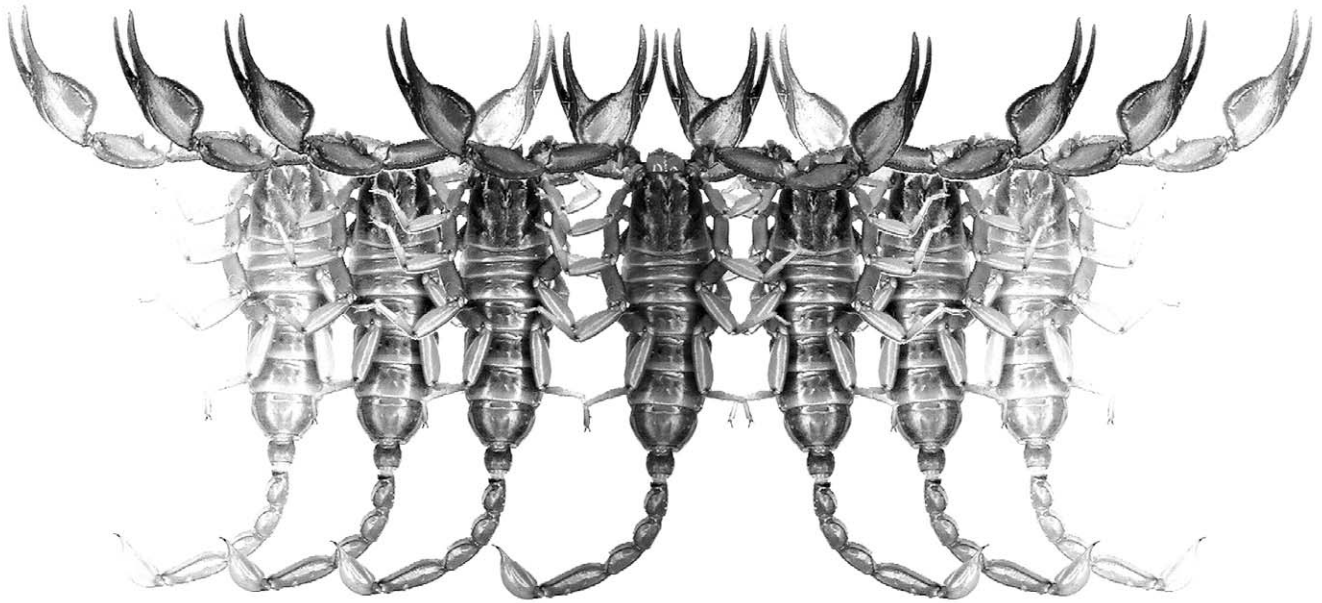


Euscorpius

Occasional Publications in Scorpiology



The First Record of Malformed Pectines in Genus *Euscorpius* (Scorpiones: Euscorpiidae)

Miroslav Šarić & Jovana Tomić

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EDITOR: Victor Fet, Marshall University, 'fet@marshall.edu'
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The first record of malformed pectines in genus *Euscorpius* (Scorpiones: Euscorpiidae)

Miroslav Šarić & Jovana Tomić

Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad,
Trg Dositeja Obradovića 2, 21000 Novi Sad, Serbia. e-mail: miroslav.saric064@gmail.com

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Summary

A teratological change in a pectinal organ of *Euscorpius* cf. *carpathicus* (C. L. Koch, 1837) from Serbia had been examined using SEM (Scanning Electron Microscopy), and compared to specimens with normally developed pectines. Possible reasons for this anomaly are discussed.

Introduction

Teratology in Scorpions

Teratological changes in scorpions have been often reported, usually in Buthidae, the most diverse and common scorpion family worldwide. Duplication of body parts has been documented in *Centruroides gracilis*, *C. sculpturatus*, *C. vittatus*, *Hottentotta alticola*, *Isometrus maculatus*, *Leiurus quinquestriatus*, and *Tityus serrulatus*. Other cases included pedipalp, tergite, and cheliceral anomalies (Teruel, 2003). Absence of one leg was reported for *Scorpio fuscus* (Scorpionidae) (David, 2012). Duplications of metasoma, tergite divisions or fusions, pedipalp chela compressions, leg malformations, sexual anomalies, and pedipalp fusions were reported by Mattoni (2005). Duplications of metasoma are known in Buthidae and Euscorpiidae (Sissom & Shelley, 1995; Lourenço & Hypolite, 2010). Two interesting duplications of metasomal segments have been observed in Buthidae: in *Tityus obscurus*, a duplication starting from metasomal segment II; and in *Centruroides nitidus*, from mesosomal segment VII (Seiter & Teruel 2014). Chela malformations had been recorded in *Superstitionia donensis* (Superstitioniidae) (Graham, 2006). Karataş & Kürtüllü (2006) reported a duplication of pedipalp of *Androctonus crassicauda* (Buthidae). Profound malformations of pedipalps had been recorded in *Ortochirus* sp. (Buthidae); and in *Hemiscorpius* sp. (Hemiscorpiidae), a malformation of telson (Jahanifard et al., 2008). A pectinal developmental anomaly in *Vaejovis lapidicola* (Ayrey, 2011) had been recorded; other pectinal malformations were observed in Vaejovidae (R. D. Farley, pers. comm.). Pectinal teratology (fusion of teeth, underdevelopment of a tooth) is occasionally observed but rarely reported (V. Fet, pers. comm.). A spectacular duplication of

pectines, combined with hermaphroditism and gynandromorphism had been recorded in *Mesomexovis punctatus* by Teruel & Baldazo-Monsivaiz (2015).

Scorpion Pectines: An Overview

Pectines are double, comb-like, sensory organs located on ventral side of scorpion mesosoma (Fig. 3). Each pecten is composed of a flexible, segmented part and a series of movable teeth. On the ground-facing surface of each tooth are dense patches of peg-shaped sensilla (Gaffin & Walvoord, 2004). During embryogenesis, pectines are formed on third opisthosomal (mesosomal) segment (O3) as limb buds closely joined with the body surface. Pectines are similar in function to the antennae of insects, and are very important sensory organs that scorpions use for orientation in environment because they are sensitive to mechanic stimuli and especially chemosensory stimuli of various volatile organic substances (Cloudsley-Thompson, 1955; Melville, 2000; Knowlton & Gaffin, 2010; Gaffin & Zhao, 2014). Pectines are highly innervated with a two large nerves (Wolf, 2008, fig. 3 and fig. 7 B) that branch into smaller nerves in each pecten that innervate individual teeth and pads around the base of a hair sensillum (usually five neurons), with 12 to 15 sensory neurons innervating each peg (Ivanov & Balashov, 1979; Foelix & Müller-Vorholt, 1983; Wolf, 2008). Main pectine nerves are passing through the body, and entering in the pectine neuropil at posterior part of the subesophageal ganglion mass. In this way signals collected by chemoreceptors on peg sensilla are quickly transferred to central nervous system, so scorpions get quick feedback information about current changes in the environment (see Wolf, 2008, figs. 1 and 3; Wolf & Harzsch, 2012, fig. 2A; Wolf, 2013, fig. 2). The pectines are



Figures 1–2: 1 (top). Serbia, Mt. Gučevo, Site 2, 44°30'20.20" N 19°09'44.30" E elevation: 237 m asl. **2 (bottom).** Female *Euscorpius* cf. *carpathicus* from Mt. Gučevo, JTPC No. 2.

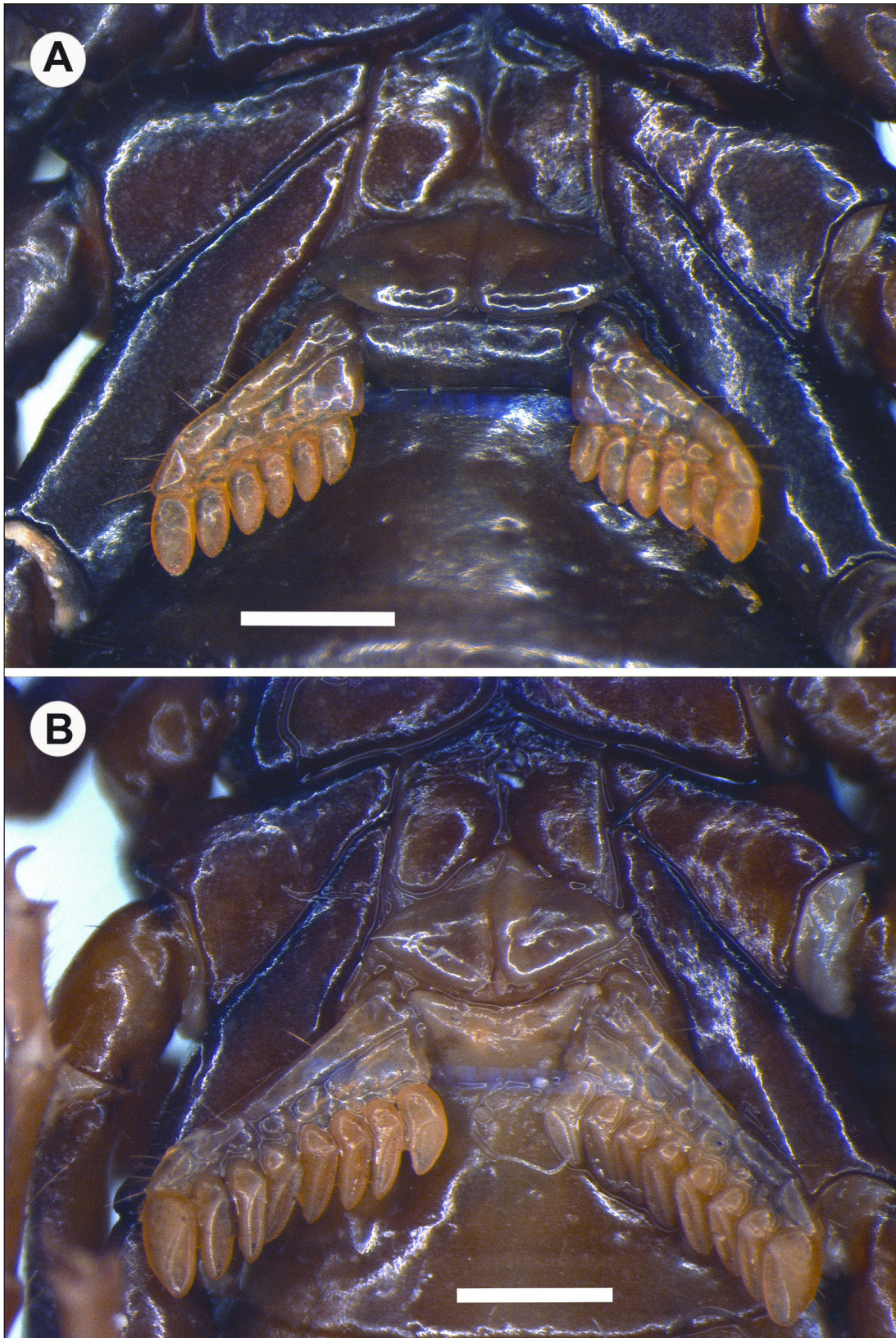


Figure 3: Normal pectines of *Euscorpius* cf. *carpathicus* from Mt. Gučevo, (A) female; (B) male. Scale bar 1 mm.

important in guiding males to prospective mates and perhaps in spermatophore exchange and in relocating stung prey (Gaffin & Walvoord, 2004). Fully developed pectines had been observed already in early Silurian scorpions (Kjellesvig-Waering, 1986; Farley, 1999, 2001a, 2001c).

Material and Methods

Terminology

Pectinal terminology used is partially from Hjelle (1990, fig. 2.4); numbering order of pectinal teeth is changed for easier orientation, counting from distal to basal.

During field work on Mt. Gučevo, western Serbia, in August 2014, 20 subadult and adult specimens were collected from three localities, each plot $200 \pm 5\text{m}^2$. The imaged specimen has been collected from "Site 2" at $44^\circ 30' 20.20''$ N; $19^\circ 09' 44.30''$ E, elevation: 237 m asl (Fig. 1). After morphological examination of all specimens, deformation of pectines had been noticed in *Euscorpius* cf. *carpathicus* female (JTPC No. 2, Fig. 2), but further taxonomic clarification is required. Photographs of specimen and locality had been taken with Sony DSC-W810, with 6x optical zoom and 20.1 megapixels. Photographs were processed in Adobe Photoshop only to adjust the brightness and contrast.

Specimen depositories

JTPC, private collection of Jovana Tomić; MSPC, private collection of Miroslav Šarić, LTZI, Laboratory for taxonomy and zoogeography of invertebrates, Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia.

Scanning electron microscopy

Right pectinal comb had been removed using ophthalmic scissors under stereo microscope and placed in 70% ethanol, and then in 90% ethanol. The comb was mounted on SEM pin stub using the standard procedure developed by the second author. After drying in JEOL-JEE-4B vacuum evaporator, specimens had been prepared for coating with gold in BAL-TEC, SCD 005 Sputter Coater. The comb was imaged at the University Center for Electron Microscopy, Novi Sad, under JEOL JSM-6460 LV scanning microscope with EDS device Oxford INCA, at resolution of 3–4 nm, the magnification range 8–300.000x, with the ability to work in a low vacuum environment to levels SEI, BEI topo, compo and shadow. For this study, magnification in range of 70–2000x, acceleration voltage of 20 eV and SEI signal were used.

Results and Discussion

After the initial examination under stereo microscope, we noticed that left pecten of one female had seven normally developed pectinal teeth (Fig. 4), while the right pecten had only first and second (distal) teeth normally developed, but third, fourth, fifth, and sixth teeth had been totally fused and seventh tooth (at the base) had been malformed. All substructures were present, including three anterior lamellae; the most basal lamella is much longer than the middle and distal lamellae (Fig. 5). The basal segment of middle lamella is much longer than the distal one; third and fourth lamellae are significantly modified. Fulcra are very modified; only the fulcrum between the sixth and seventh teeth appears to be normally shaped, while the fulcrum between fifth and sixth teeth is elongated. A fulcrum is absent between fourth and fifth teeth, and fused in a pentagonal structure between second and third teeth (Figs. 5–10). The sensorial areas are developed only in the first and second teeth, in all other teeth they are strongly modified. Peg sensilla are cylindrical blunt-pointed structures, about 5–20 μm long, and 3–5 μm wide (Figs. 12–15). On the first and second pectine teeth, next to developed peg sensilla, we noticed smaller bud-like structures (Fig. 12). We assume that after every molting, new peg sensilla are developed from these structures. Similar structures are reported in *Iurus dufourei* embryo (Kovářik et al., 2010, Fig. 240) and in *Centruroides vittatus* embryo (Farley, 2001b, fig. 16; 2011, figs. 8 and 9). Malformed teeth (Figs. 7, 8, 10, 16) are probably less effective in comparison to the first and second teeth that have fully developed sensorial areas. We counted individual peg sensilla in all teeth, and compared the results (Table 1), using percentage of peg sensilla: $\% = (a \times 100) / b$ (where a is the number of peg sensilla on a tooth, and b , number of sensilla on second tooth) (Table 2).

We conclude that this female most probably had difficulties to collect signals from the environment due to uneven distribution and lack of sufficient numbers of peg sensilla. This anomaly could affect normal movement, hunting and mating (due to lack of the ability to find a mating partner) and probably affected fitness of this individual. We have two hypotheses on possible reasons for the anomaly:

(a) This anomaly could be result of genetic changes, possibly mutation(s) in structural genes, or in Hox genes of the Ultrabithorax (Ubx) and Abdominal-A (Abd-A) groups, known as UbdA. UbdA is expressed solely in the opisthosoma, with an anterior border of expression primarily in the third opisthosomal (O3) segment (Popadić & Nagy, 2001). In addition to UbdA, in scorpions expression of other groups of Hox genes is known: Abd-B, Cscu-abd-A-1 and Antp, with detected expression of Cscu-Antp, Cscu-Ubx, Cscu-Abd-A and Cscu-Abd-B



Figure 4: Left, normally developed pectinal comb. Scale bar 1 mm.

Pectinal Tooth Number	Number of Peg Sensilla	Number of Pectinal Setae
1	59	16
2	96	4
3	32	1
4	14	1
5	13	3
6	26	3
7	37	5

Table 1: Number of peg sensilla and pectinal setae in the malformed pecten. **N.B.** Results are within error ± 5 peg sensilla.

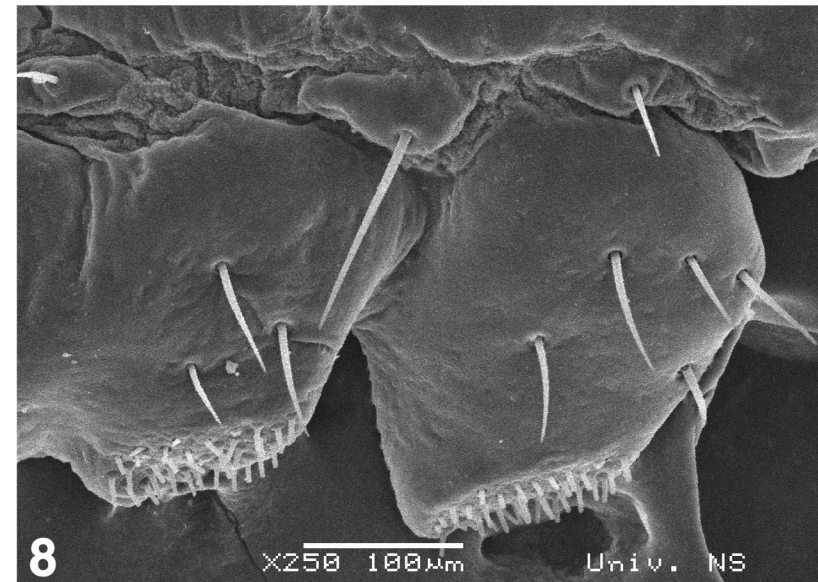
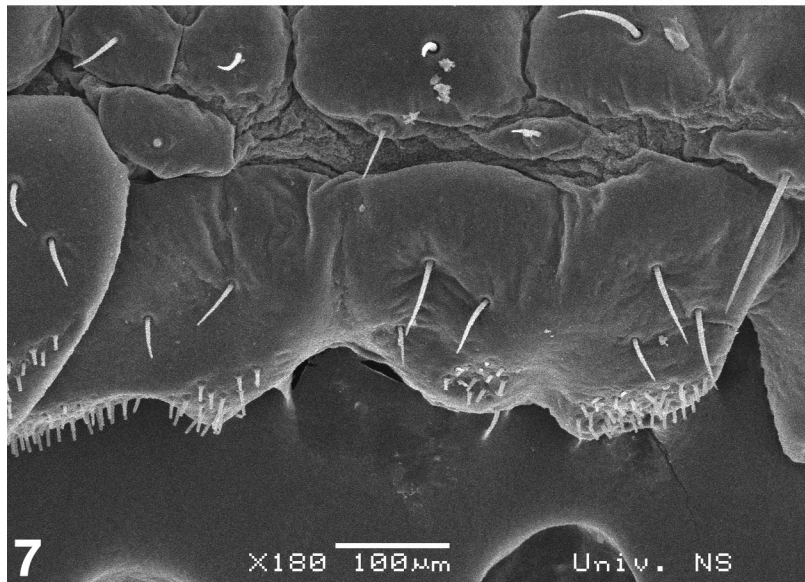
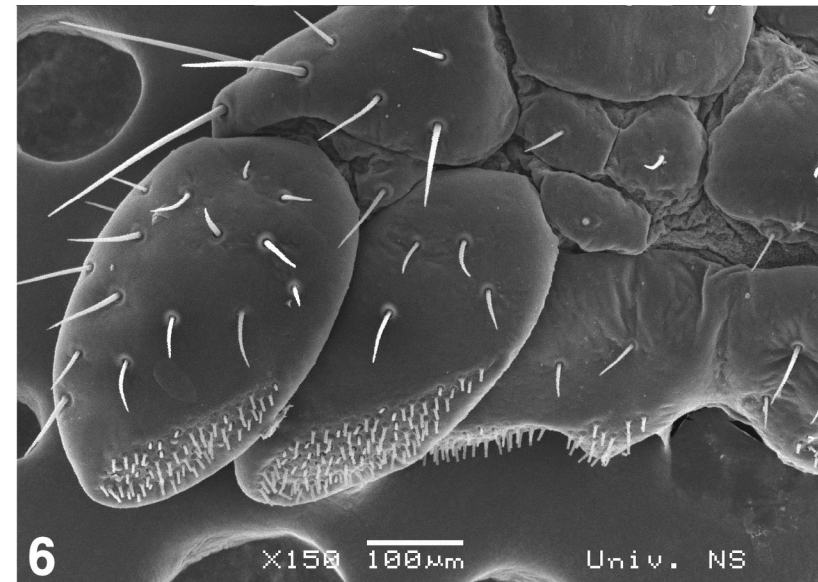
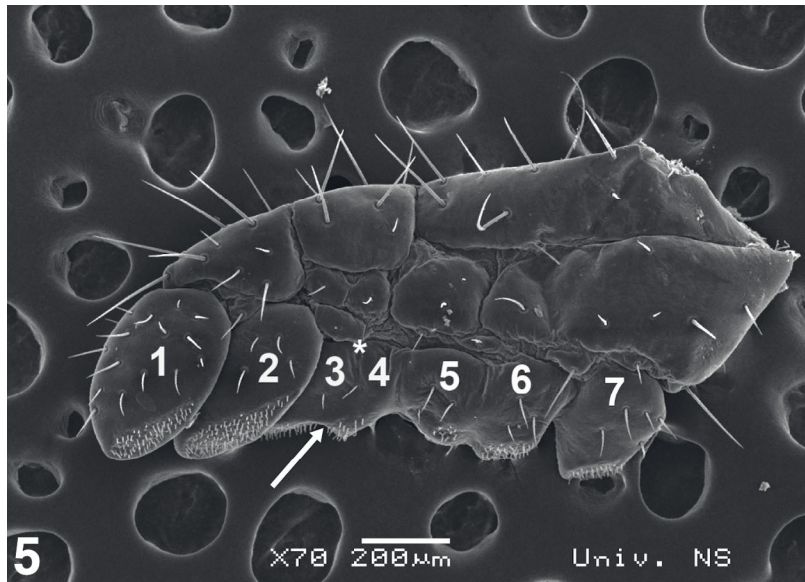
Cscu-Abd-B paralogues (Sharma et al., 2014). We think that there is possibility of genetic change in one or more of these groups of Hox genes.

(b) This anomaly could be result of environmental factors, e.g. injury at the early stages of ontogenesis or during molting (according to our observations, insuffi-

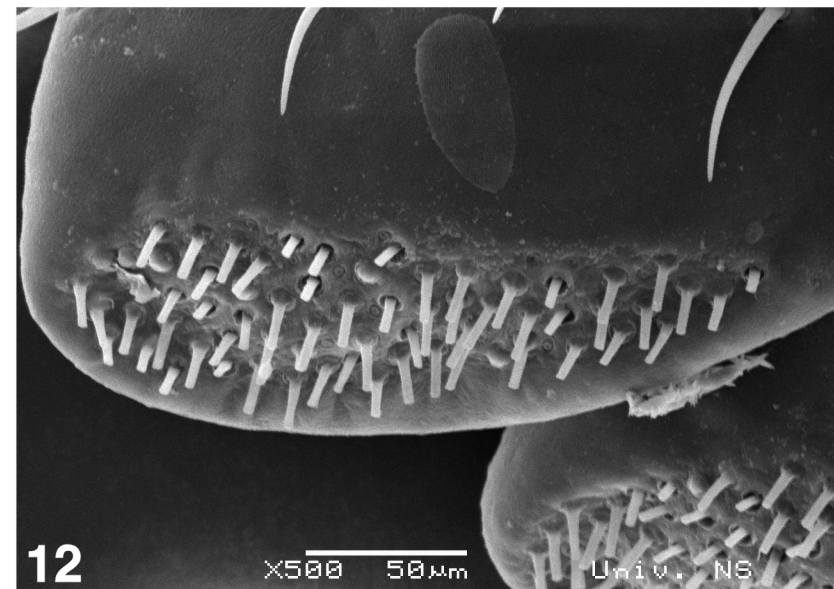
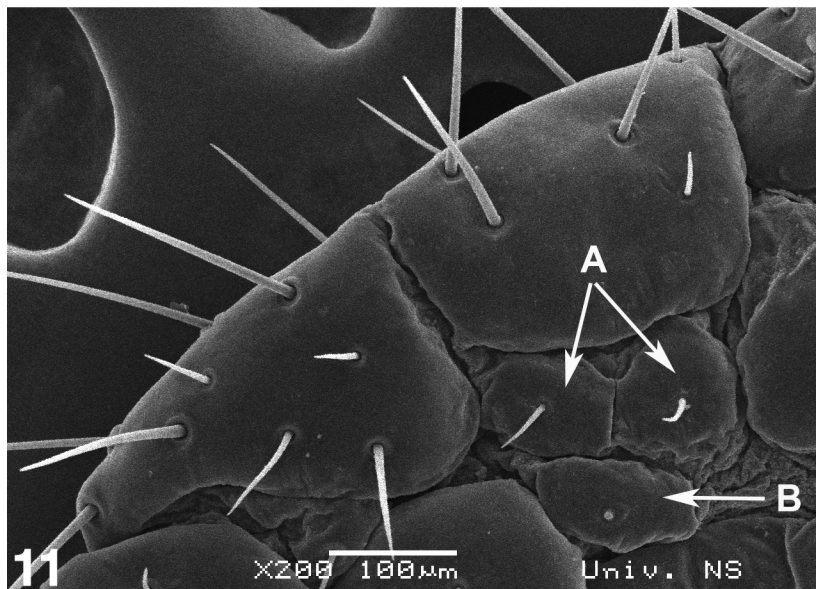
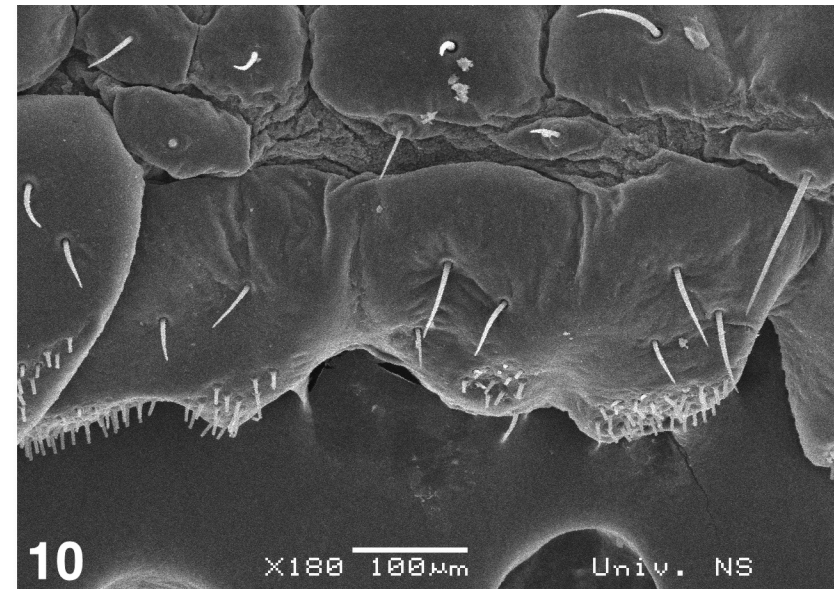
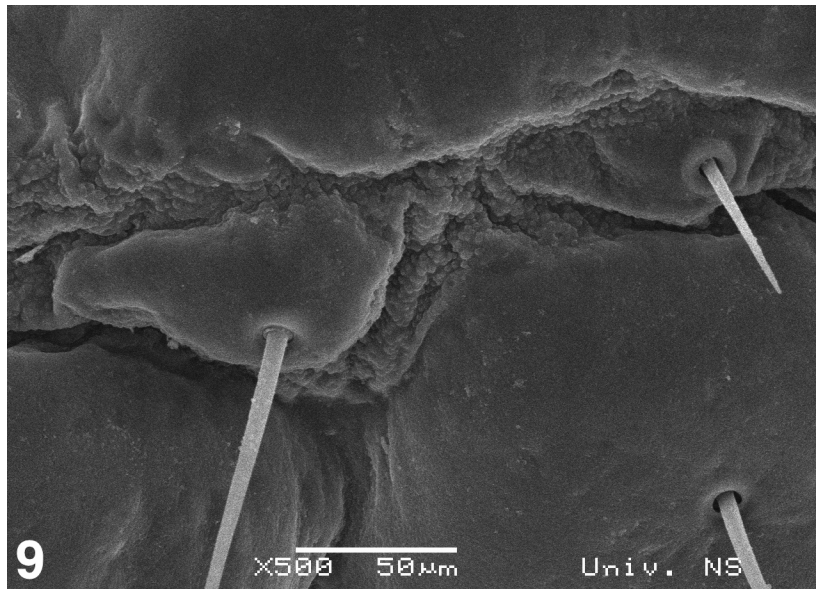
Pectinal Tooth No.	Percentage (%)
1	61.45
2	100.00
3	33.33
4	14.58
5	13.54
6	27.08
7	38.54

Table 2: Presence of peg sensilla on pectinal teeth (%). **N.B.** Second pectinal tooth with 96 peg sensilla considered as 100%.

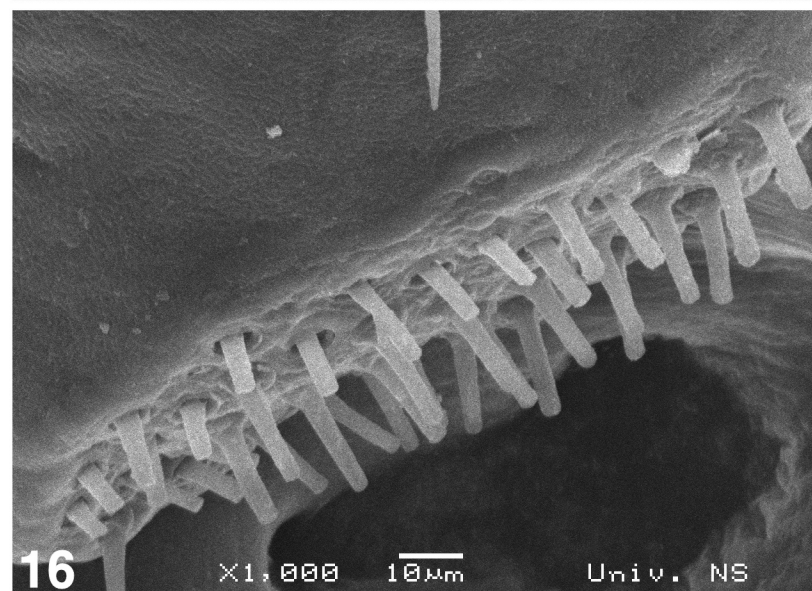
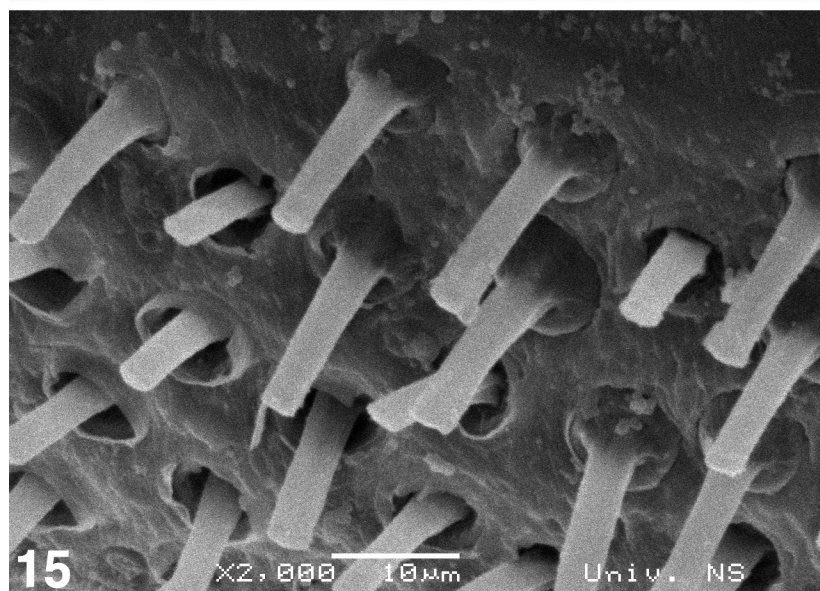
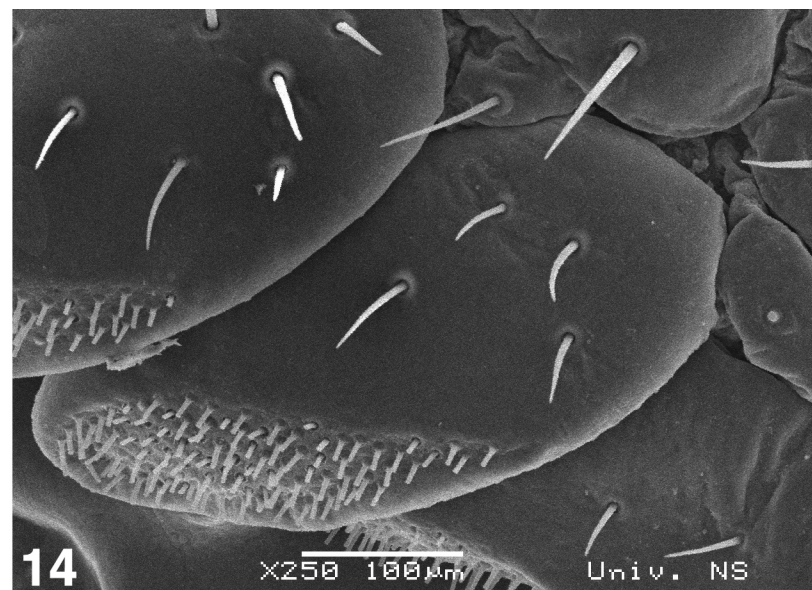
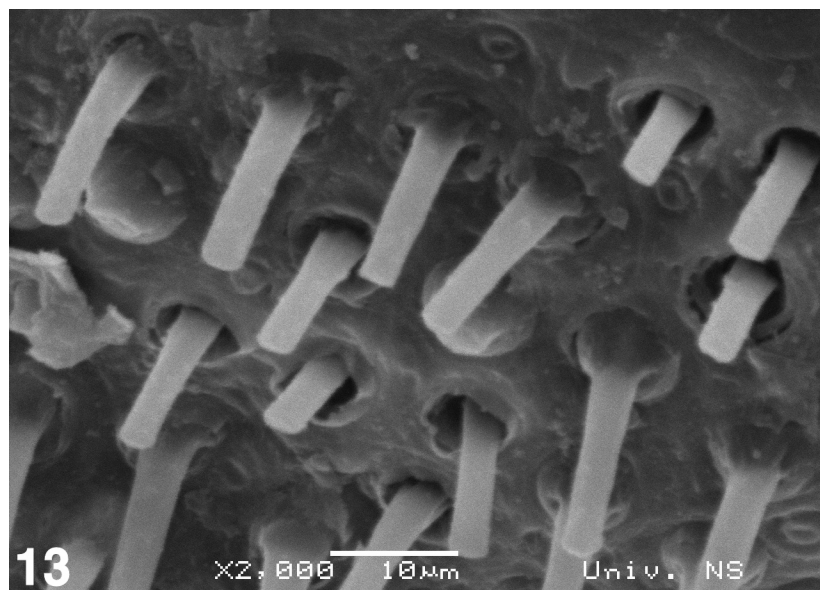
icient amount of moisture affects normal molting) or an injury due to attack of a parasite or a predator; or exposure to mutagenic/toxic substances. Other possibilities are fungal or viral infections (Gioele Tropea, pers. comm.) at the early stages of ontogenesis.



Figures 5–8: 5. Scanning electron microscopy (SEM) micrograph of the abnormal right pectine. Groove between pectinal teeth 3 and 4 indicates that two pectinal teeth had grown together in full length. In basal part groove is more pronounced (aster), then in apical part where only notch is visible between two sensorial areas (arrow). Holes in background are formed by the adhesive on the stub. 6. First and second pectinal teeth. 7. Third, fourth, fifth, and sixth pectinal teeth with malformed sensorial areas. 8. Sixth and seventh pectinal teeth.



Figures 9–12: **9.** Fulcrum between sixth and seventh pectinal teeth, normally developed. **10.** Absence of a fulcrum between fourth and fifth pectinal teeth. **11.** Distal apical segment of the pectine. **(A)** Third and fourth middle lamellae, significantly modified; **(B)** Fulcrum between second and third pectinal teeth fused in a pentagonal structure. **12.** First pectinal tooth with sensorial area.



Figures 13–16: 13. Sensorial area of the first pectinal tooth. 14. Second pectinal tooth with sensorial area. 15. Sensorial area of the second pectinal tooth. 16. Malformed sensorial area of the sixth pectinal tooth. (A hole in the background is formed by the adhesive on the stub).

Dedication

We dedicate this, our first article, to our families and to our professors Ivo Karaman and Mladen Horvatović.

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We wish to thank Mr. Miloš T. Bokorov, the Operating Manager of the University Center for Electron Microscopy, at Department of Biology and Ecology, for his help in making SEM micrographs. We thank our professors, Dr. Snežana Radenković and Dr. Jasmina Ludoški, for their help during the writing of this article; professor Dr. László Barsi for his kind help with preparation of photographs and support; Dr. Victor Fet for his help on biology of scorpions, for providing literature and his help during the writing of this article; and Gioele Tropea for his comments. Especially we wish to thank Dr. Roger D. Farley for his kind help during the writing of this article and long discussions on evolution and development of pectines. We also thank two anonymous reviewers for their valuable comments.

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