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TWO NEW SPECIES OF CHIGGER MITES (ACARI: TROMBICULIDAE) CLOSE TO *NEOTROMBICULA MINUTA*, APPLICATION OF NONLINEAR MULTIVARIATE STATISTICS

A. A. Stekol'nikov

Zoological Institute, Russian Academy of Sciences, Universitetskaya 1, St. Petersburg 199034, RUSSIA, e-mail: acari@zin.ru

ABSTRACT: Two new species of chigger mites, *Neotrombicula urartensis* sp. n. from rodents and birds and *N. kubanensis* sp. n. from rodents, are described from Caucasus. Morphological discontinuity of *Neotrombicula urartensis* and *N. scrupulosa* was demonstrated using logistic regression analysis.

KEY WORDS: Chiggers, taxonomy, parasites, Caucasus, logistic regression analysis

INTRODUCTION

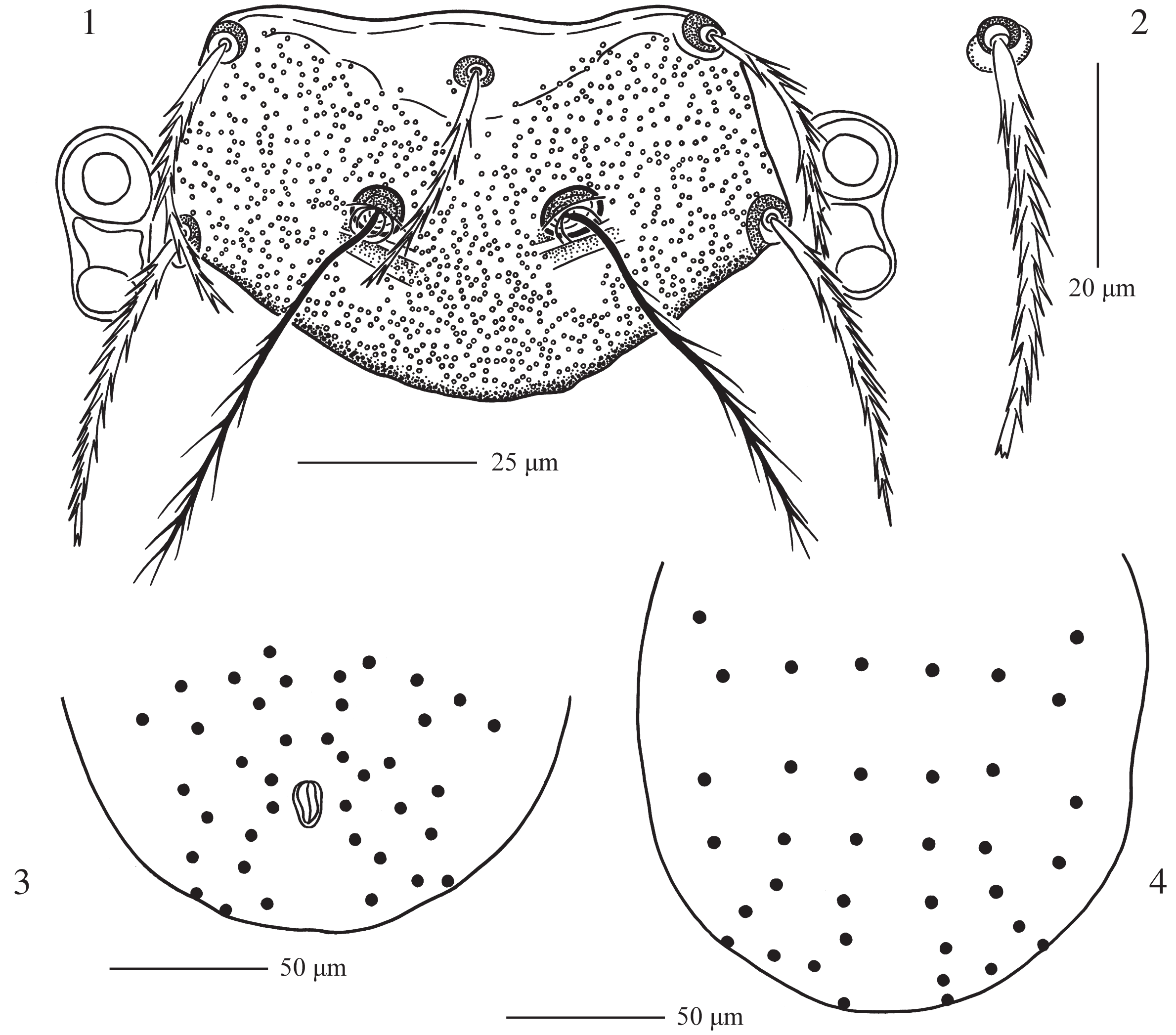
The species group *Neotrombicula minuta* Schluger, 1966 includes eight Palearctic species, including the widely distributed and variable *N. scrupulosa* Kudryashova, 1993 and several species known mainly from their type localities (Stekol'nikov, 1995). Re-examination of the collection preserved in the Zoological Institute (Saint Petersburg, Russia) revealed that *N. corvi* Kolebinova, 1971 recorded from Armenia is actually a new species, *N. urartensis* sp.n. This species is described using non-linear multivariate statistics. We also describe *N. kubanensis* sp.n. of the *minuta* group from Western Caucasus.

MATERIAL AND METHODS

Chiggers were collected into vials containing 70% alcohol and mounted in Faure-Berlese medium. Morphometric characters were obtained with an ocular micrometer. All measurements are given in micrometres (µm). Coordinates of the collection localities are acquired from the US National Geospatial-Intelligence Agency (NGA) database (http://gnswww.nga.mil/geonames/GNS/index.jsp). Altitudes of populated places were acquired from the Global Gazetteer Version 2.1 database (Falling Rain Genomics, Inc., http://www.fallingrain.com/world/), altitudes of the collection localities at the mountainsides of Semashkho and Papay Mts. were estimated using topographic maps, scale 1:150000. Type specimens are deposited in the acarological collection of the Zoological Institute of the Russian Academy of Sciences, Saint Petersburg.

Terminology follows Goff et al. (1982), with some adaptation: D_{min} and D_{max} — minimal and maximal length of dorsal idiosomal setae; V_{min} and V_{max} — minimal and maximal length of ventral setae, i.e. setae on the ventral surface of idiosoma excluding coxal and sternal setae; DS — number of dorsal idiosomal and humeral setae; VS — number of ventral setae; TaIII — length of leg III tarsus; m-t — ratio between distance from mastitarsala to the base of leg III tarsus and length of leg III tarsus. We also propose here two new indices alternative to DS and VS: $D_{1–4}$ is the number of dorsal idiosomal setae in rows 1–4 and VC is the number of ventral idiosomal setae and dorsal setae in the subsequent rows. Thus, $D_{1–4}$ + VC = NDV, as well as DS + VS. However, the boundary between the 4^{th} and 5^{th} rows is much more clear, than that between the dorsal and ventral setae. So, our new indices seem to be less subjective, than DS and VS. Moreover, the higher success of VC in the discriminating of *N. urartensis* sp.n. and *N. scrupulosa*, as compared with VS (see below), suggests that this index is more adequate.

The Mann-Whitney U test was used to evaluate the significance of the morphometric differences between *N. urartensis* sp.n. and *N. scrupulosa*. This nonparametric rank-based method is quite applicable to heteroscedastic samples of very unequal size, as in our case. The differences having p values larger than 0.01 were ignored in the differential diagnosis. Logistic (logit) regression was used to construct a diagnostic equation for discriminating these two species. At present this technique, especially in case of dichotomous dependent variable, substitutes discriminant analysis (or canonical variate analysis) in econometrics and ecology, and recently it was applied also to morphometric data in zoology, including a study on mites (Klimov et al. 2006). The logistic regression overcomes the inefficiency of the methods based on linear regression, that arises when the assumptions of homoscedasticity, linearity, and normality are violated (Menard, 2001). It is preferred when sample sizes



Figs 1–4. *Neotrombicula urartensis* sp.n. 1 — scutum and eyes; 2 — dorsal idiosomal seta of 1^{st} row; 3 — arrangement of ventral idiosomal setae in the holotype; 4 — arrangement of dorsal idiosomal setae in the holotype.

are very unequal, as in our case. The negative likelihood function serves as a loss function (a measure of the discrepancy between the observed data and the data predicted by the regression equation) in this method, and there are several procedures that allow finding its minimum and hence obtaining the best parameter estimates for the regression equation. We used here the Quasi-Newton algorithm that approximates the second-order derivatives of the loss function to guide the search for the minimum. Forward and backward stepwise, and best subset methods were used for the variable selection. All calculations were performed by means of Statistica for Windows software (StatSoft Inc., Tulsa, OK, USA), version 7.0.

SYSTEMATICS

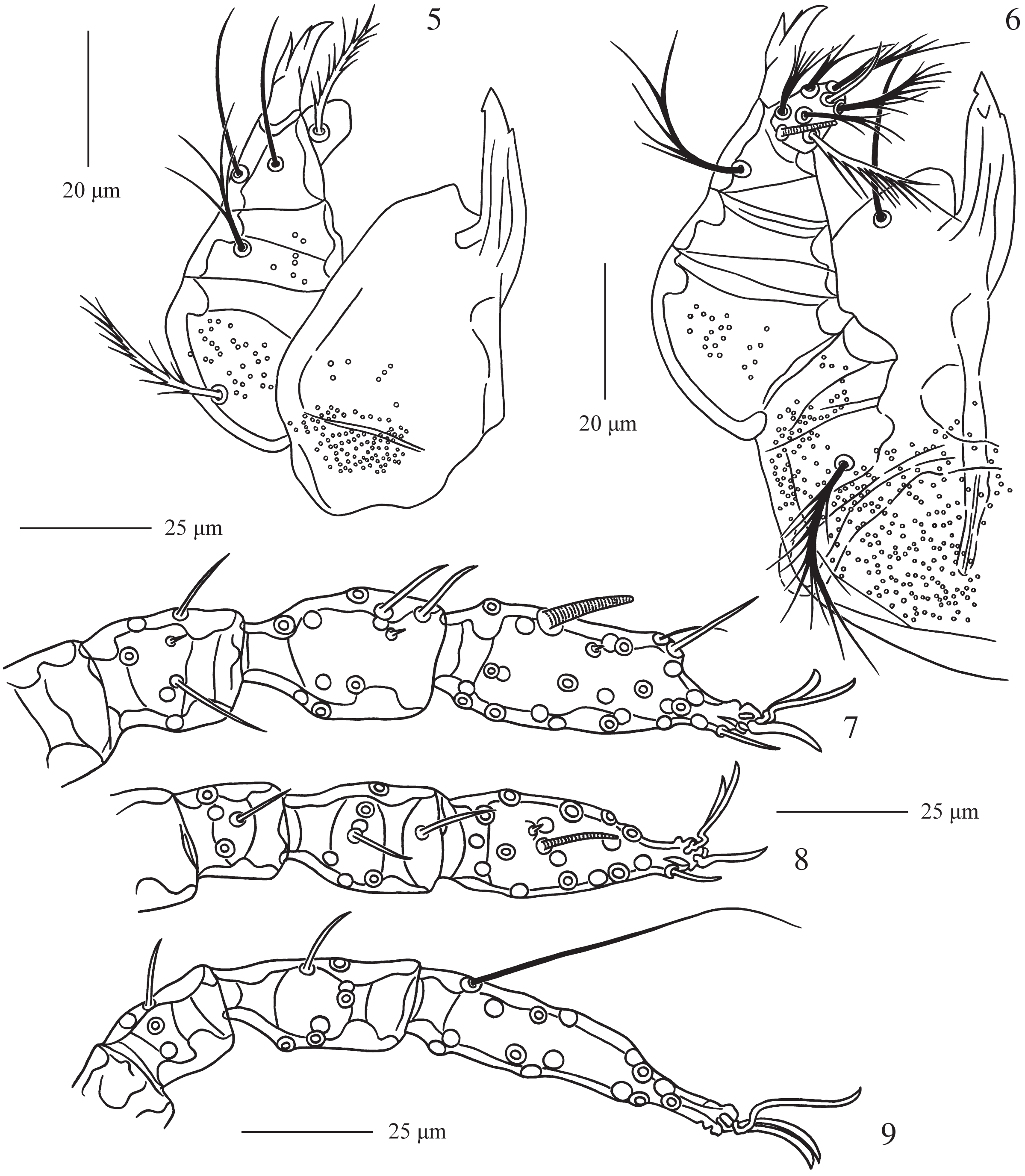
Neotrombicula urartensis Stekolnikov, sp. n.

corvi (non Kolebinova, 1971): Stekol'nikov, 1995: 262.

Figs 1–9.

Diagnosis. SIF = 7BS-N-3-2111.1000; fPp = B/B/NNB; fCx = 1.1.1; fSt = 2.2; fSc: PL > AL > AM; Ip = 828; fD = 2H-(6–8)-6-6-4-(10–16); DS = 34–43; VS = 33–42; NDV = 69–80.

Description. Larva. *Idiosoma*. Eyes 2+2. One pair of humeral setae; 32–41 dorsal idiosomal setae; there are 6 setae (sometimes 7 or 8) in 1^{st} posthumeral row, 6 setae (rarely 7) in 2^{nd} row, 6 setae in 3^{rd} row, 4 setae in 4^{th} row, 10–16 setae in next rows; 4 sternal setae and 33–42 ventral setae; total number of idiosomal setae (excluding sternal) 69–80; all setae moderately barbed. *Gnathosoma*. Cheliceral blade with tricuspid cap; gnathobase with 1 pair of branched setae; galeala nude; palpal claw with 3 prongs; seta on palpal femur feathered; seta on palpal genu with few branches; dorsal and lateral palpal tibial setae nude, ventral palpal tibial seta branched; palpal tarsus with 7 branched setae, subterminala, and tarsala. *Scu-*



Figs 5–9. *Neotrombicula urartensis* sp.n. 5 — dorsal aspect of gnathosoma; 6 — ventral aspect of gnathosoma; 7 — leg I; 8 — leg II; 9 — leg III.

tum. Densely punctate, nearly pentagonal, with rounded posterior margin; AM base posterior to level of ALs; SB slightly anterior to level of PLs (PSB–P-PL = 2.5); PL > AL > AM; sensilla flagelliform with 12–15 moderate branches in distal 3/4. *Legs*. All 7-segmented, with pair of claws and clawlike empodium. Leg I: coxa with 1 non-specialized branched seta (1B); trochanter 1B; basifemur 1B; telofemur 5B; genu 4B, 2 genualae, microgenuala; tibia 8B, 2 tibialae, microtibiala; tarsus 22B, tarsala 17–18 long, microtarsala distad of tarsala, subterminala, parasubterminala, pretarsala. Leg II: coxa 1B; trochanter 1B; basifemur 2B; telofemur 4B; genu 3B, genuala; tibia 6B, 2 tibialae; tarsus 16B, tarsala 16 long, microtarsala proximad of tarsala, pretarsala. Leg III: coxa 1B; trochanter 1B; basifemur 2B; telofemur 3B; genu 3B, genuala; tibia 6B, tibiala; tarsus 14B, nude mastitarsala.

Standard measurements are shown in Table 1.

Table 1
Standard measurements of the *Neotrombicula urartensis* type series (n = 8)

	AW	PW	SB	ASB	PSB	SD	P-PL	AP	AM	AL	PL	S	H
Holotype	73	89	28	29	29	58	26	29	36	40	50	66	43
Minimum	70	89	28	27	29	56	25	26	34	36	47	62	42
Maximum	74	92	31	30	31	59	30	31	37	43	52	70	52
Mean	72	90	29	29	29	58	27	29	36	40	50	65	47
SD	1.2	1.4	1.1	0.9	0.9	1.8	1.8	1.0	1.0	1.6	0.9	2.1	2.9

D_{min}	D_{max}	V_{min}	V_{max}	pa	pm	pp	Ip	TaIII	m-t	DS	D_{1-4}	VS	VC	NDV
34	45	30	38	301	252	295	848	73	0.173	36	24	39	51	75
30	45	25	33	281	234	265	799	65	0.141	34	24	33	45	69
34	50	30	38	304	266	295	853	77	0.200	43	27	42	56	80
33	46	28	36	291	254	284	828	73	0.164	37	25	39	50	75
1.9	1.8	1.5	2.0	8.9	9.7	9.6	28.2	4.1	0.022	3.0	1.2	3.0	3.3	6.0

Hosts. *Chionomys nivalis* (Martins, 1842) (Rodentia, Cricetidae), *Apodemus (Sylvaemus)* sp. (Rodentia, Muridae), *Coturnix coturnix* L., 1758 (Galliformes, Phasianidae).

Type material. Holotype larva (no. 1473, T–Tr.–36) and 7 paratype larvae, Armenia, Shirak Marz, Musayelyan (40°45′55″N, 43°59′27″E, 1729 m above sea level), 28 Aug. – 11 Sept. 1979, ex *Chionomys nivalis*, coll. N.A. Filippova, I.V. Panova.

Non-type material. 22 larvae (Armenia, coll. Filippova & Panova): 19 larvae, Shirak Marz, 6 km from Amasia (1863 m), 3–5 Sept. 1979, ex *Ch. nivalis*; 1 larva, Shirak Marz, Sarnakhpyur (Sarnaghbyur) (40°31′08″N, 43°55′12″E, 2038 m), 24–26 Aug. 1979, ex *A. (S.)* sp.; 2 larvae, Lorri Marz, Tumanyan (40°59′12″N, 44°39′21″E, 1135 m), 19–20 Aug. 1979, ex *A. (S.)* sp. Two larvae, Armenia, Tavush Marz, Haghartsin (Kuybyshev) (40°46′41″N, 44°57′45″E, 1060 m), 3 Aug. 1956, ex *C. coturnix*, coll. K.S. Akhumyan.

Etymology. The specific epithet refers to the *terra typica*, that is located on the former territory of the ancient kingdom Urartu.

Differential diagnosis. The new species differs from *N. scrupulosa* in having much more numerous ventral and caudal idiosomal setae (larger VC, VS, and NDV), lesser number of dorsal idiosomal setae in rows 1–4 (D_{1-4}), lesser m-t, and longer AM. There are usually 6 setae in 1st row (13 of 17 specimens measured), while *N. scrupulosa* has more frequently 7–11 setae in 1st row (only 28 specimens of 244, i.e. 11.5%, have 6 setae in 1st row). The differences of the new species from the Caucasian form of *N. scrupulosa* (including materials from Krasnodarskiy Kray, Karachay-Cherkessia, Kabardino-Balkaria, North Ossetia-Alania, Dagestan, Armenia, and North-Eastern Turkey), in addition to the above characters, include the larger scutum (PW, ASB, PSB, SD, AP, and P-PL), the longer PL and D_{max}, and the longer legs (Ip, TaIII). Main differences are summarized in Table 2.

Remarks. This material was previously identified as *N. corvi* Kolebinova, 1971 (Stekol'nikov, 1995). The latter was described by a single specimen collected in Bulgaria (Sofia Province, Godech) from *Corvus cornix* L. (Kolebinova, 1971, 1992). It has only 26 ventral idiosomal setae and NDV = 56, which makes its identity with our material unlikely. In addition, according to our measurements of the original figures, this specimen has m-t = 0.288, which is significantly larger, than in *N. urartensis* sp.n. (Tables 1 and 2). *N. corvi* is rather similar to *N. scrupulosa*, but the identity of these two species can not be established without studying material from the type locality of *N. corvi*.

Neotrombicula urartensis sp.n. was found together with *N. scrupulosa* in Haghartsin, where two specimens of the former species and 6 specimens of the latter one were collected from the same host individual. The sympatric occurrence of these two species suggests for their reproductive isolation.

Neotrombicula kubanensis **Stekolnikov, sp.n.**

Figs 10–18.

Diagnosis. SIF = 7BS-N-3-2111.1000; fPp = B/B/NNB; fCx = 1.1.1; fSt = 2.2; fSc: PL > AM > AL; Ip = 740; fD = 2H-6-6-6(8)-(2–6)-…; DS = 26–33; VS = 21–29; NDV = 47–62.

Description. Larva. *Idiosoma*. Eyes 2+2. One pair of humeral setae; 24–31 dorsal idiosomal setae; 6 setae in 1st and 2nd posthumeral rows, 6 or 8

Table 2

Differences between *Neotrombicula urartensis* and *N. scrupulosa* (min-max, mean ± standard error of mean, 99% confidence interval for mean)

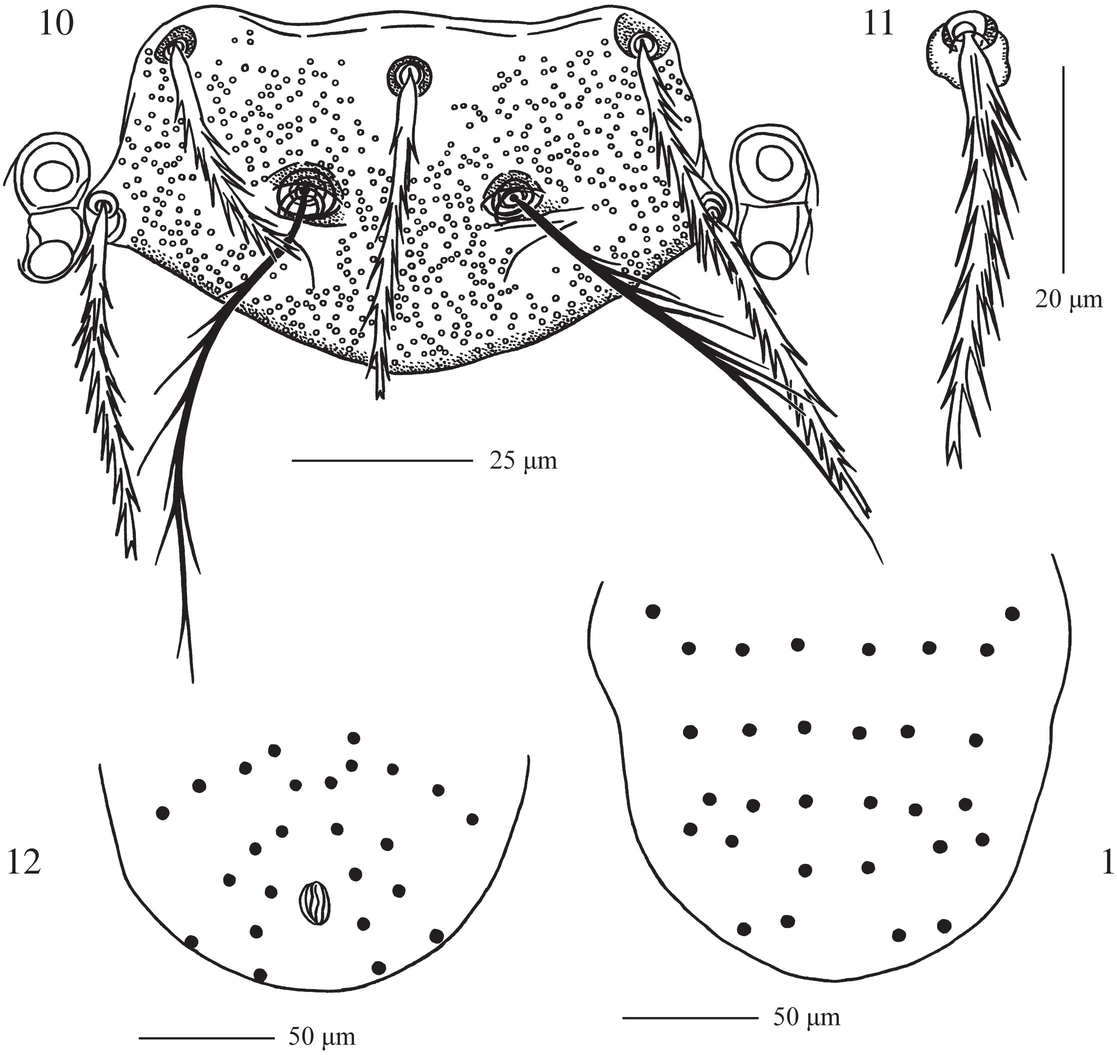
Species	*Neotrombicula urartensis*		*Neotrombicula scrupulosa*	
Sample	Type series	All	Caucasian form	All
N	8	17	114	244
PSB	29–31 29.5 ± 0.33 28.4–30.6	26–32 28.9 ± 0.38 27.8–30.0	23–33 26.7 ± 0.16 26.3–27.1	23–34 28.2 ± 0.14 27.8–28.5
SD	56–60 58.1 ± 0.44 56.6–59.7	53–61 57.3 ± 0.53 55.8–58.8	46–62 54.0 ± 0.23 53.4–54.6	46–62 55.8 ± 0.19 55.3–56.3
P-PL	25–30 27.0 ±0.63 24.8–29.2	23–30 26.0 ± 0.50 24.5–27.5	18–28 23.7 ± 0.17 23.2–24.1	18–29 24.4 ± 0.12 24.1–24.7
AP	27–30 28.8 ± 0.37 27.5–30.0	27–31 28.9 ± 0.26 28.2–29.7	23–31 27.2 ± 0.16 26.8–27.6	23–34 28.3 ± 0.13 28.0–28.7
AM	34–37 36.1 ± 0.35 34.9–37.4	30–38 35.5 ± 0.54 33.9–37.1	25–41 33.1 ± 0.23 32.5–33.7	25–41 33.4 ± 0.15 33.0–33.8
PL	49–51 49.8 ± 0.31 48.7–50.8	46–51 49.1 ± 0.36 48.1–50.2	36–51 45.2 ± 0.26 44.5–45.9	36–54 45.8 ± 0.18 45.3–46.3
TaIII	65–77 73.6 ± 1.46 68.5–78.7	65–83 75.4 ± 0.93 72.6–78.1	63–79 70.0 ± 0.31 69.2–70.8	63–85 73.2 ± 0.29 72.4–73.9
m-t	0.141–0.200 0.164 ± 0.0076 0.137–0.191	0.133–0.202 0.167 ± 0.0051 0.152–0.182	0.147–0.324 0.234 ± 0.0026 0.228–0.241	0.147–0.324 0.229 ± 0.0017 0.224–0.233
D_{1-4}	24–27 24.8 ± 0.41 23.3–26.2	23–27 24.5 ± 0.27 23.7–25.3	23–31 26.2 ± 0.13 25.9–26.5	22–34 26.3 ± 0.11 26.0–26.6
VS	33–42 38.5 ± 1.05 34.8–42.2	32–43 37.8 ± 0.81 35.4–40.2	19–35 26.1 ± 0.25 25.5–26.8	19–37 27.3 ± 0.19 26.8–27.8
VC	45–56 50.4 ± 1.18 46.2–54.5	42–56 49.3 ± 0.97 46.5–52.1	26–42 33.9 ± 0.26 33.2–34.5	26–45 35.1 ± 0.22 34.5–35.7
NDV	69–80 75.1 ± 1.30 70.6–79.7	66–80 73.8 ± 1.02 70.8–76.8	52–69 60.1 ± 0.30 59.3–60.8	52–74 61.4 ± 0.27 60.7–62.1

setae in 3rd row, usually 4 setae in 4th row, 2–8 setae in next rows; 4 sternal setae and 21–29 ventral setae; total number of idiosomal setae (excluding sternal) 47–62; all setae with long thick barbs. *Gnathosoma*. Cheliceral blade with tricuspid cap; gnathobase with 1 pair of branched setae; galeala nude; palpal claw with 3 prongs; seta on palpal femur feathered with long barbs; seta on palpal genu with few branches; dorsal and lateral palpal tibial setae nude, ventral palpal tibial seta with long branches; palpal tarsus with 7 branched setae, subterminala, and tarsala. *Scutum*. Densely punctated, nearly pentagonal; AM base posterior to level of ALs; SB at level of PLs (PSB – P-PL from – 1 to 2); PL > AM > AL; sensilla flagelliform with 7–10 long branches in distal 3/4. *Legs*. As in previous species. Tarsalae I and II 15–16 long.

Standard measurements are shown in Table 3.

Hosts. *Microtus majori* Thomas, 1906 (Rodentia, Cricetidae), *Apodemus (Sylvaemus)* sp. (Rodentia, Muridae).

Type material. Holotype larva (no. 2921, T–Tr.–37), Russia, Krasnodarskiy Kray, Semashkho



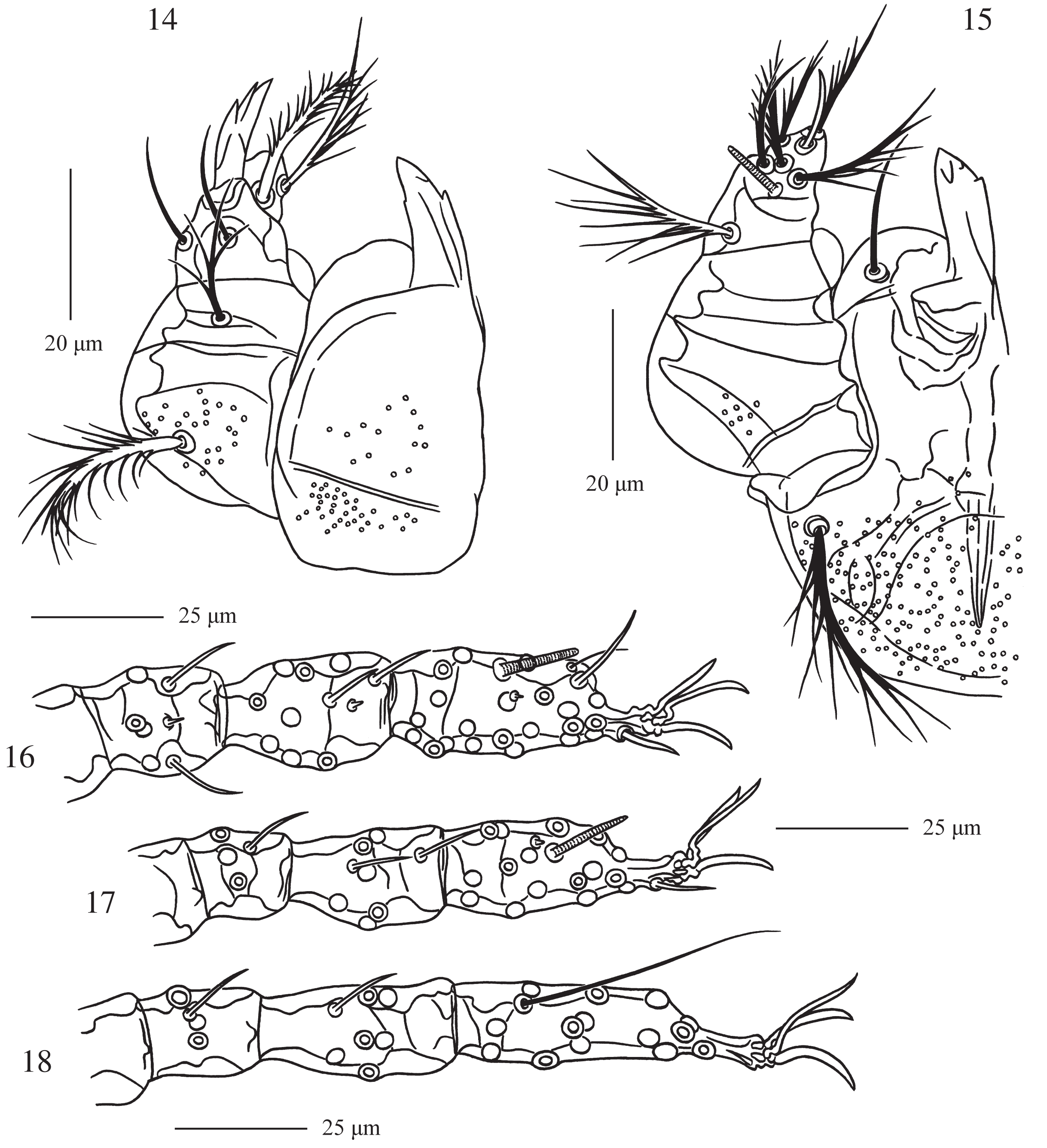
Figs 10–13. *Neotrombicula kubanensis* sp.n. 10 — scutum and eyes; 11 — dorsal seta of 1st row; 12 — arrangement of ventral idiosomal setae in the holotype; 13 — arrangement of dorsal idiosomal setae in the holotype.

Table 3
Standard measurements of the *Neotrombicula kubanensis* type series (n = 7)

	AW	PW	SB	ASB	PSB	SD	P-PL	AP	AM	AL	PL	S	H
Holotype	68	85	31	29	22	51	22	23	45	38	50	74	49
Minimum	64	81	28	24	22	46	22	22	45	35	46	67	48
Maximum	68	86	31	29	26	55	25	26	49	41	51	77	55
Mean	66	84	30	26	24	50	23	24	46	38	49	72	50
SD	1.6	2.0	1.2	1.6	1.3	2.9	1.1	1.3	1.5	2.1	1.6	3.9	2.4

D_{min}	D_{max}	V_{min}	V_{max}	pa	pm	pp	Ip	TaIII	m-t	DS	D_{1-4}	VS	VC	NDV
41	50	30	41	250	230	261	741	63	0.186	30	26	25	29	55
32	46	30	41	238	223	241	702	58	0.186	26	24	21	27	47
41	54	34	50	265	243	268	776	66	0.266	33	26	29	36	62
38	50	32	44	251	231	258	740	64	0.223	30	25	25	30	55
3.5	2.5	1.9	3.9	8.7	6.0	8.3	23.0	2.8	0.028	2.3	1.1	2.8	2.9	5.1

Mt., 1030 m, 22 Aug. 1994, ex *Microtus* sp., coll. A.A. Stekol'nikov. Six paratype larvae (Russia, Krasnodarskiy Kray, coll. A.A. Stekol'nikov): 2 larvae with same data as holotype; 2 larvae, Papay Mt., 800 m, 18 June 2002, ex *M. majori*; 1 larva, Altubinal (458 m), 29 Aug. 1995, ex *M. majori*; 1 larva, Kamyshanov (at Laganaki Massif) (1273 m), 26 Aug. 1994, ex *A.* (*S.*) sp.



Figs 14–18. *Neotrombicula kubanensis* sp.n. 14 — dorsal aspect of gnathosoma; 15 — ventral aspect of gnathosoma; 16 — leg I; 17 — leg II; 18 — leg III.

Etymology. The specific epithet derives from Kuban', the old name of Krasnodarskiy Kray, where the *terra typica* is located.

Differential diagnosis. The new species clearly differs from all other species of the *minuta* group in having much longer AM reaching posterior scutal margin (AM = 45–49 versus 25–44). The new species resembles *N. agriotricha* Stekolnikov, 1994 in having long barbs on scutal and idiosomal setae and similar arrangement of dorsal idiosomal setae, but differs from it (except for the longer AM) in having a nude lateral palpal tibial seta versus branched, smaller scutum (AW = 64–68, PW = 81–86, PSB = 22–26, SD = 46–55, AP = 22–26, versus 68–71, 86–92, 27–32, 54–60, and 27–31, respectively), the much shorter legs (Ip = 702–776 and TaIII = 58–66 versus 844–900 and 76–83), and the lesser number of idiosomal setae (VS = 21–29 and NDV = 47–62 versus 28–33 and 59–67).

DIAGNOSTICS

Contribution of different characters to discrimination between *N. urartensis* sp. n. and *N. scrupulosa* depends on the geographic origin of *N.*

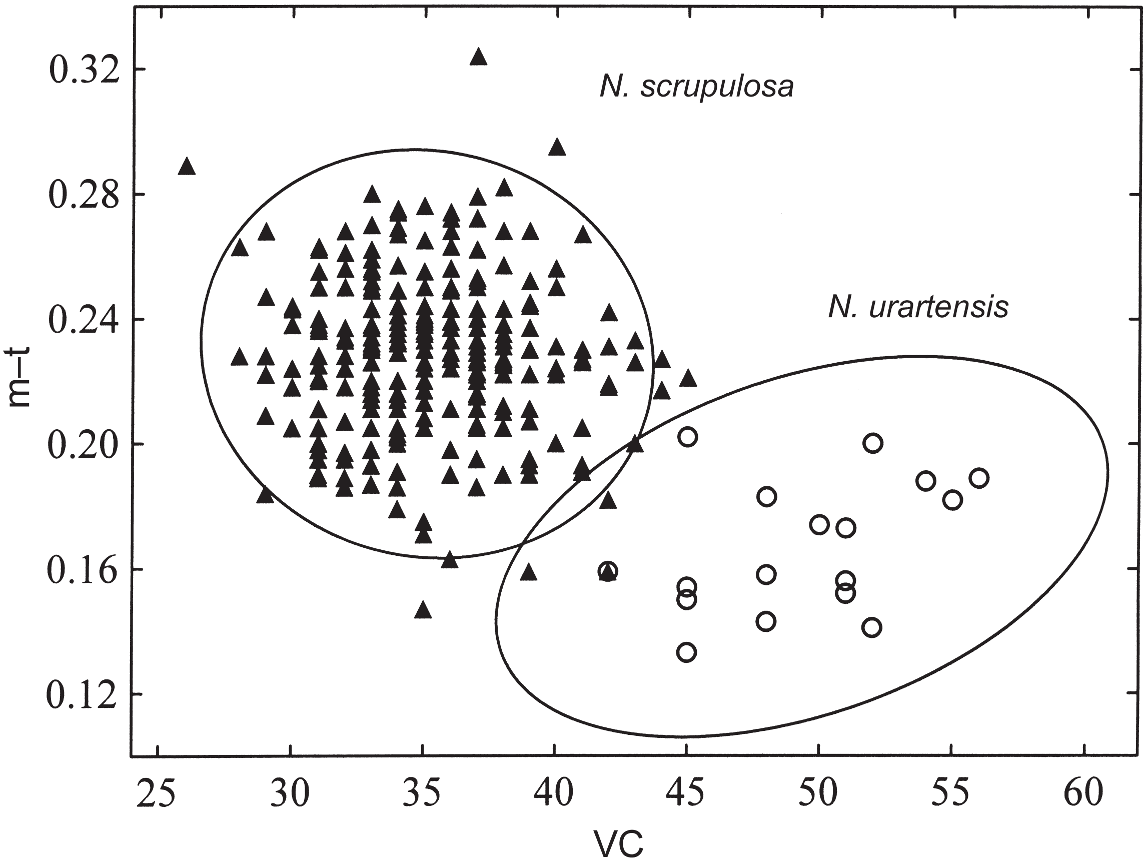


Fig. 19. Distribution of the *Neotrombicula urartensis* sp.n. and *N. scrupulosa* specimens by the values of VC and m–t.

scrupulosa samples used in the analysis. According to our data, the latter species includes three geographic forms: Caucasian (Krasnodarskiy Kray, Karachay-Cherkessia, Kabardino-Balkaria, North Ossetia-Alania, Dagestan, Armenia, and North-Eastern Turkey), mountain Central Asian (Southern Kazakhstan, Kyrgyzstan, Altai Republic, and Tuva), and plain Asian (Bashkortostan, Northern Kazakhstan, Altai Territory, and Mongolia), differing by morphometric traits. Therefore, we compare our sample of *N. urartensis* (n = 17) separately with each *N. scrupulosa* form. The following six indices show significant differences ($p < 0.01$) between *N. urartensis* and each form of *N. scrupulosa* by the Mann-Whitney test: AM, $D_{1\text{–}4}$, VS, VC, NDV, and m-t. Then, different algorithms of the variable selection within the logistic regression (forward and backward stepwise, and best subset) showed that only two indices, VC and m-t, give stable and highly significant contribution to the differentiation between *N. urartensis* and each form of *N. scrupulosa*. The difference between the species by VC and m-t is clearly demonstrated by two-dimensional scatterplot (Fig. 19). However, including these two characters in the regression model resulted in unreliable coefficient estimations, obviously because VC itself provide almost perfect discrimination. The univariate model including only VC was successful when the mountain Central Asian form (n = 82) was included in the analysis as the training sample. Final value of the maximum likelihood function was 7.5. Consequently, the value of – 2*Log Likelihood for the current model was 15.0, while that of the intercept-only model is 90.8. The chi-square value for the difference between these models is highly significant (chi-square = 75.79, df = 1, $p < 0.0000001$). The parameter estimates are given in Table 4, together with their standard errors, the respective t-values (parameters divided by standard errors) and the associated p-levels, upper and lower confidence limits, Wald's Chi-square statistics and associated p-levels for testing the significance of the parameters, and the odds ratios displayed for each parameter estimate for a unit change in the predictor variable.

Logistic regression analysis yielded the following diagnostic equation:

P(*N. urartensis*) = Exp(–56.237 + 1.268*VC)/(1 + Exp(–56.237 + 1.268*VC)).

Table 4
Results of the logistic regression

	Constant	VC
Coefficient	−56.237	1.268
Standard Error	22.294	0.506
t(97)	−2.523	2.503
p-level	0.01	0.01
−95% confidence limit	−100.484	0.262
+95% confidence limit	−11.99	2.273
Wald's Chi-square	6.363	6.264
p-level	0.01	0.01
Odds ratio	3.772E−25	3.552
−95% confidence limit	0	1.3
+95% confidence limit	0.0000062	9.706

If $P > 0.5$, the new specimen should be identified as *N. urartensis*, otherwise it is predicted to be *N. scrupulosa*. Solution of this equation give boundary value of VC between 44 and 45. Thus, according to the obtained diagnostic rule, *N. urartensis* should have VC = 45 (P = 0.69) or more, while in *N. scrupulosa* VC = 44 (P = 0.39) or less. When using this rule, only one specimen from the training sample of *N. scrupulosa* and one specimen from the training sample of *N. urartensis* were misclassified, while the classification accuracy for the rest material of *N. scrupulosa* (n = 162) was 100%. Obviously, the above diagnostic rule, based on single character, should be considered as provisional. Probably obtaining the enough large additional material of *N. urartensis* will allow calculating more reliable discriminant function.

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