Glyptotendipes ospeli, a new species from The Netherlands (Diptera, Nematocera: Chironomidae)

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CONTRERAS-LICHTENBERG, R. & I. I. KIKNADZE, 2000. *GLYPTOTENDIPES OSPELI*, A NEW SPECIES FROM THE NETHERLANDS (DIPTERA, NEMATOCERA: CHIRONOMIDAE). – *ENT. BER., AMST.* 60 (2): 21-30.

Abstract: The karyotype, larva, pupa and male and female imago of *Glyptotendipes ospeli* spec. nov. are described from The Netherlands.

Keywords: *Glyptotendipes*, systematics, taxonomy, karyology.

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Introduction

In 1989 Henk Moller Pillot collected larvae of *Glyptotendipes* in Ospel, The Netherlands, characterised by a v-shaped mark on the frontal apotome on the head capsule that differs from all known species of *Glyptotendipes*. The species is considered by Vallenduuk (1999) as *Glyptotendipes* sp. Ospel. Henk Vallenduuk (Schijndel, The Netherlands) forwarded larvae for the karyological description to Iya Kiknadze (Novosibirsk) and sent larvae and reared specimens to R. Contreras-Lichtenberg (Vienna) for the morphological description.

Material and methods

The terminology and abbreviations for the morphological description follows Sæther (1977, 1980) and for the larvae also Epler (1987). Additionally an epaulette-ratio is used in the description of the pupa: Epaulette-Ratio: (ER_1: length of epaulette 3 divided by length of epaulette 2; ER_2: length of epaulette 4 divided by length of epaulette 3; ER_3: length of epaulette 5 divided by length of epaulette 4; ER_4: length of epaulette 6 divided by length of epaulette 5.

For the karyosystematic studies eleven fourth-instar larvae of *G. ospeli* were used for analysis. Larvae were fixed in 3:1 mixture of 100% ethanol and glacial acetic acid. Squashes of salivary gland polytene chromosomes were prepared by the acetic-orcein method (Keyl & Keyl, 1959; Kiknadze et al., 1991). To map *G. ospeli* polytene chromosomes we have tried to use known *Glyptotendi-pes* species maps (Martin & Porter, 1973; Belyanina & Durnova, 1998) as standard. However, we have not found great homology between *Glyptotendipes ospeli* and the other *Glyptotendipes* karyotypes, therefore the mapping of the polytene chromosomes of *G. ospeli* was done independently. The identification of chromosome arms is according to Michailova (1989).

Glyptotendipes ospeli spec. nov.

(fig. 1-22)

Type material

Holotype: ♂, The Netherlands, Amsterdam, Oosterpark, 9.iv.1998, reared from larva hatched 30. iv. 1998, mounted together with the exuviae of the 4th instar larva and the pupa in Euparal, leg. H. Vallenduuk, 05325-19. Paratypes: 1 ♂, The Netherlands, Amsterdam, Oosterpark 9.iv.1998, reared from larva hatched 15.v.1998, leg. H. Vallenduuk, 05325-10. 1 ♀, The Netherlands; Amsterdam, Oosterpark, 9.iv.1998, reared from larva hatched 30.iv.1998, mounted together with the exuviae of the 4th instar larva and the pupa in Euparal, leg. H. Vallenduuk, 05325-11. 1 ♀, The Netherlands, Amsterdam, Oosterpark, 9.iv.1998, reared from larva hatched 8.v.1998, mounted together with the exuviae of the 4th instar larva and the pupa in Euparal, leg. H. Vallenduuk, 05325-17. 1

♀, The Netherlands, Amsterdam, Oosterpark, 9.iv.1998, reared from larva hatched 30.iv.1998, mounted together with the exuviae of the 4th instar larva and the pupa in Euparal, leg. H. Vallenduuk, 05325-13. 1 ♀, The Netherlands, Amsterdam, Oosterpark, 15.iv.1998, reared from larva hatched 23.v.1998, mounted together with the exuviae of the 4th instar larva and the pupa in Euparal, leg. H. Vallenduuk, 05350-1. 1 ♀, The Netherlands, Amsterdam, Oosterpark, 15.iv.1998, reared from larva hatched 23.v.1998, mounted together with the exuviae of the 4th instar larva and the pupa in Euparal, leg. H. Vallenduuk, 05350-3. The holotype and one ♀ paratype (05325-11) are deposited in the collection of the Naturhistorisches Museum Wien (NMW), the male paratype and the other female paratypes in the collection of the Zoological Museum Amsterdam (ZMAN). 1 larva, The Netherlands, Ospel, 2.ix.1989, leg. H. Moller Pillot, is deposited in NMW.

Description

Karyotype structure of G. ospeli

Glyptotendipes ospeli karyotype has 2n=8. Correspondingly 4 giant polytene chromosomes can be seen in the salivary gland cells as a result of tight somatic pairing of homologous chromosomes (fig. 1). Chromosomes I and II are the longest, chromosome III is shorter and chromosome IV is the shortest in the karyotype. Centromeric bands are not heterochromatinized in *G. ospeli*, so we have preliminary identified it as the most dense and intensely stained band in each of chromosomes (fig. 1, arrows).

Chromosome I (arms A and B) has several good markers: puffed fun-like telomeric end of the arm A (region 1), clear constriction in the central part of chromosome, large nucleolus (N) on region 18-19, puff (P) on region 23 and series of very slim bands at the telomeric end (region 30) of arm B. Chromosome I is divided into 30 regions on the cytomap (fig. 1).

Chromosome II (arms C and D) can be easily identified by intensely stained groups of bands on the distal part of the arm C (fig. 1, region 6, arrowhead). A puff (or fluctuated nucleolus) in region 11 is also characteristic for

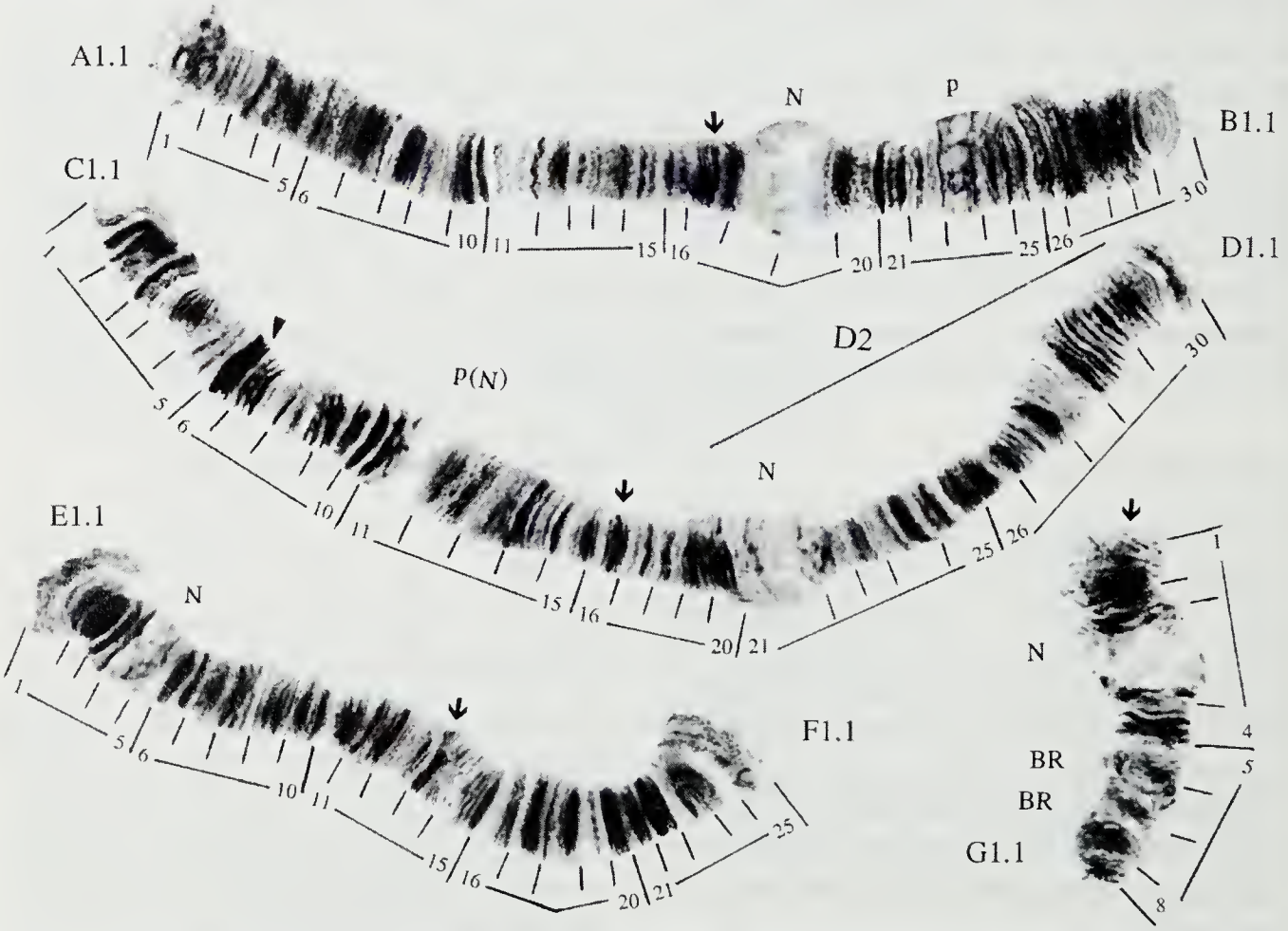

Fig. 1. Karyotype of *Glyptotendipes ospeli* spec. nov., A1.1, B1.1, C1.1, D1.1, E1.1, F1.1 and G1.1-genotypic combinations of banding sequences of corresponding chromosomal arms, N-nucleolus, BR-Balbiani rings, arrows show centromeric bands, arrowhead shows marker band group on arm C. Region covered by inversion on arm D is noted by line above the arm.

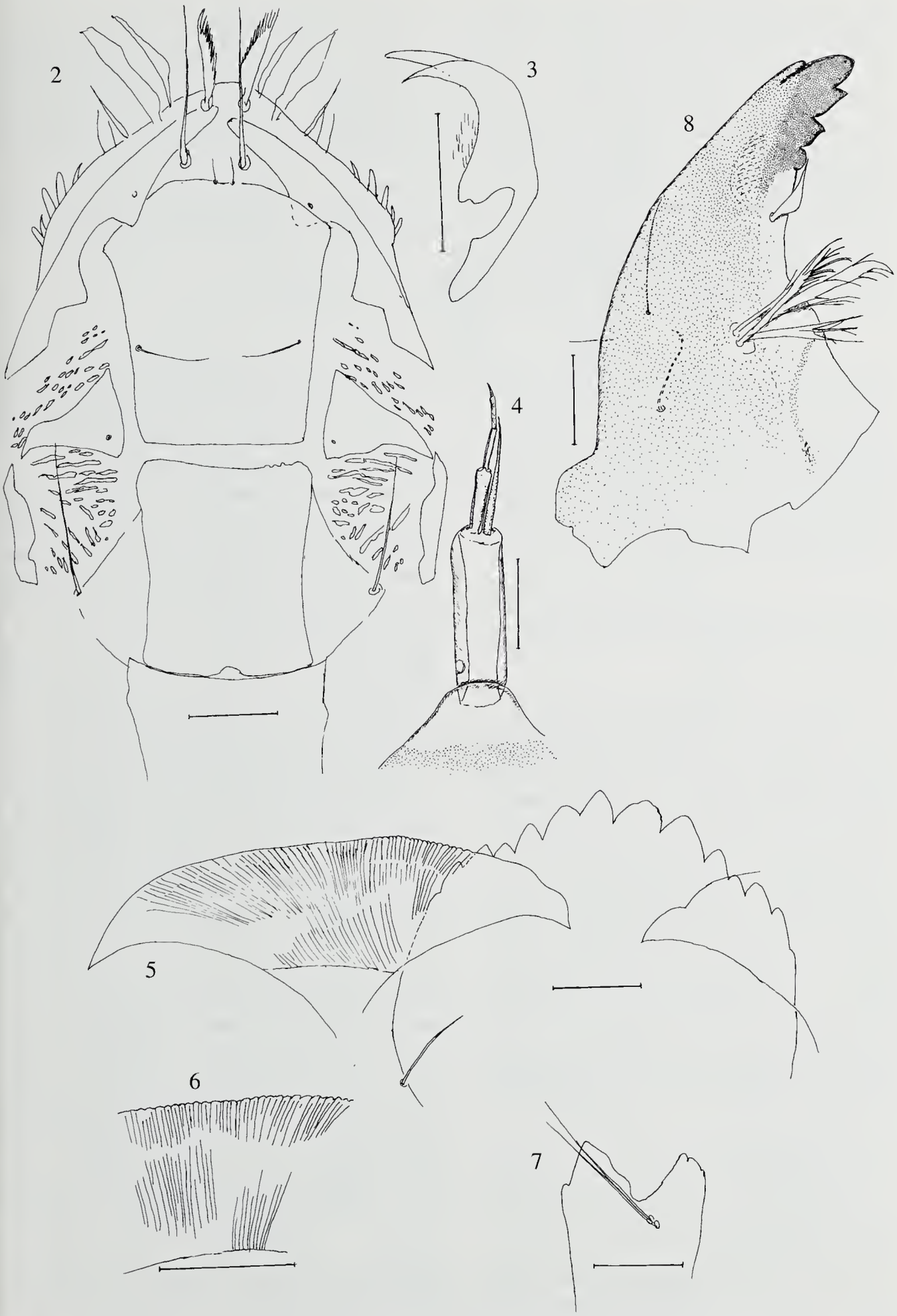

Fig. 2-8. *Glyptotendipes ospeli* spec. nov., larva. 2, dorsal view of the head-capsule; 3, premandible; 4, antenna; 5, mentum; 6, ventromental plate-detail; 7, maxillar region; 8, mandible (scale 50 µm).

this arm. A large nucleolus is situated on region 21 of arm D. Chromosome II is divided into 30 regions on the cytomap (fig. 1).

Chromosome III (arms E and F) is shorter than chromosome I and II. It bears the nucleolus on arm E (region 5). Chromosome III is divided into 25 regions on the cytomap (fig. 1).

Chromosome IV (arm G), the shortest of karyotype, has several functional active regions: the nucleolus and two Balbiani rings. It is divided into 8 regions on the cytomap (fig. 1).

Chromosomal polymorphism

Glyptotendipes ospeli has a low level of chromosomal polymorphism. Only one of 11 larvae studied had a heterozygous inversion on arm D. The region of arm D covered by this inversion is shown in figure 1.

Comparison of G. ospeli karyotype with known species karyotype in Glyptotendipes-species

Cytogenetical analysis has shown that the *G. ospeli* karyotype is similar with other known *Glyptotendipes* karyotypes on the number and general morphology of the chromosomes. Besides, *G. ospeli* karyotype is polynuclear as the karyotypes of many *Glyptotendipes*-species. At least 4 nucleoli were found in *G. ospeli*, one nucleolus on each of the four polytene chromosomes. As known, polynucleolar karyotypes are typical also for *G. barbipes* (Staeger) (3 nucleoli), *G. salinus* Michailova (3 nucleoli), *G. glaucus* (Meigen) and *G. pallens* (Meigen) (near 5 nucleoli), *G. paripes* Edwards (2 nucleoli plus several fluctuated nucleoli) (Kiknadze & al., 1991; Andreeva & al., 1998).

However, the karyotype of *G. ospeli* differs clearly from other *Glyptotendipes* karyotypes by its banding pattern. This banding pattern is strongly species-specific. Several regions of homology were found only between *G. ospeli*, *G. glaucus* and *G. pallens* karyotypes. Thus, a good homology in banding patterns was observed in region 10-16 of *G. ospeli* chromosome I and region 18-23 of *G. glaucus* chromosome I, as well as in region 1-7 of *G. ospeli* chromosome II and the corresponding regions of *G. glaucus* and *G. pallens* chromosome II according to the map of Belyanina & Durnova (1998). Chromosome IV of *G. ospeli* is similar in its general morphology with *G. glaucus* and *G. pallens*, but it differs by the distal position of the Balbiani rings as a result of inversion.

Larva, 4th instar (fig. 2-8)

Length ca 11 mm. Anterior parapods with simple claws, serrated on one side only. Larva without ventral tubuli on abdominal segment VIII but with short lateral tubuli between the abdominal segments VII and VIII.

Head with a characteristic v-shaped mark on the dorsal side of the head-capsule, gula darkened. Setae SI long, serrated, serration apically stronger than proximally; setae SII simple. Pecten epipharyngis with 14 (10-15) teeth. Chaetulae serrated, mentum with 6 lateral teeth, middle tooth 1.4 times broader than first lateral tooth and almost on the same level as lateral teeth. Paralabial plates with 50 (45-67) striae. Mentum width 196 (180-212) µm. Width of paralabial plate: 243 (234-260) µm; interplate distance (IPD) (shortest distance between the paralabial plates, according to Epler, 1987) = 51 (44-56) µm (n=5); plate separation ratio (PSR - average width of ventromental plates divided by IPD, according to Epler, 1987) 4.8 (4.2-5.4) (n=5). Antennae with 5 segments, length 192 µm, bristle of the antenna reaching to the end of the 3rd segment; ring organ on the first third of the basal segment. Mandible with a pale dorsal tooth and 4 darkened inner teeth; seta subdentalis leaf-shaped as in *Glyptotendipes pallens* (Michailova & Contreras-Lichtenberg, 1995). Premandibles with 2 apical teeth, the inner tooth broader than the outer tooth. Maxilla with well developed maxillary palp, apex of maxillary sclerite with 4 (4-5) denticles. Prementum rounded, with light inner teeth and numerous chaetulae.

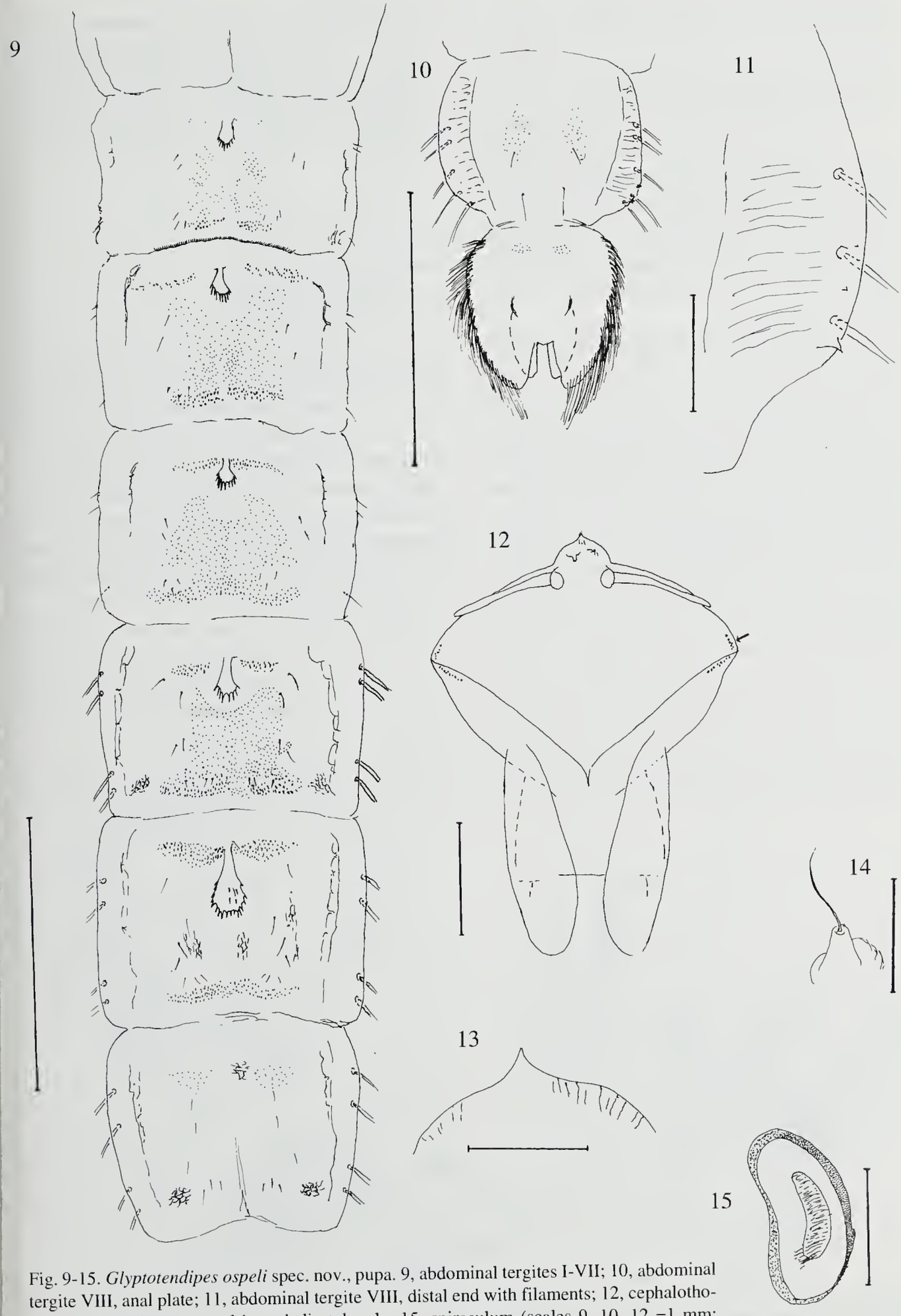

Fig. 9-15. *Glyptotendipes ospeli* spec. nov., pupa. 9, abdominal tergites I-VII; 10, abdominal tergite VIII, anal plate; 11, abdominal tergite VIII, distal end with filaments; 12, cephalothorax; 13, frontal apotome; 14, cephalic tubercle; 15, spiraculum (scales 9, 10, 12 =1 mm; 11,13, 14, 15 =0.1 mm).

Pupa (fig. 9-15; n=7, unless otherwise stated)

Length of exuviae 8.1 (7.5-8.8) mm.

Cephalothorax: Frontal apotome rounded, distally narrower than proximally. Cephalic tubercles conical, basis broadened, length of tuberculi 85 (59-99) µm (n=5), terminal seta about twice as long as the cephalic tubercle. Cephalothtorax with granulate structure, a group of distinct strong granulae in one row (fig. 12, arrow).

Abdomen: Pedes spurii B (PSB) on segments II and III. Hook-row on tergite II with 69 [57 (male holotype) -78] hooklets in one row. Epaulettes racquet-shaped, present on tergites II-VI. Length of epaulettes increasing slightly from tergite II to VI, epaulette of segment III and IV about of the same length. Length of epaulettes on tergite II: 133.5 (118.7-148.3) µm; length of epaulettes on tergite III: 176.4 (148.3-197.8) µm; length of epaulettes on tergite IV: 181.3 (158.2-197.8) µm, length of epaulettes on tergite V: 222.5 (197.8-237.4) µm; length of epaulettes on tergite VI: 275.3 (247.3-296.7) µm. ER_1: 1.3 (1.2-1.7); ER_2: 1 (1-1.1); ER_3:1.2 (1.1-1.3); ER_4: 1.2 (1.2-1.3). Shagreen on segment VI in the distal and proximal quarter of the abdominal tergite. Anal combs on segment VIII with few small and indistinct spines. Lateral filaments on abdominal segments: 4, 4, 4, 5. Anal plate proximally with very fine shagreen. Lobes of the anal plate with 165 (148-195) filaments.

Imago male (fig. 16-19; n=2)

Length (holotype): Total 6 mm. Thorax 1.9 mm. Abdomen 4.1 mm.

Head: Postoccipital region dark brown. Palps dark brown. Antennae: scapus and pedicellus dark brown; brush whitish. Frontal tuberculi present, small, length 8.9 µm, width 8.9 µm. Antennae-ratio: 3.5-4. Chaetotaxy: Temporals 17-20 on each side; Postorbitals 8-11; clypeus setae 35; cibarial setae 14-17. Maxillary palps with 5 palpomeres; length of palpomeres: Pm1: 40 µm; Pm2: 52-64 µm; Pm3: 200-204 µm; Pm4 = 164-176 µm; Pm5 = 208-220 µm.

Thorax: Dark brown, mesonotal stripes dark, shining. Chaetotaxy: (holotype): 16 acrostichals, 26 multiserial dorsocentrals on each side, 9 uniserial praealars, 30 scutellar setae.

Wings: Hyaline, brownish; venation brownish. Squama fringed. Measurements of wings:

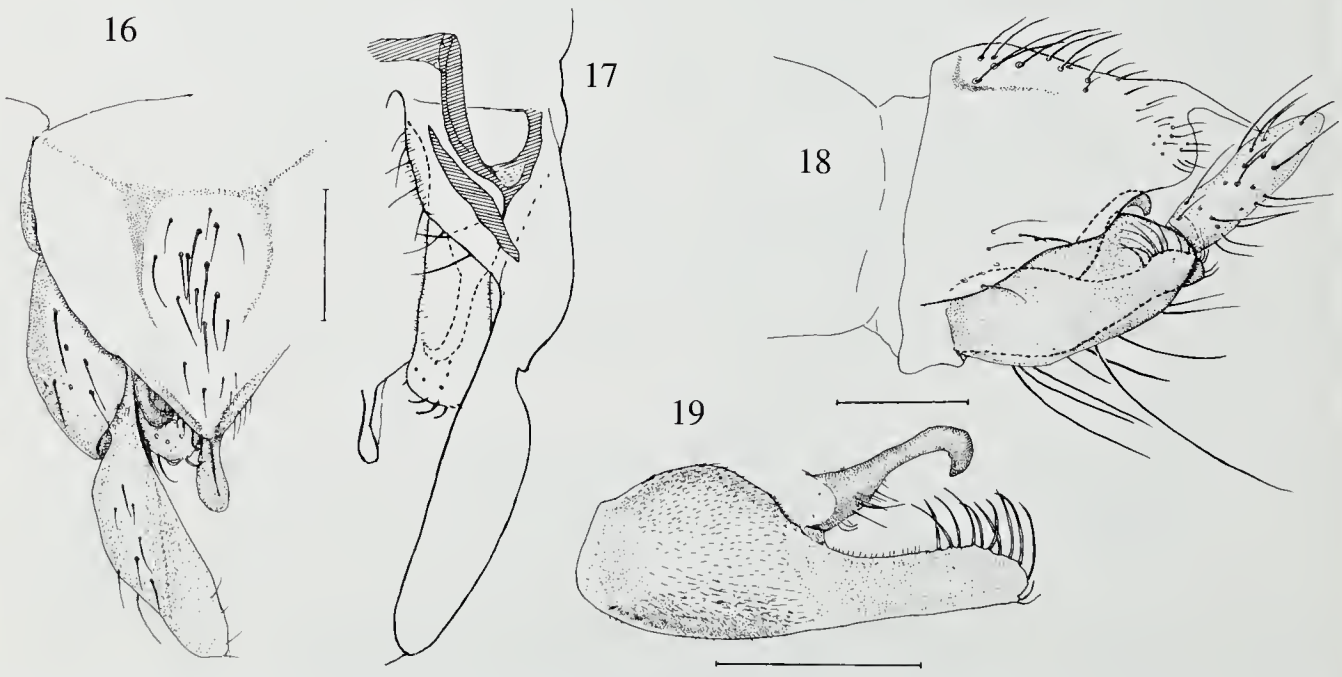

Fig. 16-19. *Glyptotendipes ospeli* spec. nov., male hypopygium. 16, dorsal view; 17, ventral view; 18, lateral view; 19, superior and inferior volsella from lateral (scale 100 µm).

Table 1: Measurements of legs of adult males of *G. ospeli* (measurements in µm, n=2)

	fore leg	mid leg	hind leg
fe	1138-1147	1019-1296	1137-1335
ti	1009-1197	1246	1256-1503
ta_1	1365-1533	564-613	811-939
ta_2	761	346-425	475-583
ta_3	653	267-326	376-475
ta_4	514	208-247	237-297
ta_5	277	158-188	168-208
LR	1.28-1.35	0.45-0.49	0.62-0.65
BV	1.59-1.78	2.66-2.89	2.42-2.55
SV	1.53-1.57	4.02-4.15	2.95-3.02

Length: 2.7-3 mm; width: 0.85 mm (holotypus). Ar-rm: 1.1-1.4 mm; Ar-FCu: 1.1-1.3 mm; VR = 0.98-0.99.

Legs: Dark brown; femora with a proximal yellow ring; articulation between Fe and Ti of all legs yellowish. Tarsi of fore legs without beard. Metatarsus of mid legs with 15 sensilla chaetica. Measurements of legs in table 1.

Abdomen: Dark brown. Tergite I: proximal margin whitish, distal part dark brown: Tergite II-IV completely dark, tergite V and tergite VI: hind margin yellowish, tergite VII: distal 1/3 yellowish, tergite VIII distal 1/3-1/4 yellowish.

Hypopygium (fig. 16-19): Anal point curved to the ventral side. Superior volsellae slender, straight, in an acute angle to the inferior volsellae. Inferior volsellae clubshaped, distal end slightly widened.

Imago female (fig. 20-22; n=5, unless otherwise stated)

Length: Total 5 (4.7-5.2) mm. Thorax 1.5 (1.4-1.6) mm. Abdomen: 3.5 (3.2-3.7) mm.

Head: Dark brown. Palps, antennae dark brown. Frontal tuberculi present, small; length 8.9 µm; width 8.9 µm. Antennae: Length of segments: Ped. 73 (68-80) µm; Am_1 100 (88-112) µm; Am_2 97 (88-104) µm; Am_3 110 (108-116) µm; Am_4 119 (116-124) µm; Am_5 131

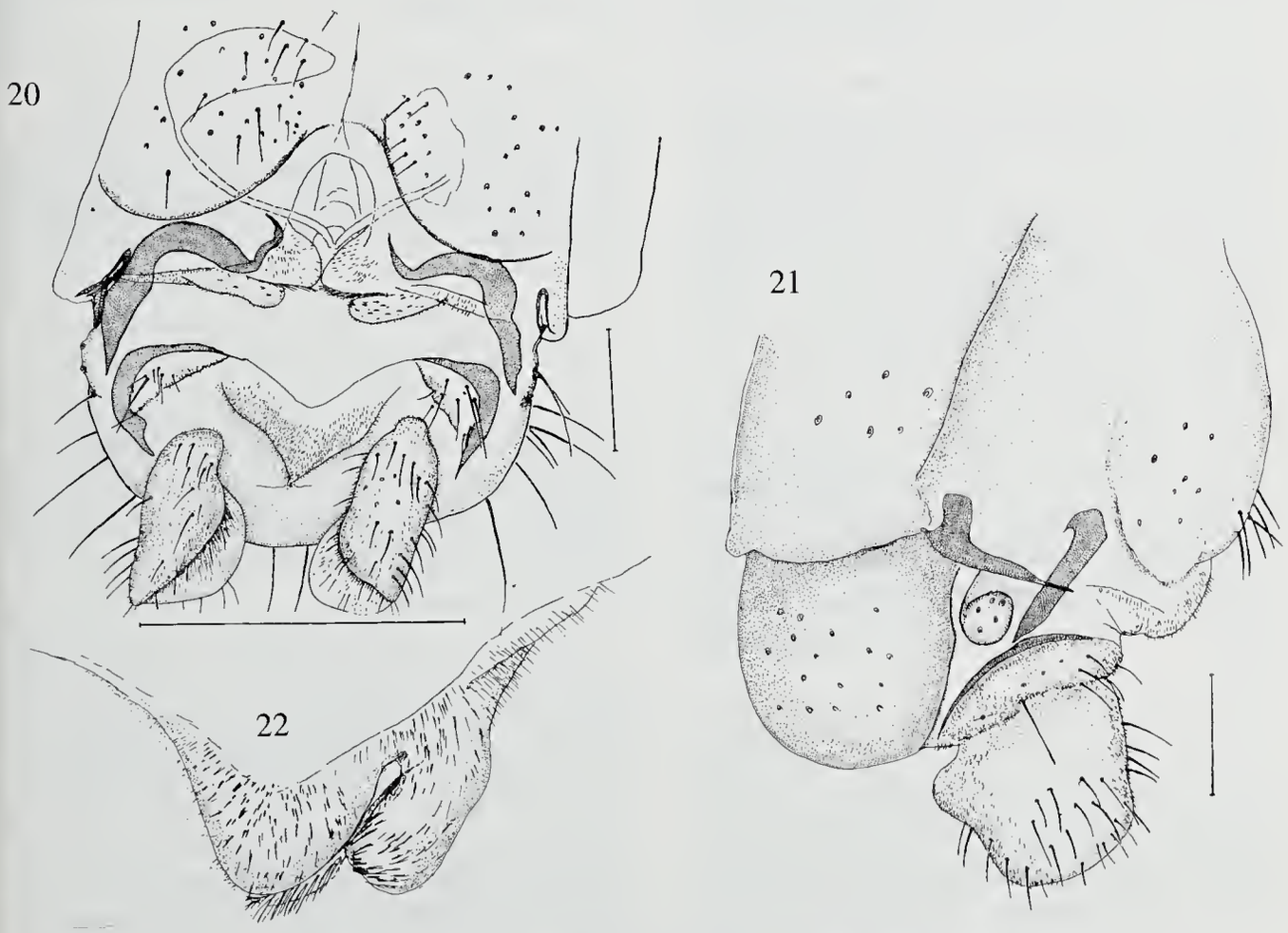

Fig. 20-22. *Glyptotendipes ospeli* spec. nov., female genital segments. 20, ventral view; 21, lateral view; 22, lobes of Gp VIII (scale 100 µm).

Table 2: Measurements of legs of adult females of *G. ospeli* (measurements in µm, n=5)

	fore leg	mid leg	hind leg
fe	1194 (1137-1236)	1342 (1266-1424)	1342 (1286-1414)
ti	1220 (1157-1276)	1327 (1256-1404)	1539 (1434-1582)
ta_1	1606 (1533-1662)	582 (554-603)	889 (841-939)
ta_2	767 (742-821)	384 (376-396)	555 (524-593)
ta_3	633 (623-673)	304 (287-316)	462 (425-494)
ta_4	516 (504-544)	219 (208-227)	276 (257-297)
ta_5	280 (267-287)	185 (168-208)	192 (178-207)
LR	1.34 (1.32-1.38)	0.43 (0.42-0.45)	0.59 (0.56-0.61)
BV	1.81 (1.78-1.85)	2.99 (2.9-3.12)	2.57 (2.5-2.6)
SV	1.48 (1.47-1.5)	4.66 (4.49-4.76)	3.19 (3.09-3.32)

(124-140) µm; Am_6 220 (204-240) µm. Chaetotaxy: Temporals 17 (13-19); postorbitals 9 (7-10) on each side (n=4); clypeus setae 42 (35-50); cibarial setae 20 (17-22) (n=4). Length of palpomeres: Pm1: 58 (44-76) µm; Pm2: 62 (56-68) µm; Pm3: 209 (188-224) µm; Pm4: 174 (160-180) µm; Pm5: 234 (228-240) µm.

Thorax: Dark brown, mesonotal stripes dark. Chaetotaxy: 19 (17-22) acrostichals (n= 3); 37 (31-42) multiserial dorsocentals on each side; 7.3 (4-8) humerals (n=3); 8.8 (8-10) praealars uniserial, in some specimens distal prals bi or triserial; 47 (43-49) scutellar setae.

Wings: Hyaline, brownish, venation brownish, costa, sc, rr, m, rm darker than cu. Squama fringed. Measurements of wings: length 3.1 (3-3.3) mm (n=4); width 1.0 (0.9-1.1) mm; Ar-rm: 1.3 (1.2-1.5) mm; Ar-FCu: 1.4 (1.3-1.5) mm; VR: 1.04 (1.01-1.05).

Legs: dark brown; femora of fore legs with a yellowish proximal ring. Metatarsus of mid legs with 44 (42-47) (n=3) sensilla chaetica, Metatarsus of hind legs with 43 (34-60) sensilla chaetica. Measuremenst of legs in tabel 2.

Abdomen: Tergites dark brown with distal margins yellowish. Genital segments dark brown.

Genital morphology (fig. 20-22): T IX short, not overlapping the ventral side, distally rounded. Sternite X with 11 (10-15) setae on each side. PgP small, distinct, distally pointed, slightly carinate, covered with microtrichia; proximal width/length ratio of PgP (according to Contreras-Lichtenberg, 1996): 1.9 (1.7-1.95) (n=3). Gc IX with 5 setae. Csa curved, medially divided into two ends, the proximal branch being longer than the distal branch. Gp VIII with VlL nearly of the same size as the DmL; the VlL are not overlapping the DmL. The DmL are triangular with a rounded tip and with short microtrichia. ApL distinct, covered with long microtrichia all over the length. Gca broad, curved, the distal and medial part with stronger sclerotization, medial ends short, slightly converging; SDu short and straight, SCa oval-shaped.

Etymology

The species is named after the locality, Ospel (The Netherlands), where it has been collected for the first time.

Diagnostic characters

The *Glyptotendipes ospeli* karyotype is strongly species-specific by its banding pattern which can be used as good character for species identification. The species is also well defined by morphological characters of larvae, pupa, male and female.

Larva: with a characteristic v-shaped mark on the frontal apotome; seta subdentalis leaf-shaped, ventromental plates with rounded tips. Pupa: epaulettes of abdominal tergite III and IV of the same length. Male: differing from the closely related *G. glaucus* and *G. pallens* (Michailova & Contreras-Lichtenberg, 1995) by the acute angle between volsella superior and volsella inferior. Female: VlL not overlapping the DmL.

***Keys for the identification of G. ospeli* spec. nov.**

Larva

1. Ventral tubuli on abdominal segment VIII missing; head capsule: dorsally with a characteristic v-shaped mark; ventromental plates with rounded tips; gula darkened over the whole area, seta subdentalis leaf-shaped ..... *G. ospeli* spec. nov.
– Ventral tubuli on segment VIII present, coloration of the head capsule not as above; front border of ventromental plates with small tips 2

2. Gula darkened over the whole area, mandible with seta subdentalis elongated, narrow ..... *G. glaucus*
– Dark colour of the gula confined to its posterior part, mandible with seta subdentalis leaf-shaped ..... *G. pallens*

Pupa

1. Length of pupal exuviae more than 10 mm, cephalic tubercles long, conical, with broadened bulb-like basis, distally pointed, terminal seta on cephalic tubercle about five times as long as tubercle; epaulettes on tergites II to VI continuously increasing in length; shagreen on abdominal tergite VI all over the length of the epaulette ..... *G. glaucus*
– Length of pupal exuviae less than 10 mm; cephalic tubercles short, subcylindrical with broadened basis; shagreen on abdominal tergite VI confined to the proximal third of the tergite 2

2. Terminal seta on cephalic tubercle about five times as long as cephalic tubercle; epaulette of abdominal tergite IV longer than epaulette of abdominal tergite III ..... *G. pallens*
– Terminal seta on cephalic tubercle more than twice as long as cephalic tubercle; epaulettes of abdominal tergites III and IV of the same length ..... *G. ospeli* spec. nov.

Imago male

1. Body length less than 7 mm; hypopygium with superior volsellae slender, straight, in an acute angle to the inferior volsellae and not parallel to the anal tergite ..... *G. ospeli* spec. nov.
– Body length 8-10mm 2

2. Hypopygium with superior volsellae curved and not parallel to the anal tergite ..... *G. pallens*
– Superior volsellae of the hypopygium in the middle part straight, parallel to the anal tergite ..... *G. glaucus*

Imago female

1. Tergite IX distally straight; sternite X with more than 20 setae; VlL larger than DmL, and overlapping them, PgP distinctly carinate, proximal width/length ratio of PgP 1.5 ..... *G. glaucus*
– Tergite IX distally rounded; sternite X with with 10-15 setae 2

2. Sternite X with 10-11 setae; VlL as in *G. glaucus*, PgP not carinate, proximal width/length ratio of PgP 2.7 ..... *G. pallens*
– Sternite X with 10-15 setae; VlL and DmL nearly of the same size; VlL not overlapping the DmL; PgP slightly carinate; proximal widt/length ratio of PgP 1.9 ..... *G. ospeli* spec. nov.

Acknowledgements

Thanks are due to Henk Vallenduuk and Henk Moller Pillot for providing the material for studies, to Odwin Hoffrichter and Peter Langton for valuable contributions when discussing the new species.

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Accepted 1.ix.1999.