A New Species of *Demodex* (Acari: Demodecidae) With Data on Topical Specificity and Topography of Demodectic Mites in the Striped Field Mouse *Apodemus agrarius* (Rodentia: Muridae)

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ABSTRACT This article describes morphological characteristics and the occurrence of *Demodex gracilentus* sp. nov., which was found in the striped field mouse *Apodemus agrarius* (Pallas, 1771) in the skin of vibrissae area. *D. gracilentus* occurred in 36.7% of the rodents examined. *D. gracilentus* is a relatively large representative of the genus (adult stages on average 292 μm in length), a slender, elongated body; characteristic feature of these mites are conical supracoxal spines on dorsal side of gnathosoma, palps with asymmetric, forked triple spines on palptarsus, and the presence of rhomboidal opisthosomal organ. So far, the occurrence of three specific representatives of the family Demodecidae has been demonstrated in *A. agrarius: Demodex apodemi* (Hirst, 1918) (=Demodex arvicolae apodemi Hirst, 1918), Demodex agrarii Bukva, 1994, and Demodex huttereri Mertens, Lukoschus et Nutting, 1983. The first one is related to common hair follicles, especially in the skin of the head, while the next one inhabits the external auditory meatus, and the last one occurs in the meibomian glands of the eyelids.

KEY WORDS Demodex gracilentus, Demodecidae, skin mite, Apodemus agrarius, topical specificity

Demodecidae (Acari: Prostigmata) of rodents has been characterized by both high host and topical specificity. Usually several mite species of this family occur in one host species, inhabiting different microhabitats in the skin or the mouth. Therefore, even as much as several synhospitalic species of demodectic mites have been found in rodents common in Europe, including, for example, four species in the brown rat *Rattus norvegicus* (Berkenhout, 1769) and the wood mouse *Apodemus sylvaticus* (L., 1758). Three specific representatives of the family Demodecidae have been found in the striped field mouse *Apodemus agrarius* (Pallas, 1771) (Izdebska 2012).

Apodemus is the common genus of murids, which includes ≈20 species of small rodents found in Europe, Asia, and Africa. The striped field mouse is a widespread species, occurring between central Europe in the west and China, and Korea in the east (Hille and Meining 1996). A. agrarius is one of the most common agricultural pests and a natural vector of diseases commonly associated with murine rodents (Okulova et al. 2012). It is also a synurbic species (Andrzejewski et al. 1978), that is, species that colonizes or is found within urban ecosystems. Parasitofauna of the striped field mouse is relatively well explored, including three species of demodectic mites reported for this mouse so far: Demodex apodemi (Hirst, 1918) (=Demodex arvicolae apodemi Hirst, 1918), Demodex agrarii Bukva, 1994, and Demodex huttereri Mertens, Lukoschus et Nutting 1983

Materials and Methods

Thirty specimens of A. agrarius from the northern Poland (53° 59′ N/18° 05′ E, 54° 15′ N/18° 14′ E) were examined for skin parasites in 2010. Skin samples $\approx 1~\rm cm^2$ were collected from the mice from several regions of the body including the head (around eyes, ears, nose, area of vibrissae, lips, cheeks, and chin), nape, belly, dorsum, limbs, and the genital–anal region. The skin samples were examined for the presence of demodectic mites using the standard method for digesting (Izdebska 2004), that is, samples were digested in 10% KOH solution, decanted, and examined using a phase-contrast microscope. Permanent preparations were made in Faure's medium. All measurements are in micrometers.

To define the level of host infestation, the main parasitological parameters were enumerated, that is, the prevalence (percentage of infested hosts), mean intensity (the average number of parasites in infested hosts), and intensity range (minimum and maximum number of parasite individuals per host in the infr-

⁽Bregetova et al. 1955; Hirst 1918, 1919; Mertens et al. 1983; Bukva 1994; Izdebska and Cydzik 2010; Izdebska et al. 2011; Izdebska 2012). The first one is related to common hair follicles, especially in the skin of the head, while the next one inhabits the external auditory meatus, and the third one—the meibomian glands of the eyelids. At present, a new species has been described in *A. agrarius* of northern Poland, inhabiting the area of vibrissae.

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Table 1. Body size (micrometers) of D. gracilentus sp. nov.

Morphologic features	δ $(n=8)$	$\ \ \bigcirc \ (n=16)$
Length of gnathosoma	25 (24-26), SD 0.7	27 (21-30), SD 2.7
Width of gnathosoma (at base)	21 (21–23), SD 0.6	22 (18–27), SD 2.1
Length of podosoma	64 (60-66), SD 2.4	67 (55–76), SD 6.7
Width of podosoma	33 (25-34), SD 3.1	32 (23-36), SD 3.8
Length of opisthosoma	207 (188–255), SD 22.3	195 (169–315), SD 34.3
Width of opisthosoma	32 (25-37), SD 4.1	28 (22-30), SD 2.4
Aedeagus	32 (31-33), SD 0.8	
Vulva	· <u>-</u>	12 (9-16), SD 2.1
Total length of body	296 (272–347), SD 24.5	289 (260–395), SD 30.3

apopulation under study). The specimens of *Demodex gracilentus* were deposited in the collection of the Department of Invertebrate Zoology and Parasitology at the University of Gdańsk.

Results

Demodex gracilentus sp. nov. (Table 1; Figs. 1-4)

Female (Holotype). Slender body, highly elongated; body length 289, width 32 (Table 1; Figs. 1B and

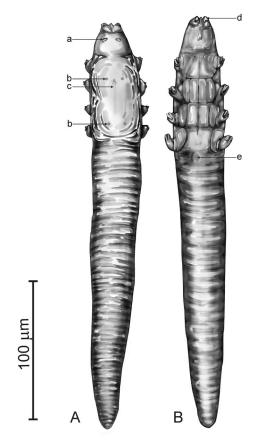


Fig. 1. D. gracilentus sp. nov. (A) δ , dorsal view, (a) supracoxal spine, (b) prodorsal tubercles, and (c) aedeagus. (B) \circ , ventral view, (d) spines on palptarsus, and (e) vulva.

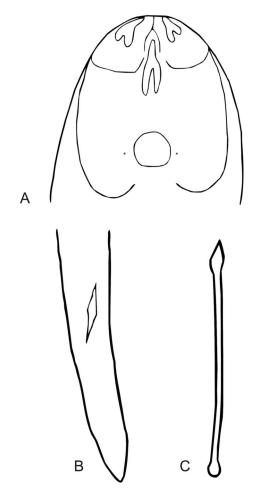


Fig. 2. D. gracilentus sp. nov. (A) Gnathosoma, δ , ventral view. (B) Opisthosomal organ, δ . (C) Aedeagus.

4B). Gnathosoma trapezoidal, longer than wider; twosegmented palps asymmetric, forked triple spines on palptarsus, the same in both sexes (Fig. 2A); dorsal side with relatively large (\approx 3.5–4 long) supracoxal spines, conical (Fig. 3A); on ventral side, oval pha-

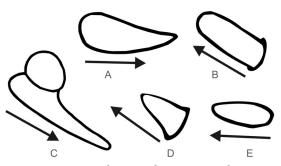


Fig. 3. Supracoxal spines of various *Demodex* species from A. agrarius and D. longior from A. sylvaticus. (A) D. gracilentus sp. nov. (B) D. longior. (C) D. apodemi. (D) D. huttereri. (E) D. agrarii. Arrows indicate the location of left spines toward the gnathosoma.

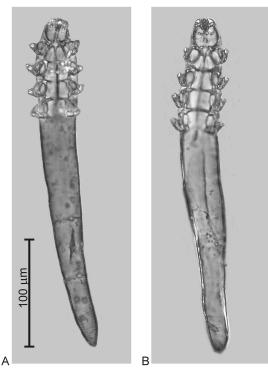


Fig. 4. D. gracilentus sp. nov. (A) δ . (B) \circ .

ryngeal bulb with small subgnathosomal setae on both sides. Podosoma narrow, trapezoidal, gradually widening toward opisthosoma; four pairs of five-segmented legs on podosoma that distinctly project beyond its edges, each with three mobile segments; epimeral plates rectangular (pair II, III, and IV) or triangular and narrowed from gnathosoma (pair I); trochanter of the first pair of legs distinctly different from the others, triangular (Fig. 1B); tarsi ending in two large, hooked claws (Fig. 1B). Opisthosoma 67% of body length, strongly elongated, gradually tapered toward end, striated. Vulva (mean 12 long) on ventral side, situated slightly below the IV pair of epimeral plates. Opisthosomal organ present, similar in both sexes (Fig. 2B).

Male. Slender body, highly elongated; body length 296, width 33 (Table 1). Gnathosoma rectangular; the

related structure elements are similar to female (Figs. 1A, 2A, and 4A); supracoxal spines somewhat larger $(\approx 4-4.5 \text{ long})$. Shape and size of podosoma similar to female; four pairs of legs developed similarly as in the female, vet slightly more massive and more protruding beyond the edge of podosoma; the first epimeral plate distinctly triangular, the others rectangular; two pairs of prodorsal tubercles on the dorsal side of podosoma, and the first pair is located at the level of the border of the first and the second pair of legs, while the second pair of prodorsal tubercles—between the third and the fourth pair of legs. An aedeagus (mean 32 long) on dorsal side of podosoma, situated at the level of the second and the third pair of legs. Opisthosoma constitutes ≈70% of body length, elongated, sharply ending, clearly striated. Opisthosomal organ present, rhomboidal (Fig. 2B).

Type Materials. HOLOTYPE: ♀ (RAaDg01), northern Poland 54° 15′ N/18° 14′ E); holotype was collected from *A. agrarius* (No. 01/10).

Etymology. The specific epithet *gracilentus* (slender and elongated) refers to the shape of the body (Figs. 1 and 4).

Distribution. Northern Poland. It is highly probable that the area of occurrence of *D. gracilentus* corresponds directly to the distribution of its host, *A. agrarius*.

Infestation and Location in the Host. D. gracilentus exhibited a prevalence of 36.7% in striped field mouse, at a mean intensity of 2.5 and intensity range of 1–6 specimens per host. All the specimens were found in the area of vibrissae. Fragments of skin possessed mainly adult specimens (8 \Im and 16 \Im). Several specimens of immature stages (four protonymphs) were also found, but their state of preservation did not allow to draw up appropriate descriptions. The observed demodectic mites did not cause any lesions in mice.

Discussion

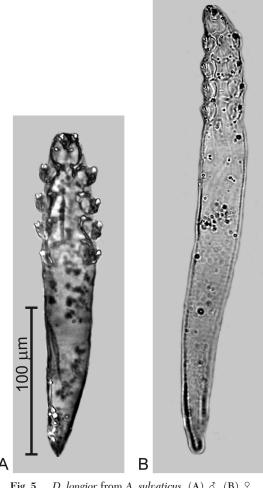
Differential Diagnosis of *D. gracilentus* and *Demodex longior*. The presently described species clearly deviates from the other species so far noted in *A. agrarius* in terms of both body size and proportions and morphological features significant in the taxonomy of Demodecidae (Desch et al. 1970, Nutting 1976, Bukva 1991), whereas morphological features, shape,

Table 2. Body sizes (micrometers) of D. gracilentus sp. nov. and D. longior according to various authors

			D. longior				
Morphologic features	D. gracilentus sp. nov.		(Unpublished data)		Hirst (1919)		
	$ \vec{\delta} \ (n=8) $? (n = 16)			∂ª	Q^a	
Length of gnathosoma + podosoma ^b	89	94	76	82	72	82	
Length of opisthosoma	207	195	143	226	128	210	
Width of body	33	32	33	29	34-35	30-32	
Ratio of body length to body width	9.0	9.3	6.6	10.6	5.8	9.4	
Ratio of opisthosoma length to body length (%)	70	67	65	73	64	72	
Total length	296	289	219	308	200	292	

^a The author gives no information as to number of examined mites.

^b For comparison, like Hirst (1919), combined length of gnathosoma and podosoma.



D. longior from A. sylvaticus. (A) δ . (B) \circ . Fig. 5.

and dimensions of this group mites are adequate to microhabitats occupied within the hosts (Izdebska 2009). Therefore, it came as no surprise that *D. longior* Hirst, 1918, described in A. sylvaticus and showing location analogous (area of vibrissae) to that of D. gracilentus, turned out to be a species that is the most similar in shape and proportions (Hirst 1919). D. longior is also the most similar to D. gracilentus in terms of taxonomically significant morphological features.

In D. longior and D. gracilentus, female have similar dimensions, whereas male of *D. longior* is smaller than males of D. gracilentus (Figs. 4A and 5A). Furthermore, male of D. gracilentus distinctly differs in proportions between particular tagmata—has longer and slender opisthosoma (Table 2). Gnathosoma of D. longior is wider than long, in D. gracilentus is longer than wide. In addition, the supracoxal spines of. D. longior are club-like shape, while in D. gracilentus conical (Table 2; Fig. 3A and B) and are located closer to the external edge of gnathosoma; spines on terminal segment of palpi of D. longior are smaller, regular, and three-armed, while in D. gracilentus—irregular, asymmetric, and forked triple. Opisthosomal organ of D. longior is fusiform, elongated (Fig. 5A), while in D. gracilentus is rhomboidal (Figs. 2B and 4A). Podosomal tubercles of the male of D. longior are located at the level between the first and the second pair of legs (the first pair of tubercles) and between the second and the third pair of legs (the second pair of tubercles), while the second pair of tubercles of the male of D. gracilentus is located distinctly lower, between the third and the fourth pair of legs. Claws of legs of the both species are double and hooked; however, in D. longior, they are smaller and more delicate than in D. gracilentus. All epimeres of D. longior are narrower and produced posteriorly, especially the first ones, while epimeres of D. gracilentus are wider; in addition, I pairs are triangular, and II-IV pairs—rectangular. Sexual dimorphism is clearly expressed in D.







Demodectic mites (\$\varphi\$, scaled) from A. agrarius. (A) D. agrarii. (B) D. huttereri. (C) D. apodemi.

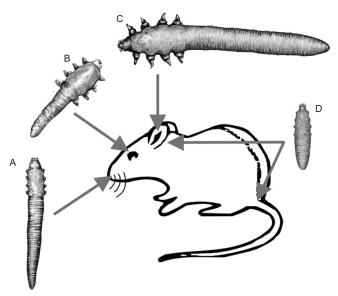


Fig. 7. Topical and topographic preferences of *Demodex* spp. (3, scaled) in *A. agrarius*. (A) *D. gracilentus* sp. nov.—area of vibrissae. (B) *D. hutterei*—meibomian glands of the eyelids. (C) *D. agrarii*—external auditory meatus. (D) *D. apodemi*—common hair follicles, especially in the head and genital–anal region.

longior, in which, the male is much smaller (almost 35%) than female, they also have different tagmata proportions (Table 2; Fig. 5). In *D. gracilentus*, both sexes have similar dimensions, differences in appearance are expressed in differ in proportions (Tables 1 and 2; Fig. 4). Genital opening of the female of *D. longior* is shorter and located directly under the fourth pair of epimeral plates, whereas in the female of *D. gracilentus*, it is longer and lower located.

Topical Structure and Topography of *Demodex* spp. in *A. agrarius*. Demodecidae is a family that usually reveal topical specificity while inhabiting strictly defined microhabitats of skin and internal organs of mammals. For example, there are known species from common and sensory hair follicles, sebaceous glands,

meibomian glands, epidermis or tissues of the mouth cavity, and anterior parts of the alimentary tract.

Therefore, *D. agrarii* (a large demodectic mite, Figs. 6A and 7C) was described from *A. agrarius* where it was found in sebaceous glands connected with the auditory meatus (Bukva 1994, Izdebska and Cydzik 2010). *D. huttereri* (Figs. 6B and 7B)—second specific species of this host—occurs in a different microhabitat, that is, in meibomian glands of eyelids (Mertens et al. 1983, Izdebska et al. 2011). Demodectic mites of similar shape live in meibomian glands of many other mammals, for example, cattle, horses, bison, or vampire bats (Izdebska 2009). In rodents, they are represented by *Demodex lacrimalis* Lukoschus et Jongman, 1974—in *A. sylvaticus* (Izdebska and Fryderyk 2012),

Table 3. Characteristic features of various Demodex species from A. agrarius (measurements in micrometers)

Morphologic features	D. apodemi (Unpublished data)		D. agrarii (Bukva 1994) ^a		D. huttereri (Izdebska et al. 2011) ^a		D. gracilentus sp. nov.	
	ð	\$	ठै	\$	3	φ	ð	\$
Length of body	144 (113-172)	170 (117-210)	513 (498-540)	587 (476-647)	241 (236-245)	364 (356-376)	296 (272-347)	289 (260-395)
Width of body (podosoma)	25 (23–26)	23 (20–26)	72 (67–79)	57 (48-64)	56 (53–59)	48 (44–55)	33 (25–34)	32 (23–36)
Ratio of body length to body width	5.8	7.4	7.1	10.3	5.0	7.6	9.0	9.0
Ratio of opisthosoma length to body length (%)	53	57	70	76	55	68	70	67
Supracoxal spines (Fig. 3)	Very large, lie flat, obliquely directed to the center of gnathosoma		Straight, slightly widened apex, upwards directed		Minute, conical, upwards and diagonally directed		Large, conical, lie flat, horizontal	
Spines on terminal segment of palps	Large, asymmetric, forked triple		Three spines, two of which are two-tined and one simple		Three minute spines, two of which are two-pointed clawlike and one simple		Asymmetric, forked triple	
Opisthosomal organ	Present in both sexes (C-shaped)		Absent		Absent		Present in both sexes (rhomboidal)	
Location/topography			Sebaceous glands, external auditory meatus, area of ears		Meibomian glands, area of eyelids		Area of vibrissae	

^a Measurements were rounded to the nearest micrometer with respect to the original results.

and *Demodex sabani* Desch, Lukoschus et Nadchatram, 1984—found in a few species of Asian rodents (Desch et al. 1984). Recently found *D. gracilentus* has a similar shape and location (Fig. 7A) as *D. longior* that lives in sensory hair follicles of *A. sylvaticus*, whereas the fourth species of demodectic mites occurring in *A. agrarius*, small *D. apodemi* (Figs. 6C and 7D), lives in common hairs follicles. Species associated with hair follicles occur in different mammals (e.g., dogs, cattle, sheep, red deer, or humans), and species morphologically similar to *D. apodemi* from *A. agrarius* were found in some mice and voles (Izdebska 2012; Table 3).

Therefore, location of demodectic mites in various regions of host's body is connected to the preferred microhabitat—for example, Demodecidae related to the glands of eyelids, ear canals, or sensory hairs of nasal area are entirely observed in head skin region, whereas demodectic mites whose microhabitats are common hair follicles may potentially settle in various regions of the body. However, even those species show strong preferences in the selection of the location, and thus, D. apodemi in the striped field mouse prefers the skin of the head and is represented in this body region by >70-80% of all specimens of this parasite (Izdebska et al. 2011). Similar preference for location by Demodex spp. in murid rodents have been observed in R. norvegicus, where Demodex ratti Hirst, 1917 and Demodex ratticola Bukva, 1995 were found in the skin of the head, and *Demodex nanu*s Hirst, 1918 and *Demodex nor*vegicus Bukva, 1995—in the skin of the genital and anal regions (Izdebska and Rolbiecki 2012a,b).

Therefore, in the case of Demodecidae, two concepts related to host location should be distinguished. The first one is topical specificity understood as a relation with a specific microhabitat—a type of tissue, an element of skin structure (e.g., hair follicle, gland). Topography is a separate concept understood as a distribution of demodectic mites in various regions of the body and topographic preferences referring to predilections for colonization of microhabitats located within specific regions of the skin (e.g., head, genital and anal regions etc.).

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