

New Species of *Demodex* (Acari: Demodecidae) With Data on Parasitism and Occurrence of Other Demodecids of *Rattus norvegicus* (Rodentia: Muridae)

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Ann. Entomol. Soc. Am. 107(4): 740–747 (2014); DOI: <http://dx.doi.org/10.1603/AN13123>

ABSTRACT This article describes the morphological features and occurrence of *Demodex ponderosus* n. sp. on brown rats, *Rattus norvegicus* (Berkenhout), in Poland. *D. ponderosus* n. sp. is a small demodecid, adults average 152 μm in length, and are oval in shape. They were found in the skin of nearly hairless regions, tail and paws, of rats. Adult mites exhibit sexual dimorphism in terms of body size and proportions. The study also compared the features of the new species with those of the four species of *Demodex* described previously from this host. *Demodex* spp. were observed in 100% of the rats examined; however, despite high infestation levels, no symptoms of parasitosis were observed in the hosts.

KEY WORDS demodecid mite, *Demodex ponderosus* n. sp., rodent, skin mite

The brown rat, *Rattus norvegicus* (Berkenhout), is a mammalian species with a strong connection to humans. They are synanthropic and cosmopolitan. In addition, they are important laboratory animals and bred for pets and pet food. These close relationships mean that rats constitute a reservoir, or chain in the life cycles, of parasites and pathogens that are significant for human and domestic animals. The rat parasitofauna is well-described (Stojcevic et al. 2004, Haitlinger and Jankowska 2005, Hancke et al. 2011, Pakdad 2012), including skin mites; sarcoptic mites—*Notoedres muris* Megnin and *Trixacarus diversus* Sellnick (Astigmata: Sarcoptidae) (Klompfen 1992), *Psorergates rattus* Fain and Goff (Prostigmata: Psorergatidae) (Fain and Goff 1986), and four *Demodex* species (Prostigmata: Demodecidae)—*Demodex norvegicus* Bukva, *Demodex ratti* Hirst, *Demodex ratticola* Bukva, and *Demodex nanus* Desch, have been observed in *R. norvegicus* (Hirst 1919; Desch 1987; Bukva 1995; Izdebska 2004; Izdebska and Rolbiecki 2004, 2012a,b). Here, we report and describe a new representative of *Demodex* in brown rats from urbanized areas of Poland.

Materials and Methods

We examined 10 brown rats, *R. norvegicus*, (4♂♂, 6♀♀) collected from urbanized areas of northern Poland (Gdynia; 54° 30' N, 18° 32' E) in July 2011. The material (rodents and mites) was collected by the authors. Skin fragments ($\approx 1\text{ cm}^2$) were collected from several body regions including head (ears, area around

eyes, sensory hairs of the nose, nose, lips, and chin region), neck, abdomen, back, limbs and genital-anal region, and tail.

Demodecids were isolated using the skin digestion method (Izdebska 2004). Samples obtained were decanted and analyzed using phase contrast microscopy (Nikon Eclipse 50i, Nikon Corp., Tokyo, Japan). Mites were measured (μm); then, photographed using a Nikon Coolpix 4500 and graphically documented in Corel Draw 12 (Corel Corp., Ottawa, ON, Canada). Demodecids were mounted in Faure's solution (Lakshminarayana 1980). Measurements were taken as follows: gnathosomal width = width at base; podosomal and opisthosomal widths = maximum width. The material is stored in the collection of the Department of Invertebrate Zoology and Parasitology, University of Gdańsk, Poland (UGP), and in the Museum and Institute of Zoology, Polish Academy of Sciences, Warszawa, Poland (MIZ).

Basic parasitological parameters, prevalence (percentage of hosts infested), mean intensity (the average number of parasites per infested host), and intensity range (minimum and maximum number of parasite individuals per host) were measured to determine host infestation levels (Margolis et al. 1982).

Results

Demodex ponderosus n. sp.
(Table 1; Figs. 1, 2, and 4CD)

Female (Holotype). Body length ≈ 163 (holotype 160) μm , with a width of 50 (holotype 51) μm (Table

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Table 1. Body size (mean \pm SD [range], in μm) of adults *D. ponderosus* n. sp.

Morphologic features	Male (n = 14)	Female (n = 33)
Length of gnathosoma	17 \pm 2 (14–23)	20 \pm 2 (15–25)
Width of gnathosoma	21 \pm 2 (16–24)	23 \pm 2 (18–28)
Length of podosoma	46 \pm 5 (35–53)	54 \pm 4 (48–63)
Width of podosoma	44 \pm 1 (43–45)	46 \pm 3 (40–50)
Length of opisthosoma	65 \pm 6 (53–75)	89 \pm 6 (73–100)
Width of opisthosoma	46 \pm 3 (43–51)	50 \pm 3 (45–55)
Aedeagus	21 \pm 2 (19–26)	–
Vulva	–	11 \pm 2 (9–16)
Total length of body	128 \pm 7 (116–148)	163 \pm 8 (145–183)

Number of hosts = 3.

1). Gnathosoma oval, base wider than long. Very fine supracoxal spines, directed outwardly, located on dorsal side of gnathosoma, on its external edge. Three-segmented palps, topped with single, archwise-bent claws. On the ventral side at the posterior part of the gnathosoma, oval pharyngeal bulb, fine subgnathosomal setae above it (Fig. 2A). Podosoma trapezoidal, very wide—length only slightly exceeding width. On the podosoma are four pairs of six-segmented legs, three pairs slightly protruding beyond its edges, legs of pair IV within its area. Epimeral plates of pairs I–III trapezoidal, posterior edge of pair IV indented archwise; vulva under the incision. Last segments of all legs end with two clenched, forked claws. Opisthosoma constitutes \approx 54% (holotype 55%) of body length; long, usually slightly wider than the podosoma, slightly narrowed to the end, insignificantly striped. Opisthosomal organ absent.

Male. Males distinctly shorter than females, more oval; \approx 128 μm in length, 46 μm in width (Table 1). Gnathosoma shape and morphological details similar to females, insignificantly smaller (Fig. 2A and C).

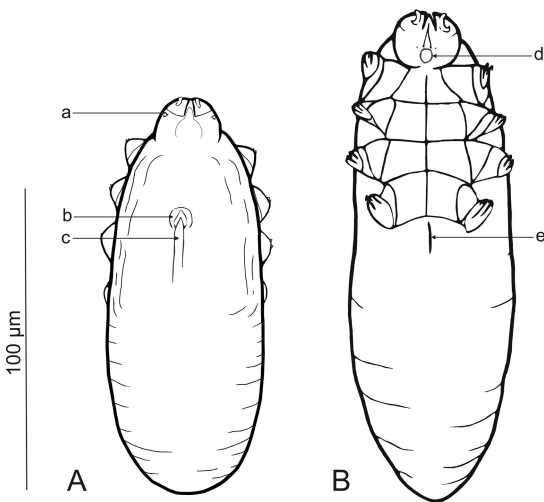


Fig. 1. *D. ponderosus* n. sp., adults. (A) Male, dorsal view, (a) supracoxal spine, (b) male genital orifice, and (c) aedeagus. (B) Female, ventral view, (d) pharyngeal bulb, (e) vulva.

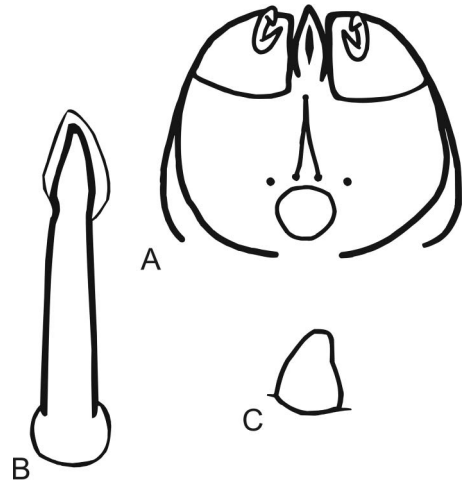


Fig. 2. *D. ponderosus* n. sp., male. (A) Gnathosoma, ventral view. (B) Aedeagus. (C) Supracoxal spine.

Podosoma wide as in females, trapezoidal, with similarly shaped and arranged legs. An aedeagus (\approx 21 in length) on the dorsal side, by epimeral plates of pairs II and IV; relatively wide, flame-shape ended (Figs. 1A, and 2B). Opisthosoma shorter than in females, constituting \approx 50% of the body length, wide, oval, poorly striped, rounded at the end. Opisthosomal organ absent.

Immature Stages. Not found. Only 6 ova were observed (Table 4; Fig. 3B).

Type Material. HOLOTYPE (\varnothing): POLAND, Pomeranian, VII–2011, Gdynia, host *R. norvegicus* (\varnothing , no. Rn30/6), col. J.N. Izdebska and L.Rolbiecki, deposited at UGP. PARATYPES (29 $\varnothing\varnothing$, 12 $\sigma\sigma$): 13 $\varnothing\varnothing$, 4 $\sigma\sigma$, host *R. norvegicus* (\varnothing , RnB/2); 9 $\varnothing\varnothing$, 1 σ , host *R. norvegicus* (\varnothing , Rn30/6); 7 $\varnothing\varnothing$, 7 $\sigma\sigma$ host *R. norvegicus* (σ , Rn3/4), other data the same as holotype. 3 $\varnothing\varnothing$ (3/2014/3–3/2014/5) and 2 $\sigma\sigma$ (3/2014/1–3/2014/2), host *R. norvegicus* (\varnothing , RnB/2), deposited at MIZ, same locality and collectors.

Distribution. Northern Poland (Gdynia). It is probable that the distribution of *D. ponderosus* n. sp. corresponds directly to that of its host, *R. norvegicus*.

Etymology. The specific epithet *ponderosus* (corpulent, portly, and stout) refers to the shape of the body.

Infestation and Location in the Host. *D. ponderosus* n. sp. was noted in 30% of the 10 examined brown rats, with a mean intensity of 15.7 and an intensity range of 11–20 individuals per host (Table 2); 47 individuals (14 males and 33 females) and 6 ova in total were found. Mites were found in the skin of thinly haired regions of the rat’s body (tail and paws). Infestations were not associated with skin lesions or other disease symptoms.

Synhospital, four other species from the family Demodecidae were observed, *D. nanus* (125 males, 253 females, 83 larvae, 66 protonymphs, and 176 deutonymphs), *D. norvegicus* (238 males, 475 females, 95 larvae, 75 protonymphs, and 304 deutonymphs), *D.*

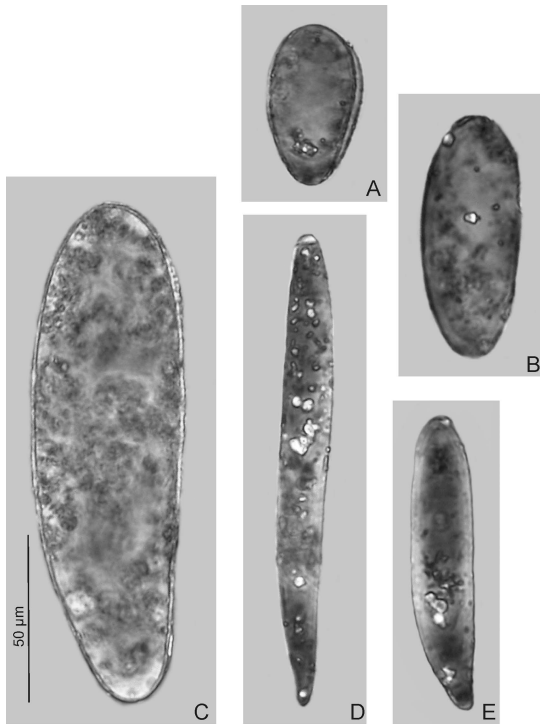


Fig. 3. Ova of *Demodex* species from *R. norvegicus*. (A) *D. nanus*. (B) *D. ponderosus* n. sp. (C) *D. ratti*. (D) *D. norvegicus*. (E) *D. ratticola*.

ratti (35 males, 26 females), and *D. ratticola* (98 males, 121 females, 15 larvae, 8 protonymphs, and 21 deutonymphs; Table 2; Fig. 4).

Differential Diagnosis of *D. ponderosus* and *Demodex criceti*. *D. ponderosus* n. sp. from *R. norvegicus* are morphologically most similar to *D. criceti* Nutting and Rauch from the hamster, *Mesocricetus auratus* Waterhouse and exhibit similar sexual dimorphism in terms of differentiation in size and shape (body proportions) of males and females. However, *D. ponderosus* n. sp. is larger and the opisthosoma of both sexes is longer than that of *D. criceti*. The gnathosoma of *D. ponderosus* n. sp. is oval, while that of *D. criceti* is trapezoidal. Particular structures within the gnathosoma are shaped and arranged differently, e.g., supracoxal spines. Also, location and shape of the male aedeagus are different (Table 3).

Discussion

Besides the well-known host specificity, topical specificity appears to be a characteristic feature in the evolution of the Demodecidae, which have developed specific adaptations to life, feeding, reproduction, and transmission in different types of microhabitats on particular host species. This led to speciation within host taxa, synhospitalic species of demodecid mites with different topography (distribution in various regions of the body), and topical specificity (relation with a specific microhabitat). For example, there are species known from common and sensory hair follicles, sebaceous glands, meibomian glands, epidermis and tissues of the oral cavity, and anterior parts of the alimentary tract. Usually, several mite species of this family occur in one host species, inhabiting different microhabitats (Izdebska 2010, Izdebska and Rolbiecki 2013a). Four species of *Demodex* have been previously reported parasitizing brown rats (Izdebska 2012). Of these, three, *D. ratti*, *D. ratticola*, and *D. norvegicus*, are morphologically similar (body proportions, presence, shape and dimorphic differentiation of opisthosomal organs, and some elements of gnathosoma); however, they also demonstrate a range of morphological differences including size and location (Tables 4 and 5). They appear to represent species of a common pedigree, where divergence has been conditioned by colonization of different microhabitats within the host. The fourth species, *D. nanus*, is smaller and morphologically distinct from the others (Tables 4 and 5). It has also been reported from another closely related host, the black rat, *R. rattus* (L.) (Desch 1987). It should be added that *D. ratti* and *D. ratticola* are both found associated with the head area of the rat; however, they probably exhibit different topical preferences when colonizing tissues and skin fragments. Also, *D. nanus* and *D. norvegicus* are usually found in the genital-anal region of host's body, where they demonstrate topical segregation with respect to skin structure and tissue (Table 5). The newly described *D. ponderosus* n. sp. differs from the previous species in terms of its structural features and location on the host (Tables 4 and 5). Although found in various body regions, all are thinly haired and often corneous.

Based on observations, *D. ponderosus* n. sp. appears to have lower infestation levels than other species (Table 2). Infestation parameters of other *Demodex*

Table 2. Infestation parameters of *Demodex* spp. in *R. norvegicus* ($n = 10$)

Species of <i>Demodex</i>	No. of individuals and ova	Prevalence (%)	Mean intensity ^a	Range of intensity ^a
<i>D. ponderosus</i> n. sp.	47 and 6	30	15.7	11–20
<i>D. nanus</i>	669 and 141	100	66.9	13–126
<i>D. norvegicus</i>	1187 and 454	80	148.4	8–449
<i>D. ratti</i>	61 and 2	60	10.2	2–25
<i>D. ratticola</i>	263 and 52	90	29.2	7–54
Total (including all parasites)	2227 and 603	100	222.7	52–566

^a Mean and range of intensity counted for the studied skin fragments.

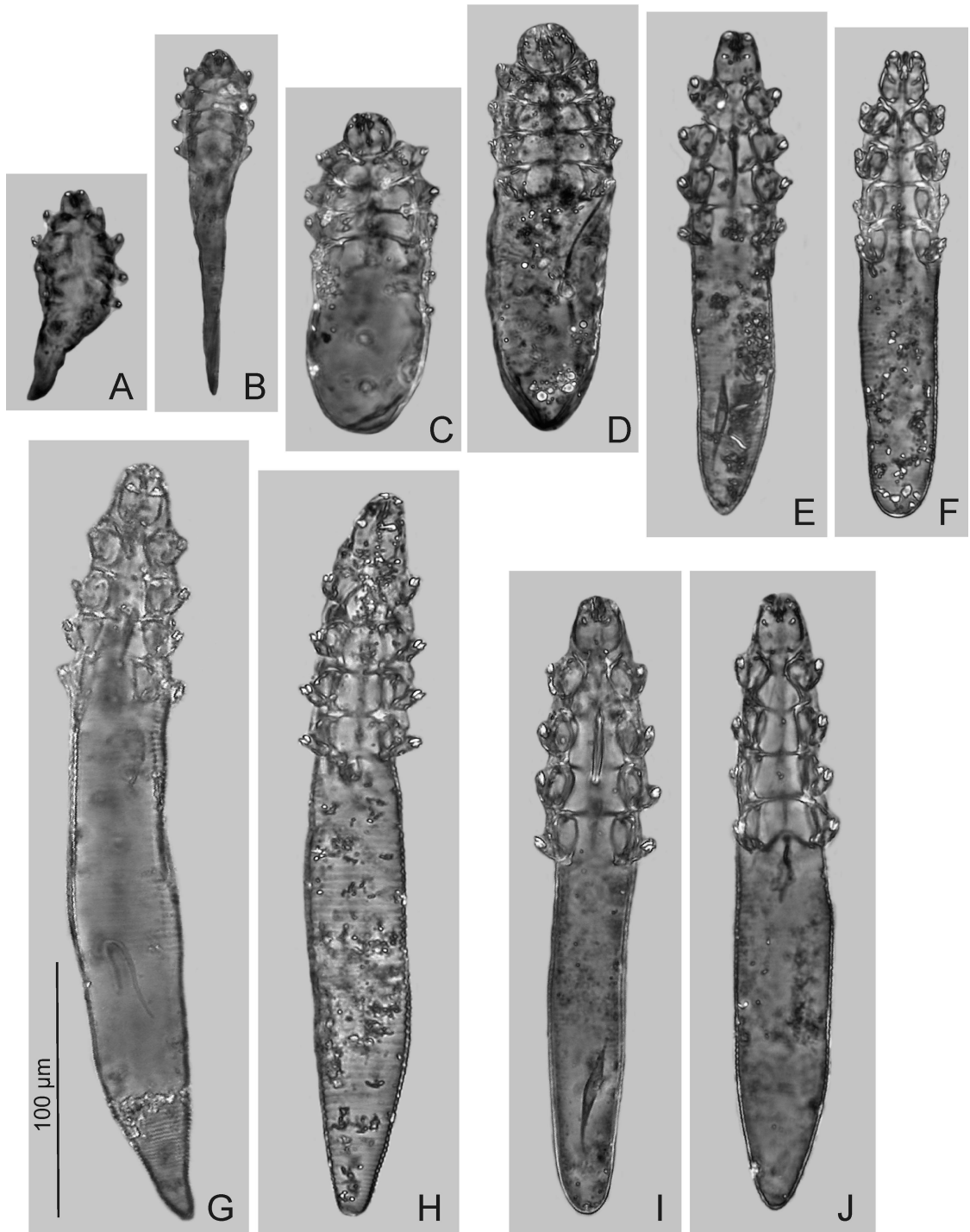


Fig. 4. *Demodex* species from *R. norvegicus*. (A and B) *D. nanus*, male, female. (C and D) *D. ponderosus* n. sp., male, female. (E and F) *D. ratticola*, male, female. (G and H) *D. ratti*, male, female. (I and J) *D. norvegicus*, male, female.

are usually high. Prevalence of *D. nanus* and *D. ratticola* often reaches 100% (Izdebska and Rolbiecki 2012a,b) with concurrently high intensity of the mites in skin fragments. In the case of *D. ponderosus* n. sp.,

only single individuals were observed, and they were dispersed on the host that does not favor their proliferation. Perhaps, for this reason, no immature stages of *D. ponderosus* were observed. Demodecid's popu-

Table 3. Differential diagnosis of *D. ponderosus* n. sp. and *D. criceti* (measurements in μm)

	<i>D. ponderosus</i> n. sp.		<i>D. criceti</i> (Nutting and Rauch 1958)	
	Male (n = 14)	Female (n = 33)	Male (n = 20)	Female (n = 20)
Length of body	(128 ± 7)	(163 ± 8)	(87 ± 4)	(103 ± 3)
Maximum width of body	(46 ± 3)	(50 ± 3)	(32 ± 1)	(34 ± 1)
Ratio of body length to body width	2.8	3.3	2.7	3.1
Ratio of opisthosoma length to body length [%]	50	54	35	45
Shape of gnathosoma	Oval		Trapezoidal	
Supracoxal spines	Minute ($\approx 1-2$), conical, directed outwardly		Greater ($\approx 3-4$), complex—two pieces, directed to the center of gnathosoma	
Aedeagus	Wide, length 21, located at the level of II and IV pairs of legs, genital orifice at the level of II pair of legs		Slender, length 16, located above then in <i>D. ponderosus</i> , genital orifice at the level of I and II pairs of legs	
Epimeral plates of IV pairs in females	Posterior edge arched		Posterior edge triangular	
Location	Skin poorly covered with hair (tail and paws)		Only epidermis, not found in the normal hair follicle	
Host	<i>R. norvegicus</i>		<i>M. auratus</i>	

Table 4. Characteristic quantitative features of *Demodex* species from *R. norvegicus* (measurements in μm)

	<i>D. nanus</i>		<i>D. ratticola</i>		<i>D. norvegicus</i>		<i>D. raffi</i>	
	Male (n = 20)	Female (n = 20)	Male (n = 20)	Female (n = 20)	Male (n = 20)	Female (n = 20)	Male (n = 20)	Female (n = 20)
Length of body	128 ± 7 (116-148)	163 ± 8 (145-183)	190 ± 15 (165-215)	182 ± 12 (165-216)	247 ± 7 (233-266)	245 ± 13 (225-267)	285 ± 20 (249-311)	282 ± 16 (258-310)
Maximum width of body	46 ± 3 (43-51)	50 ± 3 (45-55)	33 ± 4 (27-43)	35 ± 4 (29-42)	49 ± 7 (35-63)	37 ± 2 (32-43)	43 ± 7 (34-60)	38 ± 4 (34-47)
Ratio of body length to body width	2.8	3.3	5.8	5.3	5.2	6.6	6.6	7.5
Ratio of opisthosoma length to body length (%)	50	54	59	55	57	59	65	63
Aedeagus	21 ± 2 (19-26)	—	29 ± 2 (26-33)	—	32 ± 4 (25-40)	—	29 ± 3 (22-33)	—
Ovum	77 × 38 ± 10 × 6 (65-95 × 35-50) ^a	—	84 × 18 ± 8 × 3 (70-95 × 13-24) ^b	—	138 × 16 ± 12 × 3 (116-155 × 14-25) ^b	—	143 × 43 ± 11 × 4 (135-150 × 40-45) ^c	—

^a Measured 6 ova.

^b Measured 20 ova.

^c The immature stages are not found yet. Two ova were found currently.

Table 5. Characteristic qualitative features of *Demodex* species from *R. norvegicus*

	<i>D. ponderosus</i> n. sp.	<i>D. nanus</i>	<i>D. ratticola</i>	<i>D. norvegicus</i>	<i>D. rattii</i>
Supracoxal spines	Minute (1–2 μm), conical, located at the edge of gnathosoma, directed outwardly	Minute ($\approx 2 \mu\text{m}$), peglike, located at the edge of gnathosoma, directed outwardly	More prominent (3.5–4 μm), peglike, located closer to the center of gnathosoma and directed to the center of gnathosoma	More prominent ($\approx 3 \mu\text{m}$), paddle shape, located closer to the center of gnathosoma and directed to the center of gnathosoma	Conspicuously large (5–6.5 μm), arrowhead shape, located closer to the center of gnathosoma and directed to the center of gnathosoma
Epimeral plates	Distinct, meeting at midline	IV pair posteriorly confluent; male: crenulated medial edge, not meeting at midline; female: meeting at midline	IV pair posteriorly confluent; male: faintly defined, discernible near midline; female: more distinct than in male, meeting at midline	IV pair posteriorly confluent; male: faintly defined, indistinct near the midline; female: more distinct than in male, meeting at midline	Distinct, meeting at midline, IV pair posteriorly confluent
Shape of opisthosoma (Fig. 4)	Oval, wide	Narrow, strongly tapered toward end	Long, usually gradually tapered toward end	Long, usually gradually tapered toward end	Long, usually gradually tapered toward end
Opisthosomal organ	Absent	Absent	Male: spindle-shaped; female: finger-like	Male: spindle-shaped; female: finger-like	Male: reversed U-shaped; female: tubular
Vulva	Located directly below the edge of pair IV of epimeral paltes	Located clearly below the edge of pair IV of epimeral paltes	Located directly below the edge of pair IV of epimeral paltes	Located directly below the edge of pair IV of epimeral paltes	Located directly below the edge of pair IV of epimeral paltes
Aedeagus	Located at the level of the pair II and IV of epimeral plates, genital orifice at the level of II pair of legs	Located at the level of the pair I and II of epimeral plates, genital orifice at the level of I and II pairs of legs	Located at the level of the pair II and III of epimeral plates, genital orifice at the level of II pair of legs	Located at the level of the pair I and the III of epimeral plates, genital orifice at the level of I and II pairs of legs	Located at the level of the pair II and IV of epimeral plates, genital orifice at the level of II pair of legs
Shape of ovum (Fig. 3)	Oval	Oval	Club-shaped	Almost tubular, strongly extended	Oval
Location	Skin poorly covered with hair (ant. pairs)	Mainly skin of genital-anal region	Skin of head (nose, lips, and chin)	Skin of genital-anal region	Hairy skin of the body, generally head

lation structure depends on many factors, and fluctuates relative to environmental factors (seasonality) and microhabitat conditions associated with the physiological state of the host. Currently, immature stages were found in populations of *D. nanus* (adult to immature ratio is 1:0.9), *D. norvegicus* (1:0.7), and *D. ratticola* (1:0.2). While the immature stages of *D. ratticola* remain unknown, for the first time, we described the ovum of this species (Table 4).

Demodecids have rarely been the subject of study in the brown rat (Izdebska and Rolbiecki 2012a) and they have also not been included in previous elaborations of its parasitofauna, even those widely describing other groups of parasites (Pakdad et al. 2012). They have been described in elaborations concerning parasites and parasitic diseases of laboratory and domestic animals (i.a. Yunker 1973, Walberg et al. 1981). However, this does not provide an accurate picture of their diversity, infestation rates, or symptomatology, especially in the context of wild populations. On the basis of previous studies, demodecid's infestation level is much higher than that of other skin mites. In brown rats from the same area, the prevalence of sarcoptic mites was 13.3% with mean intensity 3.3 (*N. muris*: 10%, 3.7; and *T. diversus*: 3.3%, 2.0; Izdebska and Rolbiecki 2013b). In the region of Vancouver (Canada), *N. muris* were recorded only in 2.1% of rats (Anholt et al. 2014). Present observations on demodecids suggest that despite high prevalence and locally high intensity (up to a few hundred individuals per square centimeter of skin), infestations appears to be asymptomatic. It seems that the rats have a high tolerance to demodecids, as has been demonstrated in helminthes inhabiting the intestines and liver (Claveria et al. 2005). The asymptomatic nature of parasitic effects in rats suggests long-standing relationships between parasites and hosts.

However, the fact that the brown rat is a host for a range of parasites with little adverse effect on its population development or health may be worrisome, as *R. norvegicus* constitutes a reservoir for parasites and pathogens significant for human and domestic animal health that may find conducive conditions in the rat for survival, reproduction, and spread.

Acknowledgments

We thank the editor and the referees for helpful comments and suggestions on the manuscript.

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Received 1 August 2013; accepted 16 April 2014.