

TWO MORPHOLOGICALLY DISTINCT FORMS OF *DEMODEX* MITES FOUND IN DOGS WITH CANINE DEMODICOSIS FROM VLADIVOSTOK, RUSSIA

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(Received 07 August 2016, Accepted 20 January 2017)

The aim of this study was to investigate the morphology of *Demodex canis* and *Demodex* sp. *cornei* found in six dogs with canine demodicosis. A deep skin scraping technique was used for *Demodex* mite detection. Measurement data of 52 adult *D. canis* mites (26 females, 25 males and one specimen whose sex could not be determined) and 39 adult *Demodex* sp. *cornei* mites (22 females, 14 males and three specimens whose sex could not be determined) were reported. The correlation between body size of both *Demodex* species were estimated by the Student's t-test. There was a significant correlation between short-tail and long-tail forms and total body length and length of the podosoma and opisthosoma ($p < 0.05$). A significant difference was not found between the length of the gnathosoma and short-tail and long-tail forms ($p > 0.05$). *Demodex* sp. *cornei* and *D. canis*, found in dogs from Vladivostok, were smaller than species from other countries. However, the present data did not significantly differ from other studies with *D. canis* and *Demodex* sp. *cornei* descriptions.

Key words: demodicosis; dog; *Demodex canis*; *Demodex cornei*

INTRODUCTION

Canine demodicosis is one of the most well known skin diseases in veterinary practice [1]. Symptoms of demodicosis include alopecia, hyperaemia, erythema, seborrhea, follicular hyperkeratosis, crusts, pustules and dermatitis. The exact causes of mite proliferation are actually unknown. Some authors suspect that, since demodicosis frequently appears in dogs with an immunodeficient status, it is caused by immunosuppressive therapy (such hormones and cytotoxic therapy) or common immunosuppressive diseases such as diabetes mellitus and cancer. Stress can also cause demodicosis, especially in puppies [2,3]. Superficial and deep skin scrapings are used to detect *Demodex* mites.

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Mites of the genus *Demodex* (Owen, 1843), included in the family Demodecidae [4], have a small, thin, usually elongated body, with four pairs of rudimentary legs. Their bodies are divided into three distinct tagma: gnathosoma, podosoma and opisthosoma. The gnathosoma has a trapezoidal or rectangular form, and it consists of three-segmented palps, styliform chelicerae, 4-5 rounded bristles and a large hip element. The podosoma consists of four pairs of legs. The opisthosoma usually has an elongated form and has striations.

There are three distinguished *Demodex* mites in dogs: *D. canis*, *D. injai* [5] and *Demodex* sp. *cornei*.

Demodex canis (Leydig, 1859) mites were first discovered in 1844 by Simon [6] and were described and named in 1859 by Leydig [7]. All stages of the *D. canis* life cycle were described in detail by Sokolovskii [8] and French [9]. *Demodex canis* are wormlike mites, with an elongated body. Their trapezoidal gnathosoma is slightly wider than it is long. The pharyngeal bulb is horseshoe in shape. The epimeral plates are trapezoidal. The opisthosoma is long and its posterior end is pointed. The opisthosomal organ is a chitin tube present in females. Female mites are usually longer than males. Also the size of male mites can be very variable. The adult female has a total body length of 177-265 μm , and the male has a total body length of 146-251 μm [1].

Demodex injai (Desch and Hillier, 2003) was first reported in 1997 [5,10]. These mites have a long, thin body, with a total body length of more than twice the length of *D. canis* mites. The opisthosomal organ is present in both sexes.

Demodex sp. *Cornei*, firstly discovered by Mason, was in detail described in the 20th century [11-15]. *Demodex* sp. *cornei* mites have a short and wide body, the opisthosoma end is round and their total body length is 93-165 μm . Certain size and qualitative characteristics of *Demodex* sp. *cornei* (Table 1), including opisthosoma and podosoma length, total body length, ratio of opisthosoma length to total body length, shape of the opisthosoma end, shape of the epimeral plates and presence of the band-like plate in the lower edge of the fourth coxisternal plate, distinguish it from *D. canis* [1].

Table 1. Characteristics of two forms of *Demodex* mites found in dogs [1,20]

Characteristics	Short-tail form (<i>Demodex sp. cornei</i>)	Long-tail form (<i>Demodex canis</i>)
Total body length (μm)	93-165	146-265
Length of podosoma (μm)	39-66	50-81
Length of opisthosoma (μm)	36-99	74-167
Ratio of opisthosoma length to total body length (%)	38-50	50-63
Shape of body end	round	pointed
Shape of epimeral plates	rectangular	trapezoidal
Band-like plate in the lower edge of fourth coxisternal plate	present	absent
Microhabitat	hair follicles, sebaceous glands	epidermis (stratum corneum)

However, some molecular data show that *D. cornei* is probably just a morphological variant of *D. canis*, the so called “short-tail form” [16-17]. Today, descriptions of the short-body form are incomplete. *Demodex* sp. *cornei* has been described in different countries, and was recently described in dogs from Poland, Argentina, India and Thailand [1,18-21]. Reports with the descriptions of *Demodex* sp. *cornei* mites in dogs from Russia have not been published yet.

The aim of this study was to investigate the morphology of *Demodex* sp. *cornei* and *D. canis* found in dogs from Vladivostok.

MATERIALS AND METHODS

Samples, including skin scrapings, were taken from six household dogs with canine demodicosis (Table 2). The samples were investigated in the parasitology laboratory of the department of Marine Biodiversity and Bioresources, Far Eastern Federal University, Vladivostok during the year 2015. A modified deep skin scraping technique was used [2], and the scrapings were performed on the boundary of healthy and affected areas avoiding capillary bleeding. Specimens containing the mites of interest were fixed in 70% ethanol. For lightening, the skin scrapings were placed on a slide with a few drops of 10 % NaOH solution and then placed into a thermostat at +25 °C for 40 min. Preparations were examined under a phase contrast microscope with low and high magnification (10X, 40X and 100X). Approximately 91 mites were investigated. Total body length and size of each of the three tagma, the gnathosoma, podosoma and opisthosoma, were measured for each specimen. The ratio of opisthosoma length to body length, and the ratio of opisthosoma length to podosoma length, were calculated. The correlation between body size and *Demodex* sp. *cornei* and *D. canis* was evaluated via the Student’s t-test. A *P*-value obtained via the Student’s t-test of less than 0.05 was considered statistically significant.

Table 2. Summary of the anamnesis of the six observed dogs

No	Breed/sex	Age	Symptoms	Demodex mites
1	Purebred male	18 months	Localized demodicosis, alopecia behind an ear	
2	Crossbred male	5 months	Localized demodicosis with alopecia on the chest	Both forms (<i>D. canis</i> + <i>Demodex</i> sp. <i>cornei</i>)
3	Purebred male	6 years	Generalized demodicosis with erythema, deep pyoderma, alopecia	
4	Crossbred male	5 years	Generalized demodicosis with large alopecia areas on the tail and thighs, erythema on the head	
5	Crossbred female	6 years	Generalized demodicosis with alopecia, erythema and deep pyoderma on thighs and neck	<i>D. canis</i>
6	Crossbred female	8 years	Localized demodicosis with alopecia on the thighs	<i>D. canis</i>

RESULTS

Demodex sp. *cornei* and *D. canis* measurement results

Measurement data of 52 adult *D. canis* mites (26 females, 25 males and one specimen whose sex could not be determined) and 39 adult *Demodex* sp. *cornei* mites (22 females, 14 males and three specimens whose sex could not be determined) were reported (Table 3).

Table 3. Measurement data of *Demodex canis* and *Demodex* sp. *cornei*

Characteristics	<i>Demodex</i> sp. <i>cornei</i> (n=36)		<i>D. canis</i> (n=52)	
	Female (n=22)	Male (n=14)	Female (n=26)	Male (n=25)
Length of gnathosoma (µm)	15.9	15.1	16.2	16.2
	13.5-21.6	13.5-18.9	10.8-18.9	13.5-21.6
	SD 2.1	SD 2.3	SD 2.1	SD 2.2
Width of gnathosoma (µm)	14.8	14.3	15.1	13.5
	13.5-16.2	13.5-16.2	13.5-18.9	13.5-16.2
	SD 1.3	SD 1.3	SD 2	SD 1.3
Length of podosoma (µm)	40.5	39.7	40.5	40.5
	37.8-45.9	27-51.3	32.4-56.7	16.2-48.6
	SD 2	SD 5.9	SD 4.5	SD 6.7
Width of podosoma (µm)	24.3	24.3	25.9	24.3
	21.6-27	21.6-27	21.6-43.2	13.5-29.7
	SD 1.8	SD 2.3	SD 4.7	SD 3.6
Length of opisthosoma (µm)	48.6	41.8	75.6	62.1
	16.2-97.2	24.3-67.5	51.3-99.9	40.5-110.7
	SD 10	SD 9.7	SD 15.3	SD 18.4
Width of opisthosoma (µm)	21.6	23.5	20.5	18.9
	18.9-27	21.6-29.7	16.2-24.3	10.8-21.6
	SD 2.1	SD 2.5	SD 2.9	SD 3.8
Total length of body (µm)	105	98.8	126.9	118.8
	83.7-129.6	86.4-116.1	86.4-167.4	81-148.5
	SD 12.4	SD 8.7	SD 19.2	SD 19.9
Ratio of opisthosoma length to total body length (%)	44.7	42.3	60.8	52.3
	12.5-47.7	21.9-44.2	52.9-61.6	50-58.1
Ratio of opisthosoma length to podosoma length (%)	1.1	1.05	1.8	1.6
	0.9-1.4	0.8-1.5	1.2-2.2	1.6-2.7

Demodex sp. *cornei* mites had a short and wide body, and the mean total body length was 105 ± 12.4 µm in females and 98 ± 8.7 µm in males (Figure 1). The trapezoidal gnathosoma was slightly longer than it was wide. The pharyngeal bulb was horseshoe shaped, and the subgnathosoma setae were located posterolateral to the pharyngeal

bulb. The epimeral plates were rectangular in shape. The opisthosoma was short, and made up 44.7 % of the total body length (Table 3). *Demodex* sp. *cornei* mites had a rounded or slightly rounded posterior end of their opisthosoma; however, the opisthosomal organ was not present in both sexes. A band-like plate was present in the lower end of the fourth coxisternal plate. The vulva was a longitudinal slit of $4.9 \pm 0.2 \mu\text{m}$ in the ventral midline just posterior to coxa IV. The aedeagus was $18.8 \pm 2.1 \mu\text{m}$ long.

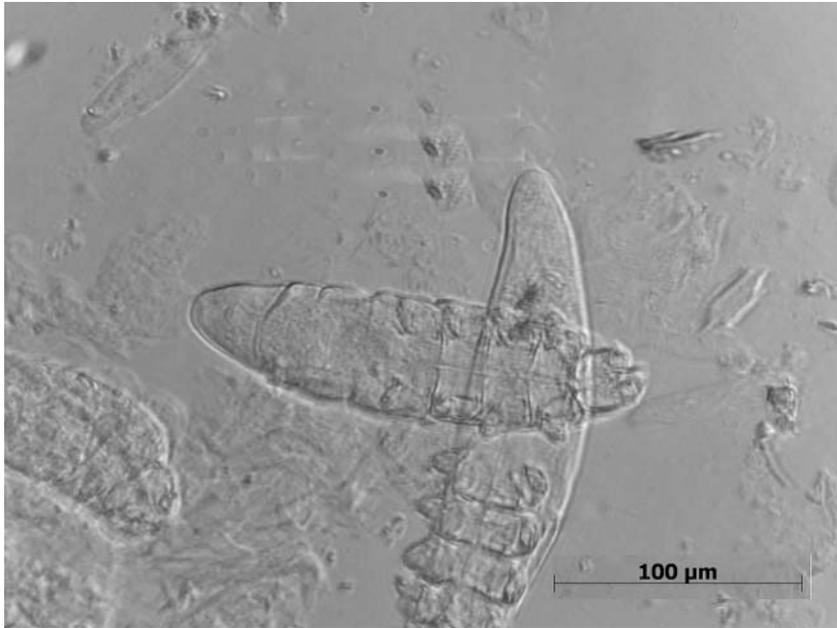


Figure 1. *Demodex* sp. *cornei* female

Demodex canis had an elongated body; the mean total body length was $126.9 \pm 19.2 \mu\text{m}$ in females and $118.8 \pm 19.9 \mu\text{m}$ in males (Table 3; Figures 2-3). The opisthosoma made up 52-60 % of the body length. Female specimens were significantly longer than male specimens. The vulva was $4.5 \pm 0.1 \mu\text{m}$ long and located in the posterior end of coxa IV. The aedeagus was $21 \pm 2.9 \mu\text{m}$ long.

Demodex sp. *cornei* and *D. canis* were distinguished by the following features:

- 1) The total length of female *Demodex* sp. *cornei* was less than that of female *D. canis*: $105 \pm 12.4 \mu\text{m}$ and $126.9 \pm 19.2 \mu\text{m}$, respectively.
- 2) *Demodex* sp. *cornei* had a short opisthosoma, with opisthosoma length less than or equal to the length of the podosoma. *Demodex canis* had a long opisthosoma tapering to the end, with the length of the opisthosoma usually longer than the length of the podosoma by 1.2-2.7 times.
- 3) *Demodex* sp. *cornei* had a rounded posterior end of the opisthosoma, and *D. canis* had a pointed end to its body.
- 4) The epimeral plates were rectangular in *Demodex* sp. *cornei* and trapezoidal in *D. canis*.

- 5) The opisthosomal organ was present in female *D. canis* and absent in both sexes of *Demodex* sp. *cornei*.

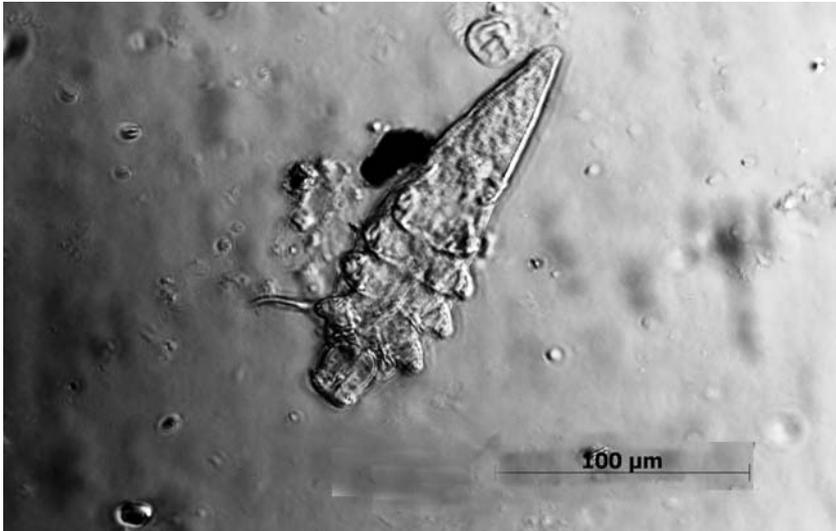


Figure 2. *Demodex canis* male

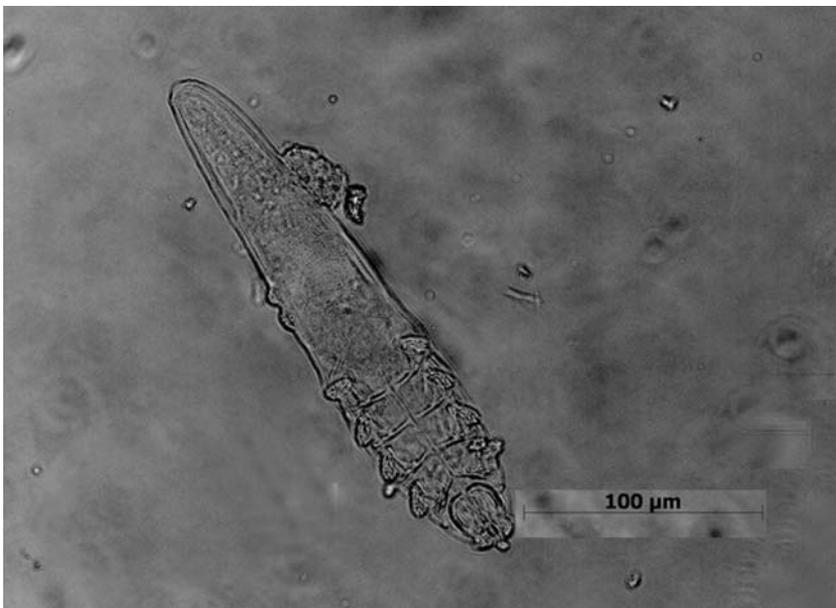


Figure 3. *Demodex canis* female

Statistical analysis results

The correlation between body size of two *Demodex* forms was estimated with the Student's t-test. A significant correlation between *Demodex* sp. *cornei* and *D. canis* was

observed for total body length and length of the podosoma and opisthosoma ($p < 0.05$). A significant difference was not found between the length of the gnathosoma and *Demodex* sp. *cornei* and *D. canis* ($p > 0.05$).

DISCUSSION

The results from this study do not significantly differ from other studies on *Demodex* sp. *cornei* and *D. canis* morphology. However, *Demodex* sp. *cornei* and *D. canis* specimens found in the present survey were smaller than both species from other countries (Table 4). The major characteristics of *Demodex* sp. *cornei*, including the rectangular epimeral plates, the short and wide opisthosoma and the rounded posterior end of the body, were present in mites from Russia. The data from this study are in accordance with other descriptions [1,19,20]. The other main qualitative feature is the band-like plate in the lower end of the fourth coxisternal plate presented in *Demodex* sp. *cornei*, absented in *D. canis* can be observed in *D. cornei* only using a scanning electron microscope [15]. So, in this survey, the band-like plate was not described.

Table 4. *Demodex* sp. *cornei* and *Demodex canis* measurement data from different regions

	<i>Demodex</i> sp. <i>cornei</i>				<i>D. canis</i>			
	Present survey	India [20]	Poland [1]	Thailand [18]	Present survey	India [20]	Poland [1]	Thailand [18]
Total length of body (μm)	98.8-105	132.21	120.8-139.4	156.9	118.8-126.9	214.32	195-226	217.83
Length of gnathosoma (μm)	15.1-15.9	19.1	19.1-19.9	23.5	16.2	18.89	25.2-26.9	24.84
Length of podosoma (μm)	39.7-40.5	61.06	52.1-53.9	60.0	40.5	60.98	63.4-63.8	60.89
Length of opisthosoma (μm)	41.8-48.6	61.48	46.2-65.4	59.25	62.1-75.6	129.68	33.3-34.6	147.50

Correlation analyses data in this study did not considerably differ from that of other report [20], except for the correlation between podosoma size and *Demodex* species, which was significant in the present survey.

Some authors reported that different types of skin scrapings can help to reveal which mites *Demodex* sp. *cornei* or *D. canis* cause demodicosis [18,20]. This is due to the different habitats of *Demodex* mites: *Demodex* sp. *cornei* lives in the superficial layer of the epidermis and *D. canis* lives in the hair follicles. *Demodex* mites in different locations have different adaptive strategies and cause different symptoms in infested animals. Therefore, two demodicosis types are distinguished: superficial demodicosis, caused by *D. canis*, and surface demodicosis, caused by *Demodex* sp. *cornei*. As a result, both the deep and superficial scrapings techniques may be useful in detecting *Demodex* mites [18]. In this study we used the deep scraping test only, and scrapings consisted of

samples of the stratum corneum and contents of hair follicles. However, *Demodex* sp. *cornei* and *D. canis* specimens were detected for both demodicosis types. In this study, infested dogs had localized and generalized demodicosis types, with alopecia, erythema and dermatitis. Pruritus was not present in the animals. Significant difference between demodicosis symptoms based on *Demodex* species was not found.

CONCLUSION

Measurement data of short-tail *Demodex* sp. *cornei* were described in Russia for the first time. These specimens do not considerably differ from other specimens described in other countries. However, a comprehensive investigation of *Demodex* mites is necessary for the control of canine demodicosis. The data can be interesting in the context of veterinary diagnostics and prognosis of demodicosis in dogs, and evaluation of its pathogenicity.

Authors' contributions

The author have made samples collection, specimens measurement, analysis and interpretation of data; have been involved in drafting the manuscript; have given final approval of the version to be published; and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Declaration of conflicting interests

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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DVE MORFOLOŠKI RAZLIČITE FORME *DEMODEX* PARAZITA KOD PASA SA DEMODIKOZOM U VLADIVOSTOKU (RUSIJA)

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Cilj studije je bio da se ispita morfologija *Demodex canis* i *Demodex* sp. *cornei*, koji su izolovani iz šest pasa kod kojih je dijagnostikovana demodikoza. Uzorci su uzimani dubokom skarifikacijom kože, oštrom kašikom. Prikazani su podaci dobijeni merenjem 52 odrasla parazita *D. canis* (26 ženki, 25 mužjaka i jedan izolat čiji pol nije mogao da se odredi) kao i 39 odrasla *Demodex* sp. *cornei* (22 ženke, 14 mužjaka i tri uzorka nedefinisanog pola). Upotrebom Studentovog t-testa, ispitivana je korelacija između veličine tela obe *Demodex* vrste. Postojala je značajna korelacija između formi sa kratkim i sa dugim repom i ukupne dužine tela kao i dužine podostome i opistozome ($p < 0,05$). Nije uočena značajna razlika između dužine gnatostoma i forme kratkih i dugih repova ($p > 0,05$). *Demodex* sp. *cornei* i *D. canis*, vrste koje su nađene u Vladivostoku, bile su manje od onih koje su opisane u drugim državama. Međutim, prikazani podaci ukazuju da ne postoji značajna razlika u rezultatima drugih studija u okviru kojih su obavljena opisivanja *D. canis* i *Demodex* sp. *cornei*.