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Systematics of the four *Notiophilus* Dumeril, 1806 (Coleoptera: Carabidae) species based on morphological and molecular data

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The research on the systematics of four species of the genus *Notiophilus* Dumeril, 1806 (Coleoptera: Carabidae) that has been based on morphological features and molecular data is presented in this article. For molecular species identification and phylogenetic analysis of four species of the genus *Notiophilus* Dum. we used dry collection specimens to get genetic information from rare of scarce material. The research has been carried out for *N. aquaticus* (Linnaeus, 1758), *N. semistriatus* Say 1823, *N. jakovlevi* Tschitscherine, 1903 and *N. semenovi* Tscitscherine, 1903. All sequences were detected for the first time. On the basis of the results, we propose to consider *Notiophilus semenovi* Tschitscherine, 1903 stat. n., previously rated as *Notiophilus aquqticus* (L.) synonymous, a valid species.

Key words: *Notiophilus, aquaticus, semistriatus, jakovlevi, semenovi*, Coleoptera, Carabidae, morphology, phylogeny, molecular data

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INTRODUCTION

Research on the systematics of the genus *Notiophilus* Dumeril, 1806 (Coleoptera: Carabidae) basing on the synthesis of morphological and molecular data practically has not been carried out to date. The present article proposes to make use of the analysis of morphological features and molecular data for 4 species of this genus, all of which putatively included into the group "*aquaticus*". The areas of their distribution overlap in definite places (in Central Asia, Siberia, the Far East, North America). The 4 species analyzed in the present article are: *N. aquaticus* (Linnaeus, 1758) widely distributed in the Holoarctic region, *N. semistriatus* Say 1823 that is found in the Eastern Palearctic region and the Nearctic region, the Altai endemician *N. jakovlevi* Tschitscherine, 1903, and the taxon that to date has been considered the synonym of *N. aquaticus* (L.) – *N. semenovi* Tscitscherine, 1903 that is to be found in Central Asia (Kazakhstan, Kyrgyzstan). Having analyzed the overlap of the regions in detail we can conclude that *N. aquaticus* (L.) has the widest distribution. In Central Asia its distribution area overlaps with the area of *N. semenovi* Tschitsch. In great part of Eastern Siberia, the far East and North America

it overlaps with *N. semistriatus* Say area, but in the Altai and surroundings the distribution area of *N. aquaticus* (L.) overlaps with those of *N. jakovlevi* Tscitsch. and *N. semistriatus* Say (Barševskis 2007). But it should be noted that in the Altai mountains, where three of the abovementioned species (apart from *N. semenovi* Tschitsch.) are distributed all together, some differences in their ecological niches have been observed (R.Dudko pers. commun.).

The present article does not consider some others, little known species of the "*aquaticus*" group from Siberia – *N. sibiricus* Motschulsky 1844 and *N. hyperboreus* Kryzhanovskij, 1995, because the accurately identified material of these species was not available. At present the specimens of these species, deposited in Zoological Institute of Russian Academy of Sciences in St.Petersburg and in Zoological Museum of Lomonosov Moscow State University in Moscow, are being studied. The morphological revision of the specimens being accomplished, the molecular analysis will be carried out for greater number of specimens from Siberia and North America. The aim of the present article is to analyze the morphological and molecular data in order to ascertain the taxonomic status of these phylogenetically close species. The results of the research testify to the fact that *N. semenovi* Tschitsch. is a valid species.

MATERIALS AND METHODS

Samples

Thirty two dry specimens of four species of the genus *Notiophilus* from the collection of the Institute of Systematic Biology, Daugavpils University (DUBC) have been studied (Table 1). All beetles have been identified by prof. Arvīds Barševskis. The number of specimens per species was a minimum of five to a maximum of sixteen. The methodology of measurements - in compliance with J.Schmidt and M.Hartman (2001). The laboratory research and measurements have been done using *Nikon* AZ100 and *Nikon* SMZ745T digital stereo-microscope and NIS-Elements 6D software.

DNA extraction

For genomic DNA extraction, dissected legs from the one side of each specimen or complete specimens were used. Legs of insects were homogenized and powdered grinding with mortar and pestle, than were placed in 1.5 ml Eppendorf tubes. Complete specimens were placed in a 1.5 ml microcentrifuge tubes without any pretreatment. In both cases, purification of total DNA was done by using of the Qiagen DNeasy Blood & Tissue Kit. The Spin-Columns Protocol for purification of total DNA from animal tissues with modifications was used. All samples were fully immersed in tissue lysis buffer ATL, added 20 mL proteinase and lysed overnight with gentle agitation at 56 ^{0}C. After incubation, complete specimens were removed from the buffer, placed in 100% ethanol for 4 hours, air-dried and replaced in the collection. Further DNA extraction for the complete specimens was done from the diggestion buffer. Subsequent procedure for the both kinds of samples included 2 min treatment with RNase A (100mg/ml) at room temperature and lysis in Buffer AL with further DNA binding on the Dneasy Mini spin columns. Previously, samples, Buffer AL and ethanol were mixed immediately by vortexing. Membrane washing procedure was done by using 500 mL buffers AW1 and AW2. For increasing the final DNA yield in the eluate, 200 mL elution buffer was used.

For DNA quantification, the spectrophotometer NanoDrop 1000 was used. Estimation of DNA quality was done by measuring the 260:280 absorbance ratio.

DNA amplification

The universal primers LCO1490 and HCO2198 (Folmer et al., 1994) were used for the COI gene fragment amplification. Primers according to Maddison et al. (2009) with some modifications were used for fragment amplification of 28S and 18S. Each 25 mL PCR reaction contained 12.5 mL AmliTaq Gold® 360 Master Mix, 1 mkM of each

primer, 1 mL extracted DNA and PCR-grade water.

The PCR protocol for COI gene was initial denaturation at 94^{0}C for 5 min, 32 cycles with 94^{0}C for 45 s, annealing at 66^{0}C for 45s, extension at 72^{0}C for 2 min and final extension for 8 min (Raupach et al., 2010). Fragment of 18S was amplified under following conditions: 94^{0}C for 3 min, 35 cycles with 94^{0}C for 30 s, annealing at 50^{0}C for 30s and extension at 72^{0}C for 8 min. The PCR temperature protocol for 28S was 94^{0}C for 3 min, 35 cycles with 94^{0}C for 30 s, annealing at 55^{0}C for 30s and extension at 72^{0}C for 8 min (Maddison, 2008). Negative and positive controls were included in each set of reactions. The amplified products were sized by electrophoresis in a 1 % agarose gel with ethidium bromide. The obtained PCR products were purified following the protocol of the QIAquick PCR purification kit (Qiagen, Hilden, Germany).

Sequencing and data analysis

Sequencing was done using an ABI 3730xl capillary sequencer (Applied Biosystems), using BigDye Terminator v 3.1 chemistry (Applied Biosystems). Sequences of CO1 (bar-coding region), 28SrDNA and 18SrDNA genes were aligned in the SeqScape v2.5. p – distance ($p=n_d/n$, where n_d is the number of nucleotide differences between two sequences and n is the total number of nucleotides compared) and *K2P* distance

$$d_{K2P} = -\frac{1}{2}\ln(1-2P-Q) - \frac{1}{4}\ln(1-2Q),$$

where P is the proportion of transitions and Q is the proportion of transvertions) (Kimura, 1991) were detected on intra- and interspecific levels.

RESULTS AND DISCUSSION

Morphological data analysis

All four species analyzed have a number of similar features. The article presents a detailed morphological description of the species *N. semenovi* Tschitsch., as in the original description this species has been described on the basis of only one female (Tschitscherine 1903). In the other species we have described the main differences from *N. semenovi* Tschitsch.

***Notiophilus semenovi* Tscitscherin, 1903 stat. nov.**

The body is elongated, 4.75-5.55 mm long. The upper part is monochrome with metallic lustre.

Head. The head is of the same width or slightly wider than the pronotum. Its width together with eyes is 1.45-1.65 mm (n=20). The upper part of the head is black, with metallic lustre. Eyes are big and hemispheric. The forehead has 6 rather parallel frontal furrows, which in some specimens are slightly irregular or some of them are doubled in the front. The outer striae, which separate forehead furrows form the rest of the forehead, are straight, very deeply impressed and wide. The stria at the eye basis is much shallower than other outer impressed forehead striae. In the middle of the eye basis it has a small setiferous puncture in both sides. Clypeus has elongated ridges of irregular form, the greatest of which is the central one. At the basis of clypeus there are two setiferous punctures. Labrum is mat, but in the front, along the setiferous punctures it is smooth, lustrous, without micro-sculpture. The centre line is of variable length. There is a convex row of 6 setiferous punctures along its rounded front margin. The antennae are dichromatic: the 1st antennomere is darker, the segments 2-4 are russet, the 3rd and the 4th segments being slightly darkened in the ends, but the other segments, beginning with the fifth, are black. The last segment of maxyllary palp and labial palp is dark. Bottom of the head is rather lustrous, without punctate, there are many distinct, smoothed, transversal plications.

Thorax. Pronotum length: 0.85-1.00 mm. Width: 1.45-1.60 mm. Pronotum is black, with bronze lustre, with concave side margins before the base. In some specimens it has a distinct band of rows of dots and reticulate micro-sculpture. Its discal part is lustrous, almost smooth with smoother, slightly uneven vestiges of plications. Along the

margins the pronotum has rough dots, which in some places turn into plications. The centre line is rather deeply impressed in its discal part, but it is practically not distinct at the base and in the frontal part. There are separate larger dots and more distinct plications. The hind angles of the pronotum are big, sharp, slightly projected sideward. Its basal impressions are widely impressed. Prosternum is lustrous, roughly dotted. Prothorax protuberance is lustrous, dotted, has a deep U-form striae at its margins. The coxae of fore-legs are black, their trochanters, tibiae and tarsi are russet or in some places transparent russet-brown. Tarsi have micro-sculpture. Femora are black and lustrous. In male the 3rd basic segments of the tarsi of fore-legs are widened and have the soles of thick silvery hair from below. Mesothorax is black and lustrous. Mid-legs are of the same colour as the fore-legs but with darker tarsi. Metathorax is black, smooth in the middle, lustrous, but heavily dotted along the sides. The episterna are dotted too. The proportion between the length and the width of the episterna is 2. The hind-legs, except trochanters, are black.

Elytra. Length: 2.95-3.25 mm. Width: 1.75-2.10 mm. The surface is monochrome black with bronze lustre. The sides of the elytra are not parallel; behind the shoulders, approximately against the dorsal setiferous puncture they are slightly concave, but then – before the top – they are slightly widened. The rows of dots on the elytra have large dots, they are slightly impressed at the base, but in the top part they become thin and some do not reach the very top. The rows of dots 5 and 6 are slightly impressed not only at the base, but often also in the dorsal part. In the elytra ends there is 1 apical setiferous puncture, rarely with one on one elytron but with two apical setiferous punctures on the other. The tops of the elytra have fine micro-sculpture. The 2nd interval of the elytra is specular, in the middle being of the same width as the following two taken together, but in the basal part in some species it is even wider that the width of the two following intervals. The dorsal setiferous puncture is situated in the 4th space between rows and is approximated to the third row of dots. The intervals 3 and 4 are about the same width. The 4th interval is noticeably wider than the 5th one. In some specimens the intervals 5 and 6 have reticulate micro-sculpture at full length or only in the base or apical parts. In some specimens they are lustrous and smooth. Transitional forms also have been observed. This feature is variable in this species.

Abdomen. The abdomen sternites are flat, lustrous, and mostly smooth. Male's anal sternite has two setiferous punctures, female's – four setiferous punctures.

Male genitalia. *Aedeagus* is convex, slightly twisted around its axis.

Differantial diagnosis. All the four species analyzed in the article are very similar morphologically.

N. semenovi Tschitsch. (Fig. 1) differs from *N. aquaticus* (L.) (Fig. 2) with different form of the pronotum, which is much more heart-shaped and with sharp base angles that are projected sideward. The elytra have more intense punctate in the rows of dots. In *N. semenovi* Tschitsch. the rows of dots with large dots usually reach at least the top quarter of the elytra, but in *N. aquaticus* (L.) they often contain small dots already from the middle of elytra, but in the apical part they often disappear at all. The colour of legs is different, too. Legs of *N. semenovi* Tscitsch. usually are dichromatic, at least the trochanters and tibiae are russet, but in *N. aquaticus* (L.) they usually are monochrome black (except some aberration, for which the fore-legs might be at least slightly russet). Looking from above the antennomere of *N. semenovi* Tscitsch. Antennae are russet but in *N. aquaticus* (L.) they are darker, almost monochrome, but from below they are a little russet. There are differences also in the constitution of male's *aedeagus*. In *N. semenovi* Tscitsch. *aedeagus* is shorter and more convex, more twisted around its axis than in *N. aquaticus* (L.).

N. semenovi Tschitsch. differs from *N. jakovlevi* Tschitsch (Fig. 3) with different form of the pronotum, which is slightly heart-shaped, with base angles of the prothorax sclerite being less

Fig. 1. *N. semenovi* Tschitsch.

sharp and not so much projected sideward. The elytra at the base have less impressed rows of dots. In *N. semenovi* Tschitsch. the rows of dots do not reach the top as distinctly as in *N. jakovlevi* Tschitsch. The surface of their elytra usually is less lustrous and has more visible micro-sculpture than in *N. jakovlevi* Tschitsch. The basic segments of antennae, tibiae and trochanters are lighter, more russet than in *N. jakovlevi* Tschitsch., whose legs in fact are black.

Fig. 2 *N. aquaticus* (L.)

Fig. 3 *N. jakovlevi* Tschitsch.

There are differences in the constitution of male's *aedeagus*, which in *N. semenovi* Tschitsch. is approximately of the same length, but less convex and twisted around its axis in a different way than it is in *N. jakovlevi* Tschitsch.

N. semenovi Tschitsch. differs from *N. semistriatus* Say (Fig. 4) in the form of the prothorax sclerite, which is more heart-shaped, more narrowed at the base and has sharp, but smaller base angles. The rows of dots on the elytra are different, as well. In *N. semenovi* Tschitsch. the 3rd and the 4th intervals each are noticeably wider than the intervals 5 and 6. But in *N. semistriatus* Say all the intervals 3-6 are approximately of the same width or only slightly narrower. Both species have slightly different form of the scutellum. There are differences in the constitution of male's *aedeagus*, which in *N. semenovi* Tschitsch. is longer, differently convex and twisted around its axis in a little different way, as well as has differently bent-down lamella than in *N. jakovlevi* Tschitsch.

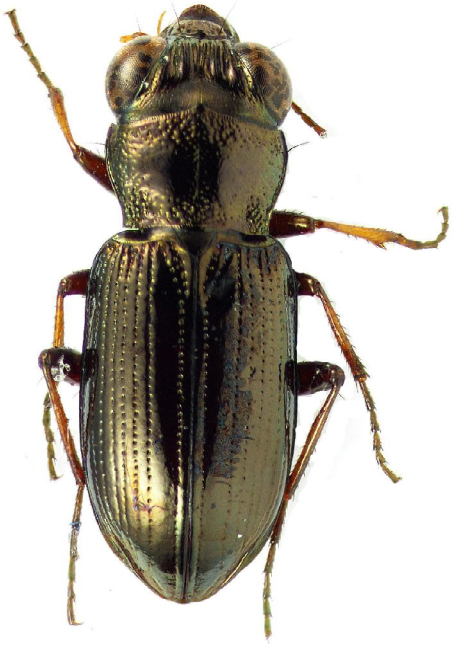

Fig. 4. *N. semistriatus* Say

Note. Till now this taxon has been considered to be a synonym of *N. aquaticus* (L.) (Kryzhanovskij et al. 1995; Lorenz 1998, 2005). A.Bar□evskis (2007) was the first to indicate that this taxon is a valid species was. Morphological features and molecular data obtained in this work support this assumption.

Results of DNA analysis

It was not possible to extract DNA from the sample NA/USA1934b. DNA concentration varied from 20 to74 ng/μL, according to the sample. In the most cases, the 260:280 absorbance ratio satisfied for pure DNA requirements and varied between 1.8 – 2.2. In a few samples only (NS/USA/1952, NSem/Ki1987 and NS/R1996) the ratio was lower than 1.8 (data not shown).

In ~50 % of the samples too little product from a single amplification was obtained. First amplification product was isolated from the gel and used as template for reamplification with a higher annealing temperature. In samples NA/USA1934a, NA/L/Da2002, NA/R/A2007 and NA/M2003 were not possible to detect amplification product even after the reamplification. Approximately 650 bp for COI in bar-coding region, 900-930 bp for 28S and 1800-1860 bp for 18S long sequences were detected in the rest of samples analyzed.
Molecular sequence data was used for the detection of the level intra and inter specific variation among four investigated *Notiophilus* species. *p*-distances and *K2P* distances for COI, 18S and 28S between *Notiophilus* species sequences presented in Table 2-4. COI was the most variable gene. Interspecific *K2P* distances and *p*-distances for COI ranged from 8.8 % to 9.4 % and from 8.2 % to 9.0 %, respectively. The least variable gene was 18S, *K2P* distances and *p*-distances varied from 3.0 % to 4.1. % and from 2.9 % to 3.9 %, respectively, while for 28S, *K2P* distances and *p*-distances ranged from 4.9 % to 5.7 % and from 4.7 % to 5.2 %, respectively. For all three markers, sequence divergence between *Notiophilus aquaticus* and *Notiophilus semenovi* considerably above the thresholds described in literature (Hebert et al., 2003; Cognato, 2006; Raupach et al., 2010), thus indicating that species mentioned above are two distinct species.

All species showed no intraspecific variation in 18S. Individuals *of Notiophilus aquaticus* sampled in Caucasus and Syberia (Na/Ra/Ca2004; Na/R/S2000; Na/R/S2002; Na/R/S2006 and Na/R/S2007) showed some intraspecific variation in 28S, ranging from 0.0 % to 1.0 %. Other species revealed no intraspecific sequence variation for 28S. For COI gene, the lowest intraspecific variations were in specimens of *Notiophilus semenovi* (from 0.0 % to 0.03 %), the highest (from 0.1 % to 2.5 %) – in *Notiophilus aquaticus*. Specimens of *Notiophilus jakovlevi* and *Notiophilus semistriatus* show intraspecific variation. The existence of *Notiophilus aquaticus* subspecies will be discussed in future reseach, involving additional specimens to evaluate these first results.

Based on our results, both morphological and molecular data in species identification, we propose to consider *Notiophilus semenovi* Tschitscherine, 1903, previously rated as *Notiophilus aquaticus* (L.) synonymous, a valid species. Nevertheless, a detailed study, including more species, as well as more specimens and more nuclear markers, is necessary for the genus phylogeny specification. A detailed study of DNA sequences of specimens from various locations, for example, from Siberia, Asia and North America is necessary to more clear understanding of the species complex and variability in so-called “aquaticus” group of species. Besides, our data revealed some interesting features into the genetic variability of 18S, 28S and COI genes of *Notiophilus aquaticus*, which will be discussed in future. In further, specimens from different locations and different molecular markers, including markers with high effectiveness on population level, should be tested.

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Table 1. Specimens used in this study

Species	Number of Specimens	Pinned	Sample ID	Originated	Sampling Year
Notiophilus aquaticus (L.)	1	Yes	NA/L/Da2002	Latvia: Daugavpils distr., Ilgas, Silene Nature park	2002
Notiophilus aquaticus (L.)	1	Yes	NA/R/K2002	Russia:Karelia, Kem. env.,taiga	2002
Notiophilus aquaticus (L.)	1	Yes	NA/R/A2007	Russia: Arhangelsk reg. Kanin penins.,	2007
Notiophilus aquaticus (L.)	1	Yes	NA/R/Ca2004	Russia: W. Caucasus, Krasnodar reg. Tuapse distr., Moldavanovka	2004
Notiophilus aquaticus (L.)	1	No	NA/L/J2002	Latvia: Jekabpils distr., Dunava, mixed forest	2002
Notiophilus aquaticus (L.)	1	No	NA/B1998	Belarus: Vitebsk, *Pinus* forest	1998
Notiophilus aquaticus (L.)	1	Yes	NA/K1990	Kazachstan: E NE Dzhungar mts. Sarg-Bukhtor	1990
Notiophilus aquaticus (L.)	1	Yes	NA/M2003	Mongolia: Arhangay Aimak, Khangay mts. Kholsayagn	2003
Notiophilus aquaticus (L.)	1	Yes	NA/R/S2007	Russia: S Siberia S Kranoyarsk reg. Badyr- taiga	2007
Notiophilus aquaticus (L.)	1	Yes	NA/R/S2006	Russia: East Siberia Irkutsk Territory 18 km N Ust -Kut Valley of Lena	2006
Notiophilus aquaticus (L.)	1	Yes	NA/R/S2000	Russia: SE Siberia, Chita reg. Kodar. Chara	2000
Notiophilus aquaticus (L.)	1	Yes	NA/R/S/2002	Russia: SE Seberia, SW Buryatia reg. E. Sayan Mts	2002
Notiophilus aquaticus (L.)	1	Yes	NA/R/FE2002	Russia: Far East S Primorje, Lazo env..	2002
Notiophilus aquaticus (L.)	1	Yes	NA/R/SKa2002	Russia:NE Siberia Kamcatka Esso env.	2002
Notiophilus aquaticus (L.)	2	Yes	NA/USA1934a NA/USA1934b	USA: Missouri St. Louis, Ranken	1934
Notiophilus semistriatus Say	1	Yes	NS/USA1952	USA: Washington Mount Rainier Nat. park.	1952
Notiophilus semistriatus Say	4	Yes	NS/R1996a NS/R1996b NS/R1996c NS/R1996d	Russia: W Altai Ivanovskyi Hrebet Mnt. 12 km S Leninogrorsk, h=2000m tundra	1996
Notiophilus semenovi Tsch.	2	Yes	NSem/Ki1991a NSem/Ki1991b	East Kirgizstan: Sary - Dzhaz riv. bas. Ashutor riv., 3400 m	1991
Notiophilus semenovi Tsch.	4	No	NSem/Ki1987a NSem/Ki1987b NSem/Ki1987c NSem/Ki1987d	Kirgizstan: Kungei Ala Too mountains Region, Kurmety River	1987
Notiophilus jakovlevi Tsch.	2	Yes	NJ/R/Bu1998a NJ/R/Bu1998b	Russia: Burayatia Hamar – Dabavs Mis. osinovka riv. 2000m	1998
Notiophilus jakovlevi Tsch.	3	Yes	NJ/R/Kr1995a NJ/R/Kr1995b NJ/R/Kr1995c	Russia: Krasnojarski reg. Aradanski xp. Buiba Per. h~1500-2000m Oiskavas riv. reg.	1995

Table 2. *K2P*-distances (%) and *p* (%) distances detected for CO1 between four *Notiophilus* species examined. *p*-distances values presented in brackets

	N. aquaticus (L.)	*N. semenovi* Tsch.	*N. jakovlevi* Tsch.
N. aquaticus (L.)	-	-	-
N. semenovi Tsch.	8.8 (8.2)	-	-
N. jakovlevi Tsch.	9.1 (8.6)	8.9 (8.5)	-
N. semistriatus Say	9.4 (9.0)	9.2 (8.7)	9.0 (8.5)

Table 3. *K2P*-distances (%) and *p* (%) distances detected for 28S between four *Notiophilus* species examined. *p*-distances values presented in brackets

	N. aquaticus (L.)	*N. semenovi* Tsch.	*N. jakovlevi* Tsch.
N. aquaticus (L.)	-	-	-
N. semenovi Tsch.	4.9 (4.7)	-	-
N. jakovlevi Tsch.	5.3 (4.9)	5.1 (4.9)	-
N. semistriatus Say	5.7 (5.2)	5.5 (5.0)	5.0 (4.8)

Table 4. *K2P*-distances (%) and *p* (%) distances detected for 18S between four *Notiophilus* species examined. *p*-distances values presented in brackets

	N. aquaticus (L.)	*N. semenovi* Tschitsch.	*N. jakovlevi* Tsch.
N. aquaticus (L.)	-	-	-
N. semenovi Tsch.	3.0 (2.9)	-	-
N. jakovlevi Tsch.	3.5 (3.1)	3.1 (2.9)	-
N. semistriatus Say	4.1 (3.9)	3.4 (3.2)	3.1 (2.9)

REFERENCES

Barševskis A. 2007. Biogeography of the genus Notiophilus Dumeril, 1806 (Coleoptera: Carabidae). Baltic J. Coleopterol. 7 (1): 121-135.

Cognato A. I. 2006. Standard Percent DNA Sequence Difference for Insects Does Not Predict Species Boundaries. Journal of Economic Entomology 99 (4): 1037-1045.

Folmer O., Black M., Hoeh W., Lutz R., Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotech 3: 294-299.

Hebert PDN, Cywinska A, Ball SL, deWaard JR. 2003. Biological identifications through DNA barcodes. Proc R Soc B **270:** 313-321.

Kimura M. 1991. The neutral theory of molecular evolution: a review of recent evidence. *Jpn. J. Genet.66(4): 367-386.*

Kryzhanovskij O.L., Belousov I.A., Kabak I.I., Kataev B.M., Makarov K.V., Shilenkov V.G. A Checklist of the Ground-beetles of Russia and Adjacent Lands (Insecta, Coleoptera, Carabidae). Pensoft Publishers, Sofia – Moscow: 32-33.

Lorenz W. 1998. A systematic list of extant ground beetles of the World (Insecta Coleoptera "Geadephaga": Trachypachidae and Carabidae incl. Paussinae, Cicindelinae, Rhysodinae), First Edition: 1 - 502.

Lorenz W. 2005. A systematic list of extant ground beetles of the World (Insecta Coleoptera "Geadephaga": Trachypachidae and Carabidae incl. Paussinae, Cicindelinae, Rhysodinae), Second Edition:530.

Maddison D. R. 2008. Systematics of the North American Beetle Subgenus *Pseudoperyphus* (Coleoptera: Carabidae: *Bembidion)* based upon Morphological, C hromosomal and Molecular Data. Annals of Carnegie Museum**77**(1): 147-193.

Raupach M. J., Astrin J. J., Hannig K., Peters M. K., Stoeckle M. Y., Wägele J-W. 2010. Molecular species identification of Central European ground beetles (Coleoptera: Carabodae) using nuclear rDNA expansion segments and DNA barcodes. Frontiers in Zoology, **7**: 26.

Schmidt J., Hartman M. 2001. Schmidt J., Hartmann M. 2001. Revision der Notiophilus – Arten Nepals (Coleoptera, Carabidae). Veroffentlichungen Naturkundemuseum Erfurt. 20: 165 – 179.

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