

## ***Demodex neomydis* sp. n. (Acari: Demodecidae) from the hair follicles of the Mediterranean water shrew, *Neomys anomalus* (Insectivora: Soricidae)**

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**Abstract.** *Demodex neomydis* sp. n. from the Mediterranean water shrew, *Neomys anomalus*, is described as a new species in all developmental stages. This demodecid is classified as a member of the genus *Demodex* Owen, 1843, but shows several morphological characters described in *Soricidex dimorphus* Bukva, 1982 and which are absent or very infrequent in other known *Demodex* species, viz., in the adult stage, a pair of shelf-like lamellae on the dorsum of the podosoma, dorso-lateral extension of the podosoma over the basal part of the gnathosoma, multiple opisthosomal organ in the male, and podosomal position of the vulva in the female. Immature stages of *D. neomydis* have unusual inflated idiosoma and dorsad deflected gnathosoma. All developmental stages of *D. neomydis* were found in the lumen of the hair follicles on the host's muzzle, causing no gross pathological response. On histological level, the main pathological change was distension of infested hair follicles by accumulations of up to a dozen mites, which appear to feed on the epithelial cells of the hair follicle walls.

The morphology of the demodecids which have to date been described from insectivores is remarkably dichotomous. As far as details of their morphology are known, four of the five forms described from insectivores do not diverge significantly from typical representatives of the genus *Demodex* Owen, 1843 as found in other mammalian orders. These *Demodex* species from the insectivores are *D. erinacei* Hirst, 1917 from the European hedgehog, *Erinaceus europaeus* Linnaeus, *D. soricinus* Hirst, 1918 from the common shrew, *Sorex araneus* Linnaeus, *D. talpae* Hirst, 1921 from the common mole, *Talpa europaea* Linnaeus, and *D. foveolator* Bukva, 1984 from the lesser white-toothed shrew, *Crocidura suaveolens* (Pallas). Another, distinctly different type of demodecid which at present is only known from soricid insectivores, is represented by *Soricidex dimorphus* Bukva, 1982 from the common shrew. This mite species and genus shows numerous morphological peculiarities including pronounced sexual dimorphism (Bukva 1982, 1993) which have not been demonstrated in *Demodex* nor in other demodecid genera.

The new demodecid described below has a special systematic position in that it may be placed into the genus *Demodex* but displays several characters which resemble those of *Soricidex*.

### **MATERIALS AND METHODS**

Ten *Neomys anomalus* Cabrera, 1907 specimens snap-trapped in South Bohemia, Czech Republic were examined under a dissection microscope for presence of demodecid

mites by scraping, squeezing with watchmaker forceps, and/or disintegrating pieces of tissues with needles in Hoyer's medium. The examined body areas were the eyelids, external auditory meatus, nasal and buccal cavities, muzzle, vertex, back, genital/anal area, and tail. Dozens of specimens of the new mite species found were transferred into a clear drop of medium, mounted into permanent mounts, and later studied with phase contrast optics.

For histological examination, pieces of skin from infested sites in two hosts were excised, fixed in 10 % neutral formalin and processed for serial paraffin sections 6–8 µm thick which were stained with hematoxylin and eosin.

### **RESULTS**

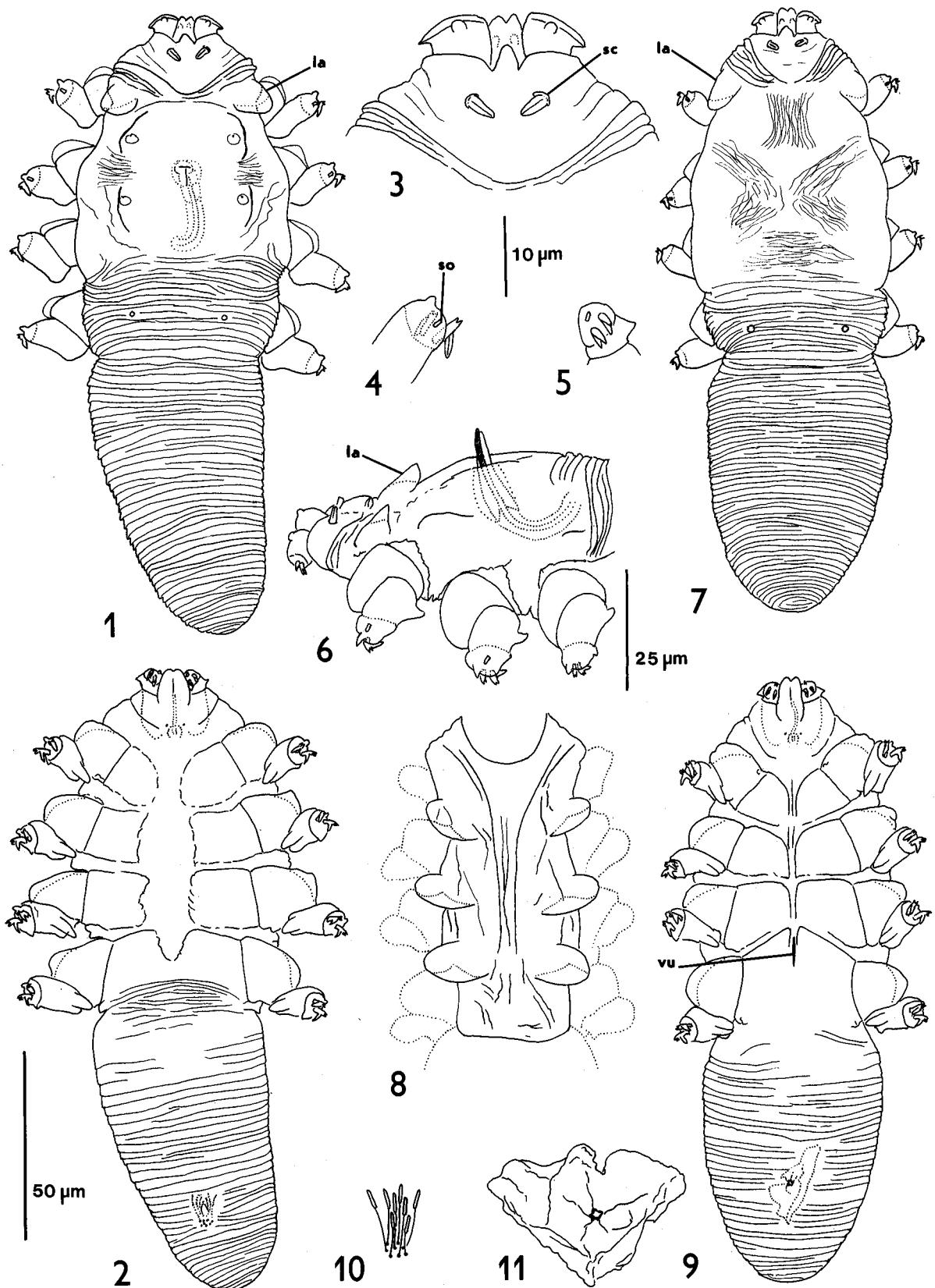
#### ***Demodex neomydis* sp. n.**

Figs. 1–29

A rather short and broad-bodied species; adults with short, broadly rounded opisthosoma and strong legs with large claws; immature stages (larva to nymph) inflated, with dorsad deflected gnathosoma.

**Male (holotype)** (Figs. 1–6, 10). Holotype body length 170 µm, with opisthosoma making up 0.47 of this value. Corresponding averages in the type series were 170.4 µm (range 150–186) and 0.44. Other measurements are in Table 1.

Gnathosoma conspicuously trapezoidal: at level with middle of gnathosomal capsule gnathosoma is wider than gnathosomal capsule long (measurements in Table 1), while it is very wide more posteriorly (Figs. 1–3). Palps (Figs. 3, 5) with two free segments; proximal segment with a round bulge dorsally, an arched ridge



**Figs. 1–11.** *Demodex neomydis*, adult stage. **Figs. 1–5.** Male, holotype. **Fig. 1.** Dorsal view. **Fig. 2.** Ventral view. **Fig. 3.** Gnathosoma, dorsal view. **Fig. 4.** Leg I, dorsal view. **Fig. 5.** Palp, ventral view. **Fig. 6.** Male, paratype, dorso-lateral view. **Figs. 7–9.** Female, allotype. **Fig. 7.** Dorsal view. **Fig. 8.** Coxisternum, the outer-most ventral podosomal structure. **Fig. 9.** Ventral view, coxisternum not drawn. **Fig. 10.** Male, paratype, opisthosomal organ. **Fig. 11.** Female, paratype, opisthosomal organ. **Explanations:** la – shelf-like lamella, sc – supracoxal spine, so – solenidion, vu – vulva. Scale bars: 50 µm – Figs. 1–2, 7–9; 25 µm – Fig. 6; 10 µm – Figs. 3–5, 10–11.

**Table 1.** Means, standard deviations, and ranges of measurements of *Demodex neomydis* (in  $\mu\text{m}$ ).

		Male (n = 20)	Female (n = 20)	
Gnathosoma	Length	21.4 $\pm$ 1.4 (19–24)	22.5 $\pm$ 0.6 (21–24)	
	Width	25.1 $\pm$ 1.4 (24–28)	24.5 $\pm$ 0.9 (23–27)	
Podosoma	Length	74.3 $\pm$ 4.0 (65–83)	71.9 $\pm$ 2.7 (68–77)	
	Width	61.1 $\pm$ 3.4 (56–67)	57.4 $\pm$ 3.8 (49–64)	
Opisthosoma	Length	74.6 $\pm$ 7.3 (57–84)	76.7 $\pm$ 5.3 (70–93)	
	Width	48.8 $\pm$ 5.6 (39–57)	50.5 $\pm$ 4.0 (41–58)	
Total length		170.4 $\pm$ 10.0 (150–186)	171.0 $\pm$ 5.2 (163–183)	
Aedeagus		27.8 $\pm$ 2.7 (22–31)	—	
Vulva		—	11.6 $\pm$ 0.4 (11–13)	
		Ovum (n = 20)	Larva (n = 10)	Protonymph (n = 7)
Length	82.1 $\pm$ 5.9 (71–91)	66.6 $\pm$ 8.3 (49–75)	81.7 $\pm$ 12.8 (68–102)	119.7 $\pm$ 23.7 (80–186)
	Width*	19.3 $\pm$ 1.8 (15–24)	43.6 $\pm$ 6.0 (31–51)	61.2 $\pm$ 10.7 (50–78)

\* Maximal transversal measurement in any (mostly lateral) view

antero-dorsally, and a sharp, recurved horn laterally; terminal segment with two strong (3  $\mu\text{m}$  long) double-tined spines and a minute single-tined spine. Supracoxal spines (Fig. 3) straight, dorso-medially directed pegs which are slightly widened towards blunt apex bearing terminal ridge; measurement in dorsal view: 4  $\mu\text{m}$  long. Distinct subgnathosomal setae anterior to pharyngeal bulb.

Podosoma with wavy lateral outlines; greatest width over legs III. Four pairs of strong legs evenly spaced along podosoma. Medial margins of coxal plates indistinct; those of coxal plates I–III widely separated, those of coxal plates IV widely separated anteriorly but fused in posterior half. Antero-lateral face of movable segment 1 of legs I–IV extends out more ventrally than dorsally. Movable segment 2 of each leg with prominent, heavily sclerotized ventro-proximal spur (on leg I, 7  $\mu\text{m}$  long). Terminal segment (Fig. 4) of each leg with a round cuticular projection and two strong claws; each claw with two tines terminally; midway along claw shaft a 3  $\mu\text{m}$  long spur with lateral keel/s. Terminal segment of legs I and II dorsally with a solenidion.

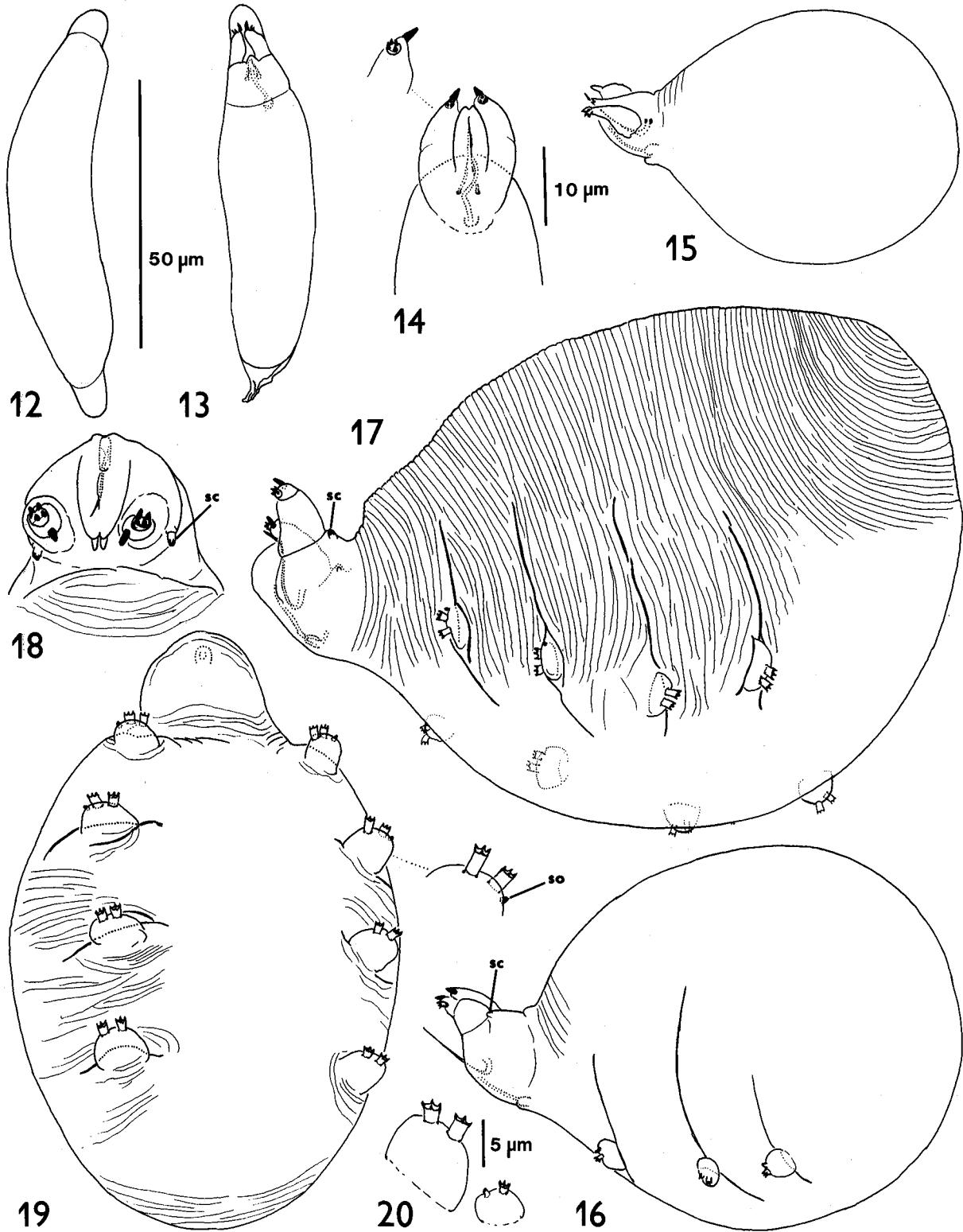
Dorsum of podosoma antero-laterally extended as two creased lobes over basal part of gnathosoma. Over legs I a pair of smooth, dorso-posteriorly extending shelf-like lamellae (Figs. 1, 6). Posterior to this an almost quadratic, flat and smooth shield demarcated by sclerotized ridges laterally; over legs II the ridge-like demarcation is interrupted by faint, close-set transverse cuticular striae. Posterior to shield, over legs IV, transverse cuticular striae as on opisthosoma.

Genital orifice a longitudinal slit at dorsal midline over legs II. Aedeagus (Figs. 1, 6) strong, much curved (in dorsal view 22–31  $\mu\text{m}$  long), with wide sheath. Two pairs of dorsal podosomal tubercles arranged subrectangularly (in holotype, centre to centre, anterior pair spaced 30  $\mu\text{m}$  apart, posterior pair 17  $\mu\text{m}$  behind anterior pair and spaced 32  $\mu\text{m}$  apart), poorly defined in shape but with a dot-like seta. Over legs IV a third pair of dorsal tubercles; minute (1.5  $\mu\text{m}$  in diameter), circular in outline, in holotype spaced 29  $\mu\text{m}$  apart.

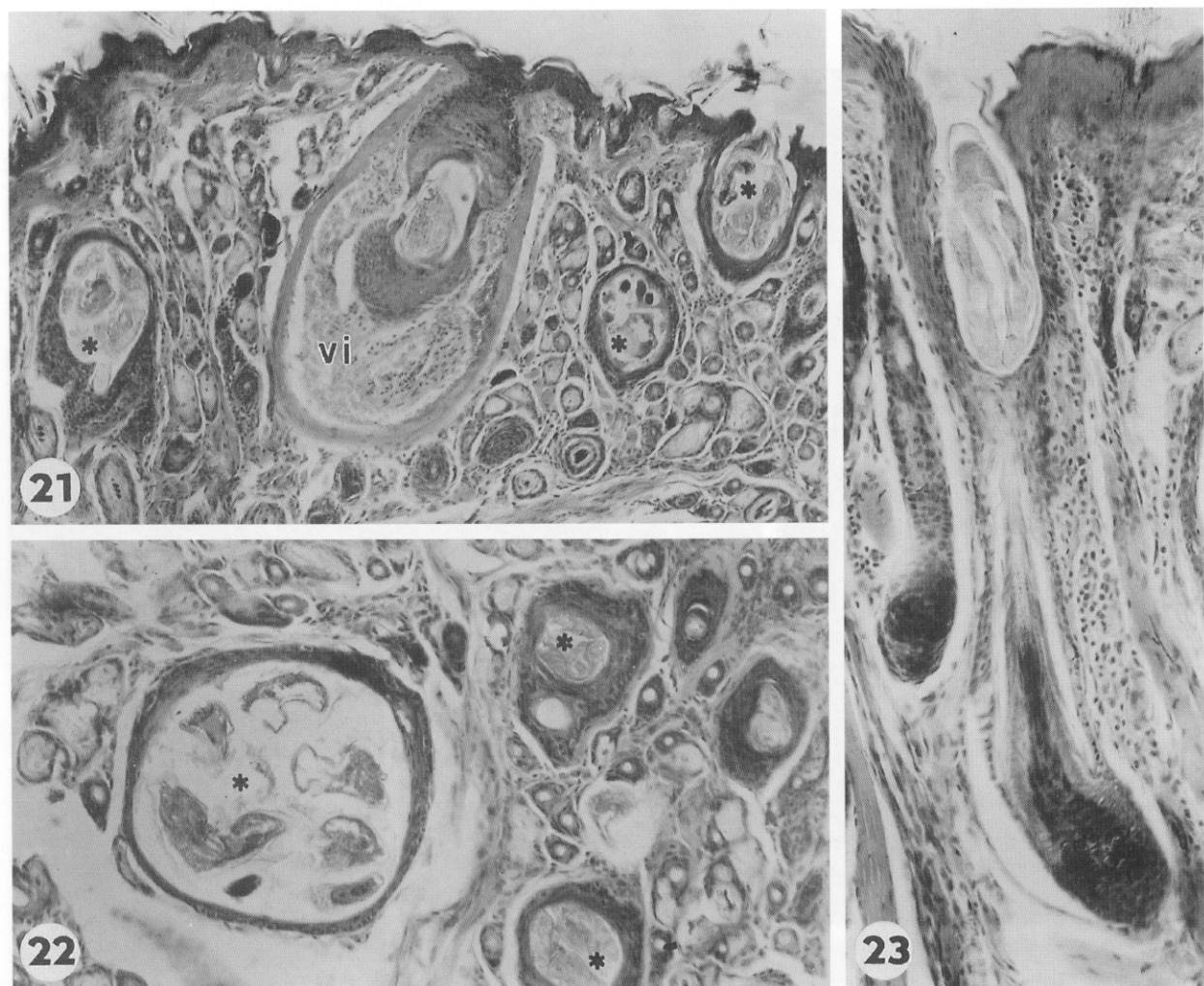
Opisthosoma as long as podosoma but narrower, almost tubular, with broadly rounded terminus. Cuticle with transverse striae which are less regular and less distinct ventrally. Opisthosomal organ (Fig. 10) multiple; a group of 8 minute cuticular pores positioned mid-ventrally 14–22  $\mu\text{m}$  from opisthosoma terminus; from each pore an approximately 8  $\mu\text{m}$  long, very narrow tubular invagination extends dorso-frontally, terminating in a minute elongate club ("stamen-like" appearance).

**Female** (allotype) (Figs. 7–9, 11). Allotype body length 168  $\mu\text{m}$ , with opisthosoma making up 0.44 of this value. Corresponding averages in the type series were 171.0  $\mu\text{m}$  (range 163–183) and 0.45. Other measurements are in Table 1.

Gnathosoma similar to male. Legs less laterally extended than in male. Coxal plates inclusive of their medial margins distinct; medial margins of coxae I–III meeting at midline, coxae IV confluent except for an 8  $\mu\text{m}$  long anterior part. Anterior margins of coxal plates IV deflected medio-anteriorly, meeting at an angle of



**Figs. 12–20.** *Demodex neomydis*, immature stages. **Fig. 12.** Ovum. **Fig. 13.** Ovum containing fully formed larva. **Fig. 14.** Larva, gnathosoma, ventro-frontal view. **Fig. 15.** Larva, lateral view. **Fig. 16.** Protonymph, lateral view. **Fig. 17.** Nymph, lateral view. **Fig. 18.** Nymph, gnathosoma, dorsal view. **Fig. 19.** Nymph, ventral view (same specimen and same position as in Fig. 18). **Fig. 20.** Protonymph containing fully formed nymph, detail of protonymphal (right) and nymphal (left) legs III. **Explanations:** sc – supracoxal spine, so – solenidion. Scale bars: 50 µm – Figs. 12–13, 15–17, 19; 10 µm – Figs. 14, 18; 5 µm – Fig. 20.



Figs. 21–23. *Demodex neomydis* in the skin on muzzle of *Neomys anomalus* (sections stained with hematoxylin-eosin). **Fig. 21.** Three mite-infested (general) hair follicles and a mite-free vibrissa follicle (x120). **Fig. 22.** Two moderately (right) and one distinctly (left) distended hair follicles containing mites (x 190). **Fig. 23.** Hair follicle with two mites in the infundibulum (x 220). **Explanations:** asterisk – hair follicle infested by mites (in each of these six hair follicles was a hair, although they cannot be clearly seen in the photographs), vi – vibrissa.

125 degrees. Free leg segments similar to male. Ventral cuticle covering coxal plates (Fig. 8) transversally undivided ("coxisternum") but with a pair of pronounced longitudinal striae which are widely spaced anteriorly, converge under legs I and then extend as parallel lines to legs IV; also present are some other, less regularly distributed striae; in inter-leg gaps three pairs of rounded lateral extensions. Dorsally, the anterior-most part of podosoma with creased cuticle and a pair of lamellae as in male. Posterior to this, cuticle of dorsum with faint, close-set fingerprint-like striae over legs I–III, and with prominent transverse striae, as on opisthosoma, over legs IV. A pair of round dorsal tubercles over legs IV similar to male.

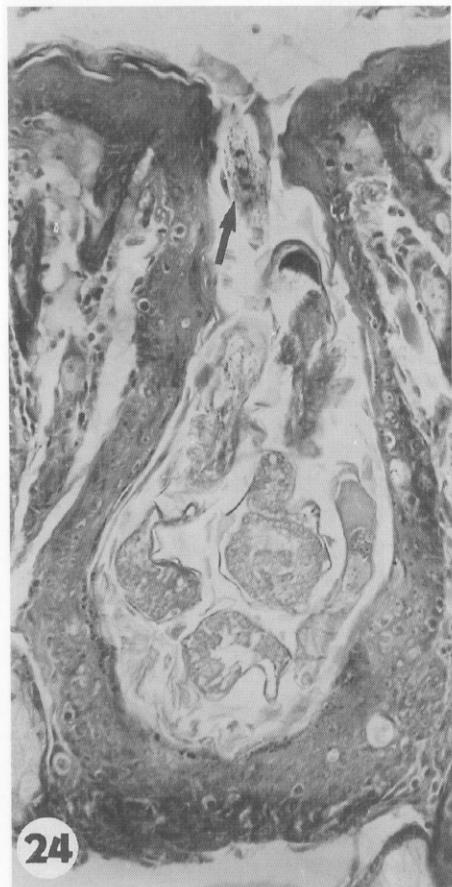
Podosoma-opisthosoma junction constricted; opisthosoma narrower than podosoma, with broadly

rounded terminus. Single, minute pore of opisthosomal organ positioned midventrally 28–34 µm from opisthosoma terminus. Invagination of opisthosomal organ poorly defined as faint (in some specimens even not discernible), variably shaped membranes extending to up to 10–15 µm distance around the pore, these membranes apparently being creased walls of a collapsed, large, very weakly sclerotized sac (Fig. 11).

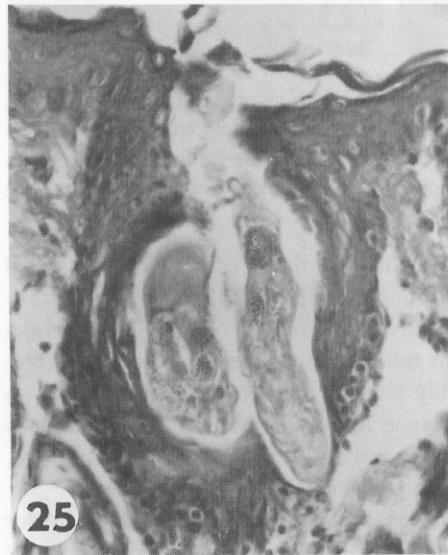
Vulva (Fig. 9) at the apex of converging anterior margins of coxae IV, a longitudinal, approximately 12 µm long simple slit obscured by flap of coxisiernum.

**Ovum** (Figs. 12–13). Non-operculate; with hyaline polar caps which protrude from both ends of central, elongate oval- to banana-shaped part containing finely granular material. Larva differentiates only from the granular material.<sup>1</sup>

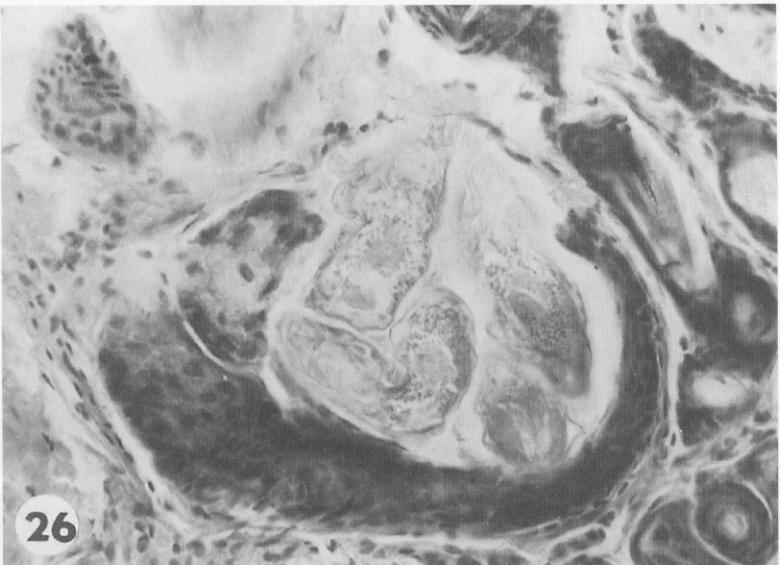
<sup>1</sup>The sequence of development was verified by findings of the subsequent-within-preceding stages as follows: ovum in female, larva in ovum, nymph in protonymph, and adult in nymph.



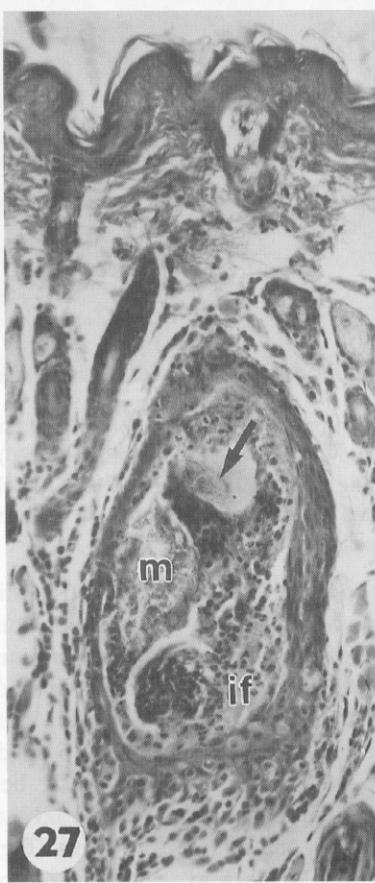
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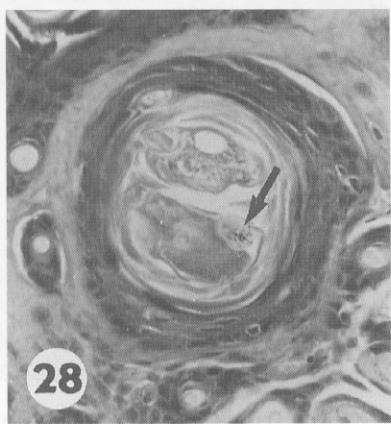
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**Figs. 24–29.** *Demodex neomydis* in the skin on muzzle of *Neomys anomalus* (sections stained with hematoxylin-eosin). **Fig. 24.** Distended hair follicle which contains several mites in various developmental stages ( $\times 220$ ). **Fig. 25.** Two mites in a distended hair follicle. Note evidence of mite feeding on the epithelial cells of the follicle wall (right) and the concavity of the follicle wall due to pressure of the mite body (left) ( $\times 340$ ). **Fig. 26.** Four mites in a distended hair follicle ( $\times 360$ ). **Fig. 27.** Distended hair follicle the lumen of which contains a hair, a mite and a rich infiltrate of polymorphonuclears ( $\times 230$ ). **Fig. 28.** Two mites in a moderately distended hair follicle. Note concentric layers of desquamated keratinized epithelium ( $\times 320$ ). **Fig. 29.** Distended hair follicle filled with necrotic material ( $\times 270$ ). **Explanations:** arrow – hair, if – infiltrate, m – mite.

**Larva** (Figs. 14–15). Probably slender shortly after eclosion (cf. Figs. 12–13) but most specimens observed were bulged (more dorsoventrally than laterally), their body outlines in lateral view being broadly oval to almost spherical with protruding gnathosoma. Palps and cheliceral stylets pointing antero-dorsad; on tapering apex of palps a comparatively strong, heavily sclerotized spine; latero-subterminally a spine terminating in four points. Segmentation of palps not apparent. Minute, not apparently sclerotized peg-like supracoxal spines at base of palps. Legs and ventral scutes absent. Cuticular striations not apparent except for a few faint striae posterior to gnathosoma.

**Protonymph** (Figs. 16, 20). Similar to larva but with three pairs of legs. Gnathosomal structures similar to nymph (see below) but smaller and less clearly discernible. Legs unsegmented, originating at distinct, transverse cuticular furrows; each leg with two minute claws, of which the ventral one is larger and terminates in several (apparently 4) points, while the dorsal one is smaller and apparently single- or double-pointed. Ventral scutes absent. Cuticular striations as in larva.

**Nymph** (Figs. 17–20). Similar to protonymph but with four pairs of legs. Gnathosoma deflected dorsad. Terminal segment of two-segmented palps with a strong, single-pointed spine terminally, and a strong, four-pointed spine (which is rather similar to leg claws) latero-subterminally. Dorso-laterally at base of palps a pair of short, peg-like supracoxal spines. Legs each at a distinct, transverse cuticular furrow; each leg unsegmented and with a pair of claws. Leg claws approximately 2.5  $\mu\text{m}$  long, each terminating in four points. Legs I and II with a short peg-like solenidion dorsad to lateral claw. Ventral scutes absent. Idiosoma with faint transverse striations dorsally and laterally.

**Host:** The Mediterranean water shrew, *Neomys anomalus* Cabrera, 1907.

**Locality:** Type series is from a host specimen taken in České Budějovice, South Bohemia, Czech Republic, 24 April 1990. Additional specimens are from another host specimen previously taken at the same locality, 14 May 1981.

**Deposition of materials:** Type collection in the Institute of Parasitology, Academy of Sciences of the Czech Republic, České Budějovice. Holotype (ringed in black), Coll. No. 1966. Allotype (ringed in red) with another 5 female and 1 male paratypes, Coll. No. 1967. Some other paratypes including larvae, protonymphs and nymphs, Coll. No. 1968. Other voucher materials are in my collection in the same Institute.

**Etymology:** The species name “*neomydis*” (genitive of *Neomys*) relates to the type host.

**Comparison with other species.** The set of characters resembling *Soricidex* as discussed below, makes *D. neomydis* dissimilar from the *Demodex* species described to date. Very broad-bodied immature stages have also been described for *D. intermedius* Lukoschus, Mertens, Nutting et Nadchatram, 1984 from the tree-shrew *Tupaia glis* (Diard)<sup>2</sup>; in this species, however, the broadness of the immatures seems only to parallel the unusual broadness of the adults (dorso-ventral inflation was not mentioned by Lukoschus et al. 1984).

**Prevalence, habitat and pathogenicity.** Of the 10 host specimens examined, *D. neomydis* was found in two. In both these hosts the infested site was the muzzle. Gross signs of infestation were not observed. Examination of serial histological sections revealed that the specific microhabitat of all developmental stages is the lumen of general hair follicles, whereas vibrissa follicles in the immediate vicinity were invariably mite-free (Fig. 21).

Depending on number of mites (up to about a dozen of mites in a hair follicle) the infested hair follicles were slightly (e.g., Figs. 22–23) to distinctly (Figs. 22, 24, 26) distended. Some mites appeared to be feeding on the epithelial cells of the follicle wall (Fig. 25). The epithelial lining of some more distended hair follicles was rather thin (Fig. 22), which might be a combined result of mite feeding and pressure atrophy due to accumulation of mite bodies (cf. Fig. 25). Other host responses observed less frequently included an increased keratinization (Fig. 28) and polymorphonuclear infiltrates (Fig. 27). Some infested hair follicles were filled with necrotic material (Fig. 29). In most hair follicles, largely irrespective of the level of infestation and host response, were intact hairs (Figs. 21–22, 24, 27–28).

## DISCUSSION

The new form is classified as a *Demodex*, since generally it corresponds with the diagnosis of this principal demodecid genus (for the latest amendment see in Desch et al. 1972). It should, however, be noted that some characters of *D. neomydis* resemble those present in *Soricidex dimorphus* (Bukva 1982, 1993) which were, in part, used also for the generic diagnosis of *Soricidex* (Bukva 1982):

- (1) In both sexes of *D. neomydis*, a pair of shelf-like lamellae on dorsum of podosoma resemble three pairs of similar lamellae in female *Soricidex*.
- (2) In both sexes of *D. neomydis*, podosoma extends antero-laterally over the basal part of gnathosoma. This extension, however, is less pronounced than in *S. dimorphus*.

<sup>2</sup>At present the tree-shrews (family Tupaiidae) are classified as a separate order Scandentia, but modern authors have classified them also as a family of Primates or Insectivora (Wilson and Reeder 1992).

(3) In both sexes of *D. neomydis*, the opisthosomal organs are very similar to those in *Soricidex*. (On re-examination, the irregular, filamentous branches adjacent to the opisthosomal organ pore described for the opisthosomal organ in female *S. dimorphus* [Bukva 1982] should be interpreted as creases on a large, weakly sclerotized sac-like invagination whose peripheral contours are not seen. Similarly, elongate bulbous tips of the male opisthosomal organ invaginations - a minute detail which was doubtful in the original description of *S. dimorphus* [Bukva 1982] - were clearly seen in some re-examined specimens.)<sup>3</sup>

(4) As in *Soricidex*, the position of vulva in *D. neomydis* is podosomal and the vulva is obscured by the posterior part of ventral cuticle of the podosoma.

(5) The amphipolar differentiation of the *D. neomydis* ovum is reminiscent of the filamentous appendages on the ends of the ovum in *S. dimorphus*. The inflated

<sup>3</sup>I have also found this multiple *Soricidex*-like type of opisthosomal organ (12 cuticular pores with very narrow tubular invaginations) in a male of an undescribed *Demodex* sp. from *Neomys fodiens* Pennant. In other respects this species has a typical *D. folliculorum*-like appearance. To the best of my knowledge, the multiple type of opisthosomal organ has not been found in *Demodex* species from non-insectivoran eutherian hosts. Similarity to male opisthosomal organs of *Demodex* from metatherian hosts has been noted elsewhere (Bukva 1982).

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appearance of immature stages in *D. neomydis* is a conspicuous character also of the nymph in *S. dimorphus*.

As far as known, with the exception of the sac-like type of the opisthosomal organ in females, the *Soricidex*-like characters enumerated above are either absent or, at the most, very infrequent in the genus *Demodex*, notably in the numerous *Demodex* species from non-insectivoran hosts. Thus the taxonomic value of these *Soricidex*-like characters should be carefully evaluated when more species of *Demodex*, *Soricidex* and other genera are known, the generic diagnoses of demodicids amended, and/or new genera/subgenera erected.

The present revelation of some similarity of *D. neomydis* to *S. dimorphus* by no means casts doubts about validity of the genus *Soricidex* Bukva, 1982: *S. dimorphus* from the common shrew still remains basically dissimilar from any known *Demodex* species. Besides, it appears that the genus *Soricidex* is not monotypic, since *Soricidex* mites found in two other soricid species (the author's unpublished findings) include at least one additional separate species.

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