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Contribution to the fauna of chiggers (Acariformes: Trombiculidae) parasitizing bats in Spain

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Original research

ABSTRACT

Four chigger mite species were collected from different bat hosts in several provinces of Spain. *Leptotrombidium europaeum* (Daniel & Brelih, 1959) and *Ascacchoengastia latyshevi* (Schluger, 1955) has been recorded for the first time on bats; *Leptotrombidium russicum* (Oudemans, 1902), *A. latyshevi* and *Willmannium cavus moldaviensis* Kudryashova, 1992 have been recorded for the first time from Spain. The latter record represents also the first finding of the genus *Willmannium* Vercammen-Grandjean & Langston, 1976 in Western Europe.

Keywords chiggers; parasites of bats; *Leptotrombidium*; *Willmannium*; *Ascacchoengastia*; fauna; Western Europe

Introduction

Chigger mites (parasitic larvae of Trombiculidae) expose different levels of hosts preferences. Many of them were recorded on many host species belonging to different classes, while some genera and species are specific parasites of amphibians, birds, reptiles or bats (Traub & Wisseman 1968; Goff 1979; Kudryashova 1998). Zajkowska *et al.* (2018) summarized all records of chigger mite species from bat hosts. Shortly thereafter, Stekolnikov & Quetglas (2019) recorded bat chiggers from Spain for the first time, reporting *Oudemansidium komareki* (Daniel & Dusbábek, 1959) from *Pipistrellus kuhlii* (Kuhl) in Menorca and *Trisetica knighti* (Radford, 1954) from *Plecotus austriacus* (Fischer) in Formentera (Balearic Islands). The checklist published by Zajkowska *et al.* (2018) erroneously recorded the American species *Speleocola secunda* Brennan & Jones, 1960 from Spain.

Our collections added four Chiroptera parasitizing chiggers to the Spanish fauna.

Material and Methods

Material was collected in three localities

- 1 — Sierra de Las Nieves National Park, Ronda, Málaga province, 36.6925°N, 5.0439°W. In June 2019, 31 bats belonging to 9 species were mist-netted there and examined for chiggers: family Vespertilionidae: *Eptesicus isabellinus* (Temminck) – 2 specimens, *Hypsugo savii* (Bonaparte) – 2, *Myotis myotis* (Borkhausen) – 3, *Myotis escalerai* Cabrera – 10, *Nyctalus lasiopterus* (Schreber) – 3, *Nyctalus leisleri* (Kuhl) – 2, *Pipistrellus pipistrellus* (Schreber) – 1, *Plecotus austriacus* (J. Fischer) – 7; family Miniopteridae: *Miniopterus schreibersii*

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Figure 1 Larvae of *Willmannium cavus moldaviensis* Kudryashova, 1992 and *Leptotrombidium europaeum* (Daniel & Brelih, 1959) parasitizing on *Eptesicus serotinus* (Schreber) examined in La Rioja province: A – on ear; B – on wing membrane. Photography by Joxerra Aihartza.

(Kuhl) – 1. One of the two adult males of *E. isabellinus* carried ca. 10 very small chiggers in plagiopatagium and uropatagium on both sides. One of the two adult males of *H. savii* was heavily infested with conspicuous reddish chiggers all over the patagium, whereas second male carried only a few chiggers in plagiopatagium and uropatagium.

- 2 — Vicinity of Palacio de Doñana, Doñana National Park, Almonte, Huelva province, 36.9907°N, 6.4435°W. In July 2019, we examined an entire maternity colony of *N. lasiopterus* (40 adult females, 10 adult males and 19 juveniles) occupying five bat boxes. Whitish chiggers were recorded on the face, eyelids and ears of only one adult female. All bats of the colony were tagged with rings and transponders; the infested female, ringed that day as K03283, could be identified as new in the colony.

- 3 — Parque Natural Sierra de Cebollera, La Rioja province, 42.0663°N, 2.6860°W. The sampling sites were La Blanca and El Achichuelo situated south to Villoslada de Cameros, by the Iregua river. In August 2020, we caught 66 bats representing 8 species (Vespertilionidae) at their drinking ponds and visually examined them for chiggers: *Barbastella barbastellus* (Schreber) – 1, *Eptesicus serotinus* (Schreber) – 6, *H. savii* – 1, *Myotis daubentonii* (Kuhl) – 4, *N. lasiopterus* – 37, *N. leisleri* – 12, *P. pipistrellus* – 3, and *Plecotus auritus* (L.) – 2. All specimens were males, except for one *E. serotinus*, *B. barbastellus*, and *H. savii*. We recorded chiggers on the ears and patagium of two *E. serotinus* males (Fig. 1).

In August 2021, we examined 26 bats at the same sites: *E. serotinus* – 1 male, *M. daubentonii* – 1 male, *N. lasiopterus* – 21 males, *P. pipistrellus* – 1 female, and *P. auritus* – 1 male and 1 female. Whitish chiggers attached on the head were recorded on three *N. lasiopterus* males.

Thus, we collected trombiculids in summer months of 2019–2021 from four species of bats belonging to the family Vespertilionidae, *E. isabellinus*, *E. serotinus*, *H. savii*, and *N. lasiopterus*. Chiggers were attached to head and wing membrane of the bats. The mites were removed using O'TOM tick twisters (H3D, Lavancia, France) or spraying TABERNIL [Tetrametine + Piperony butoxide] (Divasa-Farmavic, S.A., Barcelona, Spain) on a swab and applying it to the chiggers, and after some time removing them by pushing gently with the forceps, without need for grabbing, avoiding the possibility of damaging the mites. Samples were stored in Eppendorf tubes with 70% ethanol at room temperature. Chiggers from different bat individuals of the same species from La Rioja were combined.

Mites were mounted on microscope slides using Berlese's medium. Specimens were examined and photographed under a Leica DM2500B microscope (Leica Microsystems GmbH, Wetzlar, Germany) with a Leica DMC 4500 digital camera using differential interference contrast. Measurements necessary for identification were made using a microscope MBI-3

(LOMO plc, St. Petersburg, Russia) with phase contrast optics and ocular micrometer. We used monographs of Kudryashova (1998) and Vercammen-Grandjean & Langston (1976), a revision of the genus *Willmannium* Vercammen-Grandjean & Langston, 1976 (Kudryashova 1992), and other referenced sources for identification of chiggers.

All chigger specimens are deposited in the collection of the Zoological Institute of the Russian Academy of Sciences (ZIN).

Results

***Willmannium cavus moldaviensis* Kudryashova, 1992 (Fig. 2A)**

Willmannium cavus moldaviensis Kudryashova (1992) (original designation); Kudryashova (1998, 2004); Benda *et al.* (2019)

Material examined — Eleven larvae (ZIN 16794, 16795, 16800 – 16808) ex two males of *H. savii* Nos 190629-1 and 190629-6, SPAIN, Málaga province, Ronda, Sierra de las Nieves, fire pond Los Quejigales, 29 Jun. 2019, collected by Juan Quetglas, Sonia Sánchez-Navarro, and Carlos Ibáñez; four larvae (ZIN 16796 – 16799) ex male of *E. isabellinus* No. 190629-2, other data same; four larvae (ZIN 16920 – 16922, 16927) ex *E. serotinus*, SPAIN, La Rioja province, Villoslada de Cameros, 26 Aug. 2020, collected by Carlos Ibáñez.

Remarks — This subspecies was described from Moldova, ex *N. noctula* and *E. serotinus*. Later it was recorded from Albania, on *Vespertilio murinus* L., *Hypsugo savii*, *Pipistrellus pipistrellus*, *Pipistrellus pygmaeus* (Leach) and *Tadarida teniotis* (Rafinesque) (Benda *et al.* 2019). *Eptesicus isabellinus* is a new host species for this chigger.

Our specimens depart from the original description in having a concave (vs. almost straight) posterior margin of scutum and by shorter legs – Ip = 815 – 887 vs. 952 in the holotype (Table 1). However, the length of leg III tarsus in our material is almost the same as in the holotype – TaIIIIL = 76 – 86 (mean 81) vs. 83. In general, we do not support the practice of subspecies descriptions in the chigger taxonomy. Revision of the genus, with the possibility of raising some subspecies to the species level and synonymizing of others, is needed.

According to Kudryashova (1992), species of the genus *Willmannium* are known from Asia, Africa, and America. In Europe, three taxa belonging to this genus were recorded – *Willmannium bulgaricum* (Dusbabek, 1964) from Bulgaria, *W. cavus moldaviensis* from Moldova and Albania (Kudryashova 1992, 1998, 2004; Benda *et al.* 2019), and *Willmannium cavus cavus* Kudryashova, 1992 from Albania (Benda *et al.* 2019). Here, we record a representative of this genus in the Western Europe for the first time.

***Ascoshochengastia latyshevi* (Schluger, 1955) (Fig. 2B, C)**

Neoschoengastia latyshevi Schluger (1955) (original designation)

Ascoshochengastia (Paralaurentella) latyshevi: Kepka (1964)

Laurentella latyshevi: Muljarskaja (1968)

Ascoshochengastia (Ascoshochengastia) latyshevi: Daniel & Heneberg (1972)

Ascoshochengastia latyshevi: Sun & Wen (1984); Li *et al.* (1997); Kudryashova (1998); Literak *et al.* (2007); Stekolnikov & Daniel (2012); Moniuszko & Mąkol (2014)

Material examined — Three larvae (ZIN 17496 – 17498) ex three males of *N. lasiopterus* (No. 210811-1), SPAIN, La Rioja province, Villoslada de Cameros, El Achichuelo, 11 Aug. 2021, collected by Carlos Ibáñez.

Remarks — This species was known from Corsica (France), Austria, Czech Republic, Slovakia, Montenegro, Bulgaria, Moldova, Poland, Ukraine, Belarus, Russia, Azerbaijan, Turkey, Kazakhstan, Kyrgyzstan, Tajikistan, Mongolia, and China (Moniuszko & Mąkol 2014). Kudryashova (1998) refuted the record of *A. latyshevi* from Mongolia and reported on the presence of two closely related species from this country—*Ascoshochengastia kitajimai* (Fukuzumi

& Obata, 1953) and *Ascotoschoengastia mongolica* Kudryashova, 1998. *Ascotoschoengastia latyshevi* was recorded on many species of rodents, the soricomorph *Crocidura suaveolens* (Pallas) (Kudryashova 1998), and six species of birds (Literak *et al.* 2007). This species is recorded from bats collected in Spain for the first time. The fact of its parasitizing on *N. lasiopterus* agrees with the conclusion of Sixl (1969) on *A. latyshevi* as an inhabitant of hollow trees during the whole life cycle, since tree cavities are the typical habitat of *N. lasiopterus* (Ibáñez *et al.* 2004).

***Leptotrombidium europaeum* (Daniel & Brelich, 1959) (Fig. 2D)**

Trombicula (Leptotrombidium) intermedia europaea Daniel & Brelich (1959) (original designation)

Leptotrombidium (Leptotrombidium) europaeum europaeum: Vercammen-Grandjean & Langston (1976); Kolebinova (1992)

Leptotrombidium europaeum: Kudryashova (1998); Stekolnikov (2004, 2013); Stekolnikov & Daniel (2012); Moniuszko & Mąkol (2014)

Material examined — Four larvae (ZIN 16923 – 16926) ex *E. serotinus*, SPAIN, La Rioja province, Villoslada de Cameros, 26 Aug. 2020, collected by Carlos Ibáñez.

Remarks — This species was known from Spain, France, Austria, Czech Republic, Slovakia, Slovenia, Serbia (Kosovo), Macedonia, Montenegro, Albania, Bulgaria, Romania, Moldova, Poland, Lithuania, Latvia, Estonia, Ukraine, Belarus, Russia, Azerbaijan, and Turkey (Moniuszko & Mąkol 2014), from a wide range of rodents and insectivores, and also from the hare, *Lepus europaeus* (Pallas), and the great tit, *Parus major* L. (Kudryashova 1998). Here, it is recorded from a bat for the first time.

Table 1 Morphometric (AW – TaIIIW, µm) and meristic (DS – NDV) traits of *Willmannium cavaus moldaviensis*.

	Spain (n = 7)		Moldova		Spain (n = 7)		Moldova
	Range	Mean	Holotype*		Range	Mean	Holotype*
AW	59-68	64	67	D _{min}	23-32	28	29
PW	73-92	82	88	D _{max}	41-49	44	40
SB	26-31	29	34	V _{min}	18-23	21	23
ASB	28-32	30	32	V _{max}	34-41	38	38
PSB	13-16	14	13	pa	283-304	297	333
SD	41-48	44	45	pm	254-277	264	295
P-PL	5-13	8	7	pp	279-310	295	324
AP	30-36	33	34	Ip	815-887	856	952
AM	35-45	40	45	TaIIIL	76-86	81	83
AL	27-34	32	29	TaIIIW	14-19	16	16
PL	44-53	49	45	DS	37-42	40	42
H	41-46	44	38	V	40-41	41	40
				NDV	77-83	80	82

* after Kudryashova (1998). Abbreviations: AW – distance between anterolateral scutal setae; PW – distance between posterolateral scutal setae; SB – distance between sensillary (trichobothria) bases; ASB – distance from the level of sensillary bases to extreme anterior margin of scutum; PSB – distance from the level of sensillary bases to extreme posterior margin of scutum; SD – length of scutum (ASB + PSB); AP – distance between antero- and posterolateral scutal seta on one side; AM – length of anteromedian scutal seta; AL – length of anterolateral scutal setae; PL – length of posterolateral scutal setae; H – length of humeral setae; D_{min} – length of the shortest dorsal idiosomal seta; D_{max} – length of the longest dorsal idiosomal seta; V_{min} – length of the shortest ventral idiosomal seta; V_{max} – length of the longest ventral idiosomal seta; pa – length of leg I (including coxa, excluding claws); pm – length of leg II (including coxa, excluding claws); pp – length of leg III (including coxa, excluding claws); Ip – sum of leg lengths (pa + pm + pp); TaIIIL – length of leg tarsus III; TaIIIW – width of leg tarsus III; DS – number of dorsal idiosomal setae (excluding scutal); V – number of ventral idiosomal setae (excluding coxal and sternal); NDV = DS + V.

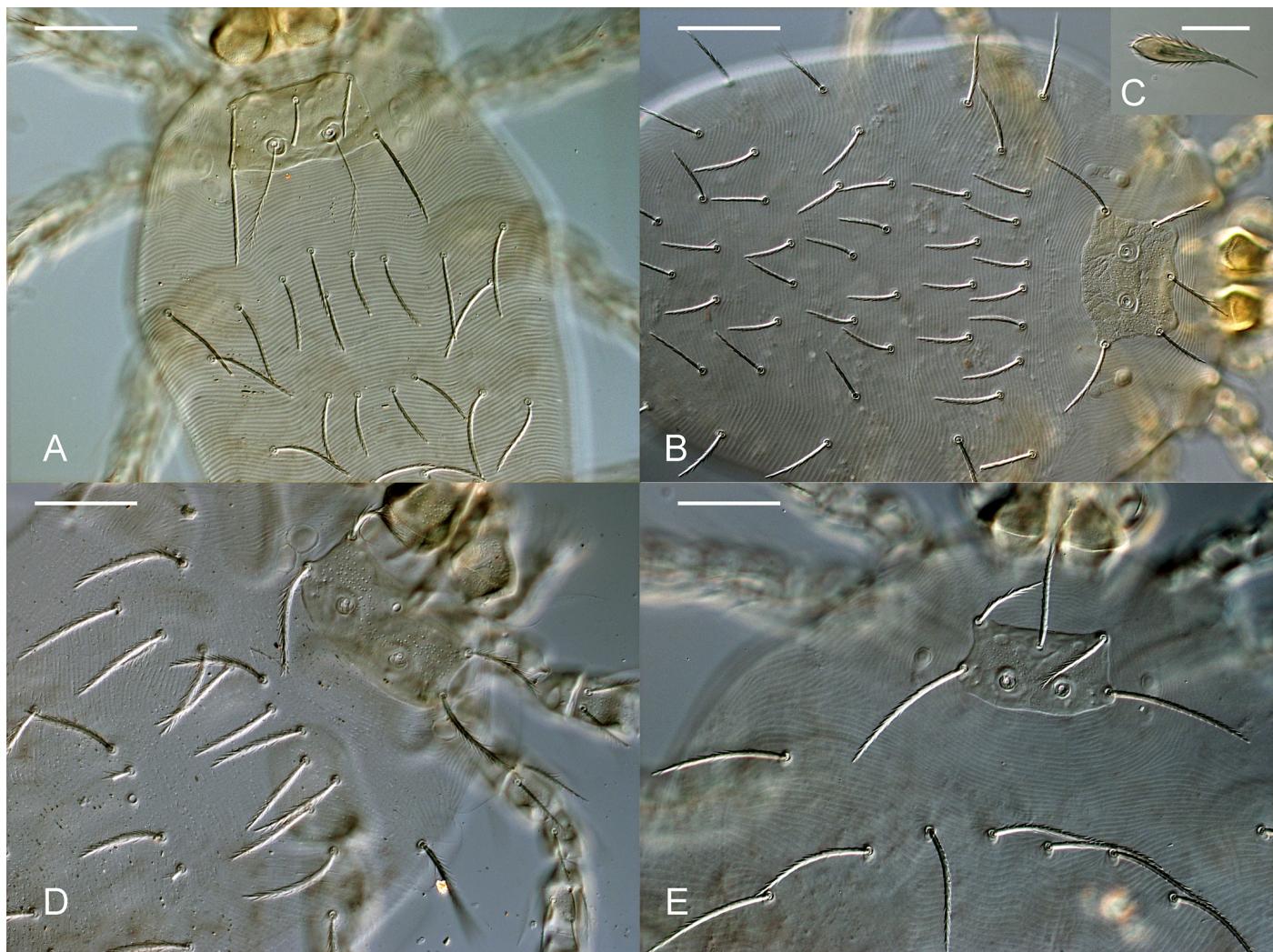


Figure 2 *Willmannium cavus moldaviensis* Kudryashova, 1992, larva ZIN 16794: A – anterior part of idiosoma with scutum. *Ascoschoengastia latyshevi* (Schluger, 1955), larva ZIN 17496: B – anterior part of idiosoma with scutum (sensilla lost); C – sensillum. *Leptotrombidium europaeum* (Daniel & Brelih, 1959), larva ZIN 16925: D – anterior part of idiosoma with scutum (anteromedian scutal seta and sensilla lost). *Leptotrombidium russicum* (Oudemans, 1902), larva ZIN 17505, Villoslada de Cameros: E – anterior part of idiosoma with scutum (sensilla lost). Scale bars: 50 µm (A, B, D, E), 20 µm (C).

***Leptotrombidium russicum* (Oudemans, 1902) (Fig. 2E)**

Thrombidium russicum Oudemans (1902) (original designation); Oudemans (1909)

Trombicula russicum: Fuller (1952)

Leptotrombidium (*Leptotrombidium*) *russicum russicum*: Vercammen-Grandjean & Langston (1976)

Leptotrombidium russicum: Kudryashova (1998); Mąkol *et al.* (2010); Stekolnikov (2013); Benda *et al.* (2019)

Leptotrombidium (*Leptotrombidium*) *russicum*: Kolebinova (1970, 1992); Fernandes & Kulkarni (2003)

Material examined — 12 larvae (ZIN 17499 – 17510) ex *N. lasiopterus* (No. 210811-1), SPAIN, La Rioja province, Villoslada de Cameros, El Achichuelo, 11 Aug. 2021, collected by Carlos Ibáñez; six larvae (ZIN 16809 – 16814) from face of adult female of *N. lasiopterus* (No. 190716-2, Ring k03283), SPAIN, Huelva province, Almonte, Palacio de Doñana, 16 Jul. 2019, collected by Juan Quetglas and Carlos Ibáñez.

Remarks — For the complete synonymy and full bibliography on this species before 1976 see Vercammen-Grandjean & Langston (1976). This species was known from Ireland, France, Belgium, The Netherlands, Austria, Switzerland, Germany, Czech Republic, Slovakia, Albania, former Yugoslavia, Hungary, Bulgaria, Romania, Moldova, Poland, Ukraine, Russia, Azerbaijan, Kazakhstan, Kyrgyzstan, Tajikistan, and India, on many species of bats and, occasionally, on yellow-necked mouse, *Apodemus flavicollis* (Melchior) (Kudryashova 1998; Moniuszko & Mąkol 2014; Fernandes & Kulkarni 2003; Benda *et al.* 2019). Here, it is recorded from Spain and on *N. lasiopterus* for the first time.

The metric characters of specimens collected from Villoslada de Cameros are very close to the typical *L. russicum*. However, the specimens collected in Almonte are significantly larger (Table 2). Seven of the nine specimens examined had the arrangement of dorsal idiosomal setae characteristic for *L. russicum* ($fD = 2H-8-6-6-4-2$); one specimen from Villoslada de Cameros had $2H-8-7-6-4-2$ and one specimen from Almonte had $2H-10-6-7-4-3$. Only one specimen from Villoslada de Cameros and one from Almonte had the numbers of all idiosomal setae characteristic for *L. russicum* according to Vercammen-Grandjean & Langston (1976) and Kudryashova (1998) ($DS = 28$, $V = 22$, and $NDV = 50$). Other specimens have $V = 23 - 28$ and $NDV = 51 - 57$.

Contrary to Kudryashova (1998) and Stekolnikov (2013), sensillary bases in our specimens were slightly posterior to the PLs ($P-PL - PSB = 0 - 6 \mu m$). The ratio $PW/AP = 2.77 - 3.64$ in our material and 3.35 according to Vercammen-Grandjean & Langston (1976).

Discussion

Leptotrombidium russicum was described by a single specimen collected from an unidentified bat near Kiew (currently Kyiv, Ukraine) (Kudryashova 1998). The holotype is deposited in Rijksmuseum van Natuurlijke Historie (RMNH; currently, Naturalis Biodiversity Center, Leiden, Netherlands). Fuller (1952) noted that this specimen is nearly fully engorged, its scutum is tipped forward, and the posterior scutal margin is hidden under a fold of the cuticle. Vercammen-Grandjean & Langston (1976) provided a complete re-description of *L. russicum* on the base of the holotype, one specimen from Maastricht (Netherlands) deposited in the Oudemans collection (RMNH), and literature data. Kudryashova (1998) re-described this species on the base of new materials from Moldova, Kyrgyzstan, and European Russia (Moscow, Tver, and Tula Oblast); her measurements and morphological drawings were in good agreement with those of Vercammen-Grandjean & Langston (1976). Fernandes & Kulkarni (2003) also gave full morphological and morphometric data for an Indian sample of *L. russicum*; these data are consistent with previous descriptions, except for the barbs of scutal setae, which look fairly long on their drawing of the scutum.

Many records of this species from hosts other than bats were misidentifications (Vercammen-Grandjean & Langston 1976; Kudryashova 1998). In part, the species *Leptotrombidium subrussicum* Kolebinova, 1970 is morphometrically similar to *L. russicum* (Stekolnikov 2013) and could be misidentified. This species was described from rodents on Corsica (Kolebinova 1970) and later recorded from rodents, soricomorphs, and one bird in Bulgaria (Vercammen-Grandjean & Langston 1976; Kolebinova 1992). We also found a series of specimens belonging to *L. subrussicum* in the collection of ZIN collected from rodents in North and Western Caucasus (Russia) and in Transcaucasia (Armenia) and originally identified as *L. russicum* or *L. fulleri* (Ewing, 1945).

There are several species of *Leptotrombidium* that may be specific to bats, including four described from Romania and one from the UK (Stekolnikov 2013). *Leptotrombidium russicum* differs from all of them in having fewer idiosomal setae and $fD = 2H-8-6-6-4-2$ vs. $2H-10-8-8-...$ or $2H-8-6-6-6-....$ One larva from France (Ariège) identified by Kolebinova (1970) as *L. russicum*, with $fD = 2H-9-8-8-8-6-4-2$, probably belongs to another species. However, our finding of *L. europaeum* on *E. serotinus* from La Rioja demonstrates that some European

species of *Leptotrombidium*, usually parasitizing rodents and soricomorphs, can also infest bats. Therefore, we may expect the joint occurrence of morphologically similar species, such as *L. subrussicum* and *L. russicum*, which can be represented by different intraspecific forms, on same individuals of bats in Europe. Two size forms of *L. russicum* were described in the present work; *L. subrussicum* also shows a wide range of size variations, probably depending on altitude (Vercammen-Grandjean & Langston 1976).

A recent work on bat-infesting chiggers in Poland reported the presence of four different *Leptotrombidium* identified by mitochondrial cytochrome c oxidase subunit I gene (COI) sequences. One was identified morphologically as *L. russicum*, whereas the identity of other three forms remain unclear (Zajkowska & Mąkol 2022). Interestingly the genetic distance between the form designated as *Leptotrombidium* sp. 2 and *L. russicum* was only 5.6 – 5.9%. This distance is rather close to the threshold of the intraspecific variation in Trombiculidae, which attained 4.6% by the COI sequence (Zajkowska & Mąkol 2022). Further morphological examination of these samples is required to establish the nature of the observed molecular

Table 2 Morphometric (AW – TaIIIW, µm) and meristic (DS – NDV) traits of *Leptotrombidium russicum*.

	Villoslada de Cameros (n = 5)	Almonte (n = 4)	Holotype*	Moldova (n = 7)**	
	Range	Mean	Range	Mean	Range
AW	57-64	62	68-71	70	68
PW	68-76	72	81-88	84	80
SB	25-29	27	30-34	32	28
ASB	27-31	29	31-34	33	25
PSB	13-16	14	14-18	17	19
SD	41-45	43	49-52	50	44
P-PL	14-17	16	17-24	21	-
AP	21-26	23	23-28	26	21
AM	54-59	57	61-69	65	52
AL	34-39	37	40-45	42	36
PL	63-72	67	77-83	79	68
H	57-66	61	68-78	73	60
D _{min}	47-50	49	57-66	61	48
D _{max}	57-67	62	70-79	74	58
V _{min}	28-38	33	37-40	38	33
V _{max}	53-60	57	59-66	63	54
pa	254-265	259	279-301	292	235
pm	225-239	235	254-266	262	200
pp	259-272	264	297-306	301	241
Ip	738-770	757	830-873	855	676
TaIIIL	68-74	70	77-79	79	-
TaIIIW	16-17	16	16-19	18	-
DS	28-29	28	28-32	29	28
V	22-28	24	22-25	24	22
NDV	50-57	52	50-57	53	50
					50-51

* after Vercammen-Grandjean & Langston (1976); ** after Kudryashova (1998). Abbreviations as in Table 1.

forms more definitely.

Noteworthy is that our material on *L. russicum* was collected from *N. lasiopterus*, a bat usually inhabiting tree cavities (Ibáñez *et al.* 2004). This allows us to conclude that hollow trees can be at least one of possible habitats where the life cycle of *L. russicum* takes place.

Stekolnikov *et al.* (2014) presented the list of 19 chigger mite species known from Spain at that time, but omitted *Ericotrombidium ibericense* (Vercammen-Grandjean & Langston, 1976) from this list. However, we suppose that the previous record of *E. hasei* (Feider, 1958) could have been *E. ibericense*. Later, Stekolnikov & Quetglas (2019) added two more species to the chigger fauna of Spain. Currently, 25 chigger species are recorded from Spain, including the results of the present study. Six species were collected from bats and three – *L. russicum*, *O. komareki*, and *T. knighti* – only parasitize bats.

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