Acta Zoologica Academiae Scientiarum Hungaricae 51 (4), pp. 279–285, 2005

# ACHAETA ANTEFOLLICULATA SP. N., A NEW ENCHYTRAEID SPECIES (OLIGOCHAETA, ENCHYTRAEIDAE) FROM THE ROCK GRASSLAND OF THE SAS-HEGY IN HUNGARY

#### K. DÓZSA-FARKAS & G. BOROS

Department of Systematic Zoology and Ecology of the Eötvös Loránd University Pázmány Péter sétány 1/C, H-1117 Budapest, Hungary, E-mail: dfk01@cerberus.elte.hu

The description of *Achaeta antefolliculata* sp. n., a new species found in Sas-hegy, a small, protected dolomite hill in Budapest, during the investigation of the enchytraeid fauna, is given. This new species of small size differs from all other *Achaeta* species the presence of five pairs of epidermal follicles (glandular organs), slightly protruding into the body cavity at the anterior part of the body between the head and segment VI.

Key words: Oligochaeta, Enchytraeidae, Achaeta, new species, dolomite rendzina, Hungary

## INTRODUCTION

The botanical investigation of Sas-hegy in Budapest started already at the beginning of the 19th century. Zoological studies in the area mainly focussed on arthropods and vertebrates. A general overview of previous research results regarding both plants and animals was presented by LOKSA (1977). Sas-hegy was designated as a protected area in 1958, today it is a part of the Danube-Ipoly National Park. We started to study the enchytraeid fauna, which had not been investigated before, in 2003. Research on its seasonal dynamics still continues in 2005. Though the number of species turned out to be low, besides three common species, a previously unknown *Achaeta* species was also found, which is described in this article.

#### MATERIAL AND METHODS

Sas-hegy is a typical, small dolomite hill with narrow ridges and steep slopes in the 12th district of the Hungarian capital, Budapest, surrounded by the city. Its size is approximately 30 hectares with a maximal height of 254 m above sea level. Due to its shallow rendzina soil (pH = 7.3) with high heat absorption capacity there is only a low amount of water available for plants in the hill. Rare sub-Mediterranean and continental plant species exist on the southern slopes such as *Seseli leucospermum*, *Draba lasiocarpa* and *Fumana procumbens* in the open, and *Carex humilis, Chrysopogon gryllus* and *Pusilla grandis* in the closed rock grassland developed in less steep areas. *Ephedra distachia* reaches the northern limit of its distribution at this site, while on the northern slopes an ice age relict, *Sesleria sadleriana*, can be found. From among reptiles, *Coluber caspius* and *Ablepharus*  *kitaibelii* are especially valuable. The invertebrate fauna of Sas-hegy is also very diverse. Its spider fauna, which has been the most intensively studied invertebrate group, consists of nearly 170 species (BALOGH 1935, BLEICHER *et al.* 1999, SAMU & SZINETÁR 2000). Rare Lepidoptera, Coleoptera and Hymenoptera species were also found there. At the foot of the hill as well as at higher altitude loess patches increase habitat diversity. The original vegetation cover of the area was changed by the introduction of syringa (*Syringa vulgaris*) at the end of the 19th century, which gradually spread into new areas later, and the introduction of Austrian pine (*Pinus nigra*).

Samples were collected from five biotopes:

1. Closed rock grassland (Festucetum pallenti–Brometum pannonici)

2. Open rock grassland (Seseli leucospermi–Festucetum pallenti)

3. Scrubbery

4. Moderately disturbed Festuco pallenti-Brometum pannonici

5. Secondary loess grassland

Both quantitative and qualitative soil samples were collected. Animals were extracted from the soil with the O'Connor's wet funnel method (O'CONNOR 1962).

As preservation only caused minor changes in body characteristics, worms were investigated and measured both alive and preserved. The animals were anaesthetized in 30% ethanol, then preserved in 70% ethanol with a percentage of formaldehyde in the solution (1 drop of 4% formaldehyde in 1 ml 70% ethanol). Most preserved specimens were stained with a mixture of borax-carmine and bromphenolblue and mounted in euparal. Important structures were drawn and photographed by a Zeiss Axioskop 2 microscope, using DIC (differential interference contrast) illumination and an Olympus Colour View digital camera with DP-Soft software and motion picture sequences were also taken with a Sony CCC-IRIS. DXC 107 p colour video camera attached to the microscope.

The type material is deposited in the corresponding author's (DózSA-FARKAS, K.) collection at Department of Systematic Zoology and Ecology, Eötvös Loránd University, Budapest. The material was collected by G. Boros.

### RESULTS

The following species were found in the investigated area: *Fridericia maculatiformis* DÓZSA-FARKAS, 1972, *F. tubulosa* DÓZSA-FARKAS, 1972, *Buchholzia* sp. juvenile, *Enchytraeus buchholzii* complex and the new species, *Acheta antefolliculata* sp. n. With the exception of loess soil, the new species was found at all sampling sites. It was most abundant at site 2, in the open rock grassland (*Seseli leucospermi–Festucetum pallenti*).

#### Achaeta antefolliculata sp. n.

(Figs 1–13)

Holotype: A 4 (1939) stained whole mount.

Type locality: Hungary, Sas-hegy, open rock grassland (biotope 2), 09.01.2005.

 $Paratypes: P. 79.1 \ (1941) - P.79.2 \ (1941) \ seven \ specimens, \ whole \ mounted \ on \ two \ slides, \\ biotope \ 2, \ 23.04.2004, \ P.79.3 \ (1942) - P.79.13 \ (1952) \ fourteen \ specimens, \ whole \ mounted \ on \ 11 \ respectively.$ 

slides, biotope 2, 09.01.2005, P.79.14 (1953) two specimens, whole mounted on one slide, biotope 2, 28.10.2004, P.79.15- P.79.20 56 adult + 5 juvenile specimens in 70% ethanol: P.79.15 (1954) 35 specimens, biotopes 2 and 3, 23.04.2004, P. 79.16 (1955) one specimen, biotope 1, 23.04.2004, P.79.17 (1956) two specimens, biotope 4, 28.04.2004, P.79.18 (1957) six specimens, biotope 2, 28.04.2004, P.79.19 (1958) seven adult and 5 juvenile specimens, biotope 3, 28.04.2004, P.79.20 (1959) five specimens, biotope 3, 09.01.2005.

Type locality of paratypes : Hungary, Sas-hegy, open rock grassland (biotope 2), closed rock grassland (biotope 1), scrubbery (biotope 3) and moderately disturbed *Festuco pallenti-Brometum pannonici* (biotope 4).

Etymology: Named for the epidermal follicles, which are present only in the anterior segments from the head to segment VI.

Description - Very small species, 2.4-2.8 (3.5) mm long with a diameter of 0.14-0.19 mm at VIII, 0.16–0.22 mm at XII, 20–21 segments, whitish colour. Holotype (fix): 2.7 mm long, diameter 0.19 mm at VIII and 0.22 at clitellum, segments: 20. Bottle-shaped cells (setal follicles) absent, but on the dorso-lateral surface of the segment I and segments III, IV, V and VI there are one pair of oval epidermal follicles, which are fixed in the body wall (slightly protruding into the body cavity and unlike in many Achaeta species possessing bottle-shaped cells, they do not bend when the animal is moving) (Figs 1f, 7, 8). The length : width ratio of follicles is about 22–30 : 12–30 µm. The epidermal follicles stain well in borax-carmine and contain cells inside. In some of the follicles this mass of cells is concentrated in the inner part of the follicle, facing the body cavity. The organ as a whole, seems to be a gland, however, an orifice has not been observed either in live, or in stained specimens. To reveal the structure and possible function of the organ, histological sectioning is indispensable. One pair of lens-shaped, hyaline epithelial cells are present dorsally, in the same transversal section where the epidermal follicles are positioned but the lens-shaped cells are not always conspicuous. Clitellum extends over XII–1/2 XIII, it is covered by 20 regular, transverse rows of gland cells. The hyaline cells are arranged in four more or less regular longitudinal fields dorso-laterally, usually with longitudinally oriented irregular rows of small granular cells in between (Fig. 13). Clitellum is broken in a thin line dorsally between the hyaline fields. Ventro-laterally only granulated gland cells are present in rows (Fig. 11). Gland cells are absent between the two penial bulbs. The hyaline glands field is not always conspicuous because the hyaline cells are probably only enlargened and look characteristic during the reproducting stage (Fig. 13). Often, even with egg-bearing animals, granulated cells dominate. The brain is about twice as long as wide (length  $87-95 \ \mu m$ ), its posterior end is slightly concave (Figs 1b & 8b). Head pore small, nearly on tip of 0. Dorsolateral oesophageal appendages in V poorly developed (Fig. 1a). First and second pairs of pharyngeal glands (septal glands) broadly united dorsally, the third pair has a narrow connection, all with ventral lobes (Figs 1, 9pg, 7). One pair of secondary pharyngeal glands in V (Figs 1 & 9spg). The oesophagus joins the intestine with a sharp bend in VII (Fig. 10e). Chloragogen cells start from V, a dense layer from VII onwards containing brown-yellow globules; the length of cells is 20-25 µm (Fig. 1c). The septa of IV/V and V/VI are thickened. The dorsal vessel originates in VII with pulsating expansion in VII and VI (Fig. 1dv). Blood is colourless. Coelomocytes 30-50 µm (viv) and 18-30 µm (fix) long, oval with sharp ends and hairs on the tips, they have a hyaline border and irregular inner structures (Fig. 4). Nephridia of the usual type of the genus (Figs 1n & 6) two pairs in front of the clitellum at VI/VII, VII/VIII. Postclitellar, mostly 3-4 pairs in the last segments. Seminal vesicle absent. Sperm funnel  $\times 1.5-2$  as long as wide (about  $43-55 \mu m \log (viv)$ ) and about 1/4-1/3 as long as body diameter, collar distinct, narrower than the funnel body (Figs 5 & 12). The sperm duct is 5  $\mu$ m, thick and short, about ×4–5 as long as the length of the funnel. Spermatozoa ca.  $40-45 \,\mu m$  long and heads ca.  $15 \,\mu m$  (viv). The small



**Figs 1–6.** Achaeta antefolliculata sp. n.: 1 = anterior segments (0–VIII) dorsal view (a = oesophageal appendages, b = brain, c = chloragogen cells, dv = dorsal vessel, f = epidermal follicles, n = nephridia, oe = oesophagus, pg = primary pharyngeal glands, ph = pharynx, s = spermatheca, spg = secondary pharyngeal gland ), 2 = brain, 3 = spermatheca, 4 = coelomocytes (oblique and upper view), 5 = sperm funnel, 6 = nephridium



**Figs 7–9.** Achaeta antefolliculata sp. n. (photographs of stained whole mounts): 7 = segments 0–VII of the holotype epidermal follicles marked with arrows, 8 = anterior segments, dorsal view, follicles marked with arrow, b = brain, 9 = segments IV–VI, dorso-lateral view; arrow: spermatheca, pg = primary pharyngeal glands, spg = secondary pharyngeal glands



**Figs 10–13.** *Achaeta antefolliculata* sp. n. (photographs of stained whole mounts): 10 = male copulatory organ (penial bulb and glands), lateral view (marked with arrow), 11 = male copulatory organ, ventro-lateral view (marked with arrow), and the granular gland cells of clitellum in rows, 12 = sperm funnel (marked with arrow), 13 = hyaline gland cells with some granular cells in dorsal view

male copulatory organ (16–23  $\mu$ m diameter, fix) in XII surrounded by some indistinct glands (Figs 10 & 11). Distance between male pores: 55–60  $\mu$ m (fix). Spermathecae free, restricted to V. No glands at the spermathecal pores. Ampullae spherical with some sperm in it. Spermathecal ducts about twice as long as the ampullae (Figs 1s, 3 & 9).

Remarks. Achaeta antefolliculata is similar to the Achaeta group devoid of bottle-shaped cells (setal follicles) and with the spermathecae restricted to V, but it is clearly distinguished from the other members of the group by the 5 pairs of dorsolateral epidermal follicles (with a different shape from the usual "setal follicles") present in the first six segments only and differs from them by the presence of one pair of secondary pharyngeal glands in V.

\*

*Acknowledgements* – This research was financed partly by the Hungarian Scientific Research Fund (OTKA 034864). Some research equipments (microscope and digital camera) were also provided by the OTKA (M27225 and M045482).

#### REFERENCES

- BALOGH, J.(1935) A Sas-hegy pókfaunája. Faunisztikai, rendszertani és környezettani tanulmány. (The spider fauna of the Sas-hegy. A faunistical, taxonomical and ecological study.) Sárkány Nyomda Rt. Budapest, 59 pp.
- BLEICHER, K., SAMU, F, SZINETÁR, CS. & RÉDEI, T. (1999) A budai Sas-hegy Természetvédelmi Terület farkaspókjainak (Araneae, Lycosidae) vizsgálata hatvan évvel ezelött és napjainkban. *Természetvédelmi Közlemények* 8: 11–119.
- LOKSA, I. (1977) A Sas-hegy növény- és állatvilágának jellemzése. In: PAPP, J. (ed.): A budai Sas-hegy élővilága. Akadémiai Kiadó, Budapest, 99 pp.
- O'CONNOR, F. B. (1962) The extraction of Enchytraeidae from soil. Pp. 279–285. In: MURPHY, P. W. (ed.): *Progress in Soil Zoology*. Butterworth, London.
- SAMU, F. & SZINETÁR, CS. (2000) Rare species indicate ecological integrity: an example of an urban nature reserve island. Pp. 177–184. In: CRABBÉ, P. (ed.): *Implementing Ecological Integrity*. Kluwer Academic Publishers.
- Revised version received November 8, 2005, accepted November 30, 2005, published December 29, 2005