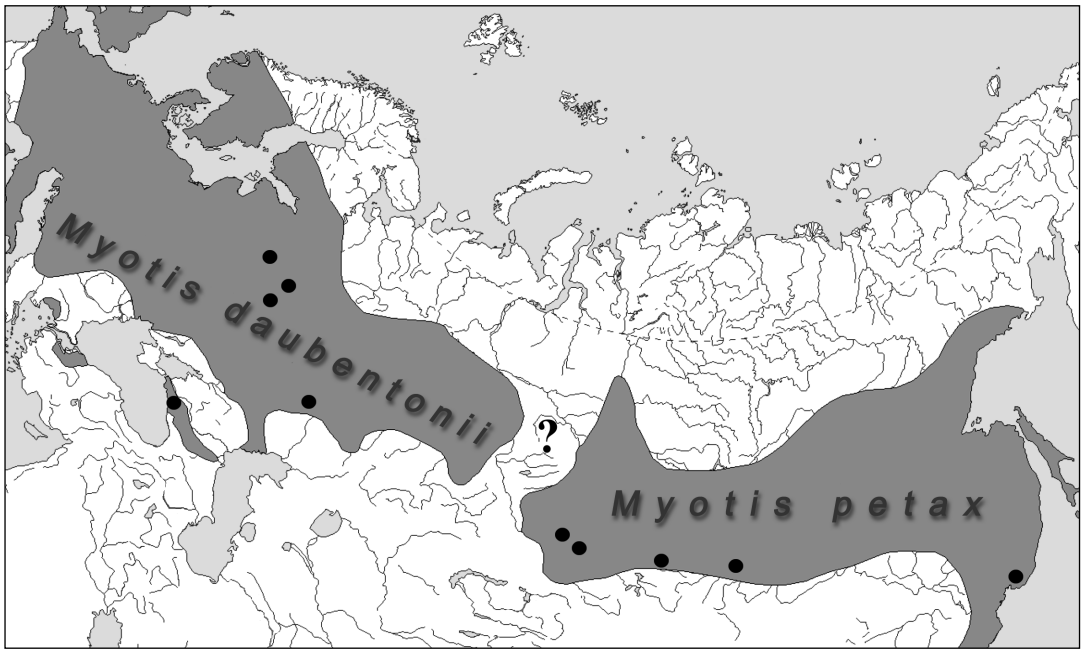

FIG. 5. The vast transpalearctic range, thought to be occupied by one single species, Daubenton's bat, in fact should be divided into two parts: between *M. daubentonii* in the west and *M. petax* in the east. The geographical 'border' between both species, or areas of their possible sympatry are not known, but are likely to occur in the Omsk region. Genetic material was sampled from the areas marked with dots

enough for distinguishing between the two species.

Remarks on Ecology

Myotis daubentonii is well known for its strong adherence to open water and typical feeding behaviour, when the bat makes low and steady flights close to water surface, taking its prey from or very close to it (e.g., Jones and Rayner, 1988; Kalko and Schnitzler, 1989). The same behavioural pattern was observed several times in *M. petax* too (e.g., in the vicinity of Baikal). At the same time, our recent observations in the same area in August 2004 have shown that the most common species in the region, demonstrating the same or similar feeding behaviour in most habitats, is *M. ikonnikovi*, and that it may be often taken for 'Daubenton's bat' if one relies on visual observations only. Moreover, none of *M. petax*, which we observed in the Republic of Altai (Matveev, 2004), behaved in this way. All bats of the same colony used to fly above the meadow or in the birch grove, separating this meadow from Teletskoye Lake and the river. They usually made their flights 2–6 m above ground, or sometimes right above the grass, making sudden loops or steep dives from time to time. With all this, none could be seen feeding above the adjacent water reservoirs. The same behaviour has been observed in some places in Transbaikalia (Kruskop, 2003), while our latest observations in the vicinity of Baikal have also shown the presence of the typical '*daubentonii*-like' feeding pattern. Then it appears that *M. daubentonii* and *M. petax* are not identical in their feeding ecology and behaviour as well. The trophic dependence upon open water is less pronounced in the latter, than in its sibling species. However, this issue still requires a special investigation in the field, as well as reassessment of already existing literature data in the view of our latest findings. The difference in the observed frequencies in the two species was not that prominent: the maximum output in the case of *M. petax* was around 47–48 kHz, while 45 kHz in *M. daubentonii*.

Distribution

To speak of the distributional patterns of both species, the most topical issue now in this regard is to find a 'border' between their geographic ranges, or an area of possible sympatry (in other words the eastern bound in the distribution of *M. daubentonii* and the western — for *M. petax*): both could actually occur in Omsk region — to certain extent the area representing a gap in the reliable records (Fig. 5). The last time '*M. daubentonii*' was found there was more than half a century ago (Shukhov, 1949), while the task-oriented search by the group of the regional specialists (Kuzmin *et al.*, 2000) in the course of the last 20 years has provided no results. However that may be, this is another task for future accurate investigations in the field, as well as for the reassessment of the existing museum material.

Conclusion

Based on the above (including the holotype examinations), the name *Myotis petax* Hollister, 1912 must be accepted as a senior name valid for what was formerly known as the 'eastern complex of forms of *M. daubentonii*' (with *ussuriensis* Ognev, 1927, *loukashkini* Shamel, 1942, *abei* Yoshikura, 1944, and *chosanensis* Tiunov, 1997 as junior synonyms). Such allocation of *abei* comes from the recent revision of its status by Tsytsulina (2004).

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APPENDIX I

Material studied

The following museums and depositaries have been covered in our research: ISU — Irkutsk State University (Irkutsk, Russia), KMHN — Kirov Regional Museum of History and Nature, ZISP — Zoological Institute of Russian Academy of Sciences (Saint Petersburg, Russia), ZMMU — Zoological Museum of Moscow State University (Moscow, Russia). Additional designations, such as 'Rus' and 'Vtn' followed by the number, refer to the series of tissue samples from the personal collection of the first author (underlined)

Myotis daubentonii s.str. — Russia, Western Siberia, Tobolsk: ZMMU (S-13700, S-13702–13708, S-84018); Russia, the Urals, Bashkiria: ZMMU (S-84023–84026, S-84031, S-113000); European Russia, north-western Caucasus: Rus-100, ZISP 80845– 80846, ZMMU (S-166222, S-167383, S-171260, S-173331–173332, S-173335, S-173337, S-173339– 173340, S-174669); European Russia, the Moscow region: Rus-74, ZMMU (S-13715, S-13717, S-13723–13728, S-101137–101138, S-101175, S-101181, S-101189–101190, S-101194–101199, S-101204, S-101209–101210, S-165838); European Russia, the Tver' region: Rus-(18–19); European Russia, the Samara region: ZMMU (S-174784)/ Rus-57; European Russia, Volgograd region: ZMMU S-167240; European Russia, the Leningrad region: ZISP 42697; European Russia, the Tula region: Rus-14; Estonia: ZMMU S-49494–49496, ZISP (33467, 33469); Belarus, Vitebsk region: ZMMU S-166186–166187; Czech Republic: ZMMU S-74768–74769.

M. petax — Japan: ZISP 59102–59103; Mongolia, Dalai Nor: ZMMU S-103842–103843; China, Manchuria: ZMMU S-84014–84015; Russia, the Kuriles: ZMMU S-60140–60142; Russia, Sakhalin: ZMMU (S-52492, S-175244); Russian Far East, Khassan: ZMMU (S-86494–86496, S-86498, S-86502–86506, S-86508, S-104320, S-104331, S-104333–104334, S-10438–10440, S-104342–104344, S-104346, S-104349–104350, S-104352–104355, S-104357–104359, S-104362); Russian Far East, Vladivostok: ZMMU (S-103863, S-103865–103879, S-103883–103884), ZMMU (S-173255)/Rus-123; Russia, Upper Amur flow: ZMMU S-175362– 175363; Russia, Eastern Siberia, the Chita region: ZMMU

S-167579; Russia, Eastern Siberia, Irkutsk: ISU (358, 376); Russia, Western Siberia, Tuva: ZISP (64465–64466, 64468–64477, 79613), ZMMU S-167740–167742, ZMMU (S-168637)/Rus-125, KMHN (0135/1, 0740/1–0743/1); Russia, Buriatia: ZISP (66108, 66111, 66114), Rus-21, ZMMU (S-103844–103847, S-103849–103850, S-103852–103856, S-103858, S-103860); Russia, Western Siberia, Altai: ZMMU (S-33154, S-61858, S-103861–103862, S-154255), ZMMU (S-173291)/Rus-4, ZMMU (S-173292)/Rus-5, ZMMU (S-173296)/Rus-9, ZISP (64460, 64462, D-5, D-10, D-12), Rus-31; Russia, Western Siberia, Khakassia: ZISP (59597–59602, 64461, 64463–64464, A-80, B-4, B-65, B-80–81, Г-7, Г-11, Г-14); Russia, Western Siberia, the Yenisei river: ZISP (Ъ-1, Ъ-6–7, Ъ-32, Ъ-36, Ъ-42); eastern Kazakhstan, Katon-Karagai: ZMMU S-144925–144927.

M. macrodactylus — Japan: ZISP 59100–59101; Russian Far East: ZMMU (S-18958, S-41734, S-86359, S-104365–104366, S-104370–104375).

M. capaccinii — Former Yugoslavia: ZISP 35045–35048; Bulgaria: ZISP 48035–48036; Albania: ZMMU S-74670; ?Southern Europe: ZISP 50096; Turkey, Anatolia: ZISP 5891.

M. blythii — European Russia, north-western Caucasus: Rus-51.

Eudiscopus denticulus — Vietnam, Cat-Tien: ZMMU (S-172558)/Vtn-11.

Appendix II

Inter-SINE-PCR-derived binary matrix

Moscow Rus-74
0001101000000011010011100000001000000000000101111001000110001010000100111000000100010010100110001100011000000100000110000010100100001001010100101000000010010001000011000011000001000001100000010100100001001010100101000000010010001000

Tula Rus-14
0001101000011011010011001010001000000000000010110010001100010100001000010000001000100101001100011000110000001000001000001010010000100101011000101000000010010001000011000011000001000001100000010100100001001011000101000000010010001000

Tver' Rus-18
0001101000000011010011110000001000000000000010110010001100010100001000010010001000100100011000110000011000000100001100000010100100001001010100101000000010010000001000011000011000001000001100000010100100001001010100101000000010010000000

Tver' Rus-19
0001101000000011010011010010001000000000000010110010101100010100001000010010001000000101000110000011000000100001000000010100100001001010100101000000010010000000000

Krasnodar Rus-100
0001101000000011010000010010001000000000000010110000001100010100001010110010001000000101000110000011000000100001000000010100100001001010100101000000010010000000010

Samara Rus-57
0001101000000011010011000000000000000000000010110001001100010100001000000010001000000101000110000011000000100001000000010100100001001010100101000000010010001000011

Barnaul Rus-31
0100010100000011101010000000000000010100000001001100011000100101000000000000101100000010010001100000011001001010000001000011000100001001010100100100001000001000010000000

Gornoaltaisk Rus-4
0100010000000011111010000000000000010100000001001100000001010010100010000001011000001001000110000001100100101010000100001100010000000101010010010000100000100010000000

Gornoaltaisk Rus-5
0100010000000011111010000000000000010100000001001100000001010010100010000001011000001001000110000001100100101010000100001100010000000101010011010010000000001010000000

Gornoaltaisk Rus-9
0100010000000011111010000000000000010100000001001100000000100110100010000001011000000001001110000011000001010100001000001100010000000101010010010000000010000000001100

Buriatia Rus-21
0010010000000011101010000000000000010100000001001100000000100101000000000001011000000001001100000011001001010000001000011000100000001010100100100000000100000001000110

Tuva Rus-125
0000010010000111010100000000000010001010000010011000110001001001110100000010110000001001000111010000011000001010000001010011000100000001010000010001100
Primorie Rus-123
0000010000000111010100000000000000101010000010011000110001001000000000000010110001000010001110100001101000101000100100101100010000100000101000100010100
Myotis blythii Rus-51
1001100000000110010000000101010101010100000000101100001100100000001000001001000010001000100000000000010001100000001000100010000100000110001000010000101000010000101001000
Eudiscopus denticulus Vtn-11
0000000001100000001100000000100000000000111010000000000000000000000010000001000001100000000000100101100001100000111000000000000100001100010000000000000000000010000001