

Description, phenology and ecology of *Paraphaenocladius voltheus* n. sp., a characteristic inhabitant of alder spring forests 1. Description of the male and female imago

Alexander Klink and Henk Moller Pillot

With 1 figure, 13 photos and 5 tables

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Paraphaenocladius voltheus n. sp. belongs to the *irritus* group (Saether and Wang (1995)). Male and female are described from several localities in alder spring forests in the Netherlands. The male differs from the other known species by the exceptional high antennal ratio (1.3). The female 9th tergite is undivided with an incurved apex. Also the combs on the pleurites and the incision lateral on tergite 8 are not mentioned from other known females of this genus. The pupa is unknown and the larvae can be separated from the only other known species (*P. pseudirritus*) by the smaller apical eye, larger head capsule and more slender 1st antennal segment. Adults were only caught in April and larvae from November to early spring, indicating a true summer diapause.

1 Introduction

In the past decade, the first author has spent much time to collect specimens of Chironomidae for the reference collection of Naturalis Natural Museum Leiden Netherlands. This database contains a voucher collection approx 1700 Chironomidae prepared on microscopic slides embedded in DMHF sirup. Of all these specimens non-destructive DNA extraction and amplification has been conducted at Naturalis. About 400 specimens yielded a complete DNA sequence of the CO1 gene. In the CLC Main Workbench software (Qiagen Aarhus Denmark) trees were constructed to find relationships between species in the genera and to see if unexpected results could add to the present taxonomical knowledge. As of now this resulted in a first article describing the larva of *Mollerella calcarella* based on the DNA match between the known adult and the unknown larva (Klink et al. 2020).

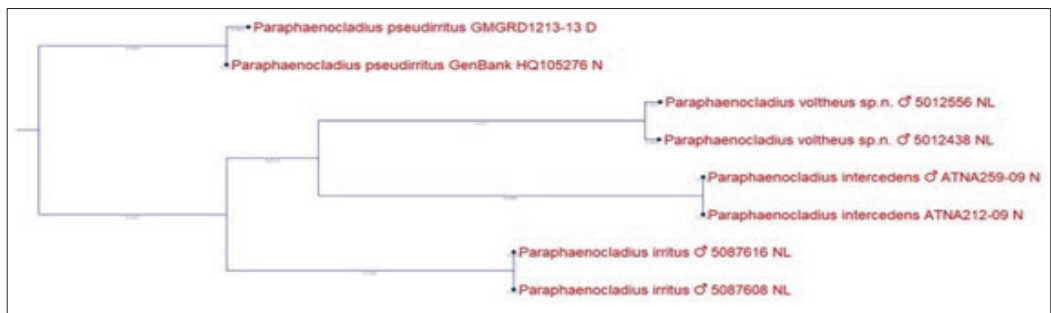


Fig. 1: Figure 1. Cladogram of the *irritus* group of *Paraphaenocladius* according to Saether and Wang (1995). The Naturalis material has a number starting with 50. The final character(s) show the land of origin (D = Germany; N = Norway and NL = the Netherlands)

The 4 species differ between 10,3-13,2 % in base pairs, meaning enough genetic distance (1-1.6 %) to assume that they indeed are true species (Resp. Ekrem et al. 2010 and Gadawski et al., 2022) *P. voltheus* shows the most affinity with *P. intercedens* and *P. irritus*

In the present study the sequences of the *Paraphaenocladius* in the Naturalis collection contained a DNA sequence not present in the BOLD database. The males clearly belong to the *irritus* group (Saether and Wang 1995) and in figure 1 the cladogram of this group is presented.

2 *Paraphaenocladius voltheus* sp. n.

2.1 Material, methods and morphology

Material (Tab. 1) is collected by the authors (AK and HMP), Rink Wiggers (RW) and David Tempelman (DT) at the following Dutch locations (with Amersfoort coordinates between parentheses) and the collection method and rearing procedure when applicable.

Tab. 1: Locations of the material examined

Location	Municipality	Date	X Coord.	Y Coord.	Method	Emerging	♂♂	♀♀	larvae	leg.
Voltherbroek	Dinkelland	Apr-1-2017	259903	489401	reared	Apr 2-24 2017	36	39		HMP
Agelerbroek	Dinkelland	Apr-23-2019	259109	489566	reared	Mar 2020		1		HMP
Lemselermaten	Dinkelland	Apr-1-2017	256168	485316	sweeping net		5			HMP
Oude Gooren	Someren	Apr-13-1982	176155	383458	sweeping net		86			AK
Oude Gooren	Someren	Mar-23-2019	176155	383458	reared	?	2	2		HMP
Halder	Sint-Michielsgestel	Mar-18-2009	150300	407200	reared	?	11	6		HMP
Strijperheg	Heeze-Leende	Mar-23-2020	165017	369707	reared	Mar 2020				HMP
Berkenputten	Heeze-Leende	Apr-15-2019	165639	370272	reared	Apr 15-20 2019	1	4		HMP
Berkenputten	Heeze-Leende	Mar-23-2020	165639	370272	reared	Mar 23 to Apr 15 2020	32	30		HMP
Urkhovense Zeggen	Eindhoven	Mar-5-2020	65988	382916	reared	Mar 14 to Apr 6 2020	3	8		HMP
De Mortelen Smalzij	Oirschot	Apr-4-2020	150712	395431	sweeping net		swarm			DT
Sang	Someren	Nov-14-2019	172847	383163	hand net				17	RW
Sang	Someren	Dec-28-2019	172847	383163	hand net				57	HMP

DNA has been extracted from males originating from Voltherbroek, Lemselermaten and Oude Gooren, but only the former two locations yielded DNA sequences for barcode analyses at the Boldsystems (http://www.boldsystems.org/index.php/IDS_OpenEngine).

The extraction of DNA and sequence determination were conducted by Naturalis Dutch Museum of Natural History Leiden Netherlands.

Derivatio nominis

The imaginal stages have been collected or reared from Voltherbroek, an alder carr near the German border near Ootmarsum (Overijssel).

The morphological nomenclature follows Saether (1980).

Description male imago

In parentheses number of individuals. Total length 2.8-3.4 mm (4). Wing length 1.6-2.3 mm (5)

Head. Eyes bare and with a dorso-medial extension; antennal ratio 1.30-1.63 (22); Maxillary palp with 5 segments. Length: (µm) 28 (1) 28-34, 70-96, 112-120, 124-144 (all 2); stipes 160 µm (1); temporals 9-12 (3) not grouped in inner and outer verticals; postorbitals 4 (3).

Thorax (n = 3). Anteprenotals lateral 6-8; acrostichals 19-25 in 3 groups; dorsocentrals 23-30 in 1-3 rows; prealars 7 and scutellars 10.

Wing (photo 2) (n = 3 unless otherwise stated). Length 1.6-2.3 (5); C-extension 60-74 μm (4) ending not proximal to M3+4 with 6-8 non-marginal setae ending posterior to apex M3+4 and Cu1 clearly sinuous at apex; Sc, RM, M,Cu and anal lobe devoid of setae. R with 20-27 setae; R1 with 14-15; R4+5 with 30-46 setae; M1+2 with 67-76; M3+4 with 33-37 setae; Cu1 with sinuate apex and with 18-20 setae; Pcu with 8-10; An with 10-22 setae. Cell m proximal from RM with 1-3(-6) (10); r3+4 appr. 250; m1+2 appr. 150; m3+4 with appr. 80-00, cu+an with 36-56 setae; squama with 7-18 setae (11).



Photo 1: Male

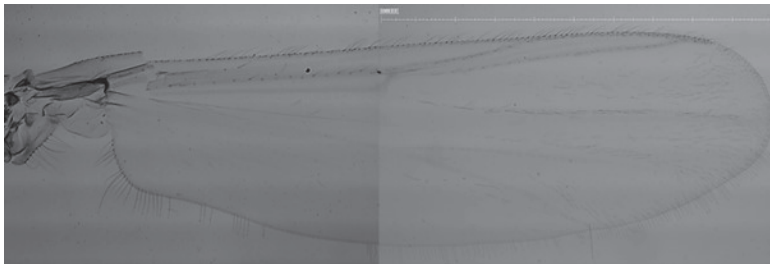


Photo 2: Male wing

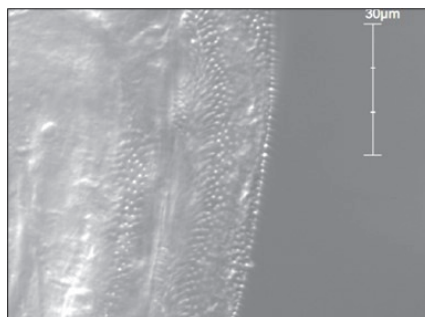


Photo 3: Male, setation on pleurites

Abdomen (n = 1). The tergites of the males contain a large central field of evenly distributed long setae. More lateral the tergites are bare and at the dorsal edge of the pleurites a number of lateral setae are placed in a straight longitudinal row. On the sternites the long setae are

separated into 3 groups. The medial group is clearly separated from the medio-lateral group and the lateral group at the ventral edge of the pleura. The pleurites of the males are covered with microsetae (photo 3), which is in great contrast with the pleural armament of the females (photo 9).

Numbers are the total on tergites and sternites

Legs (n = 3 unless stated otherwise). Pulvilli are vestigial.

Hypopygium (n = 2). Anal point (31-32 μm long), slender triangular with a broadly rounded apex, completely covered with microtrichia and large setae scattered around. Virga absent. Gonostylus with a megaseta (15 μm long (1)). Crista dorsalis triangular with exceptionally large apex, about as high as the megaseta. Inferior volsella (photo 5) symmetrically tapering to broadly rounded apex and without a basal hump. The dorsal side is devoid of microtrichia and with about 9 evenly scattered normal setae.

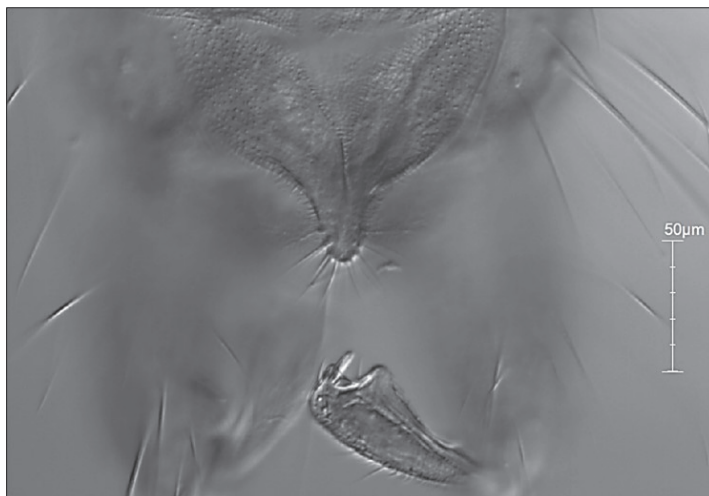


Photo 4: Male genitalia, with focus on anal point and crista dorsalis

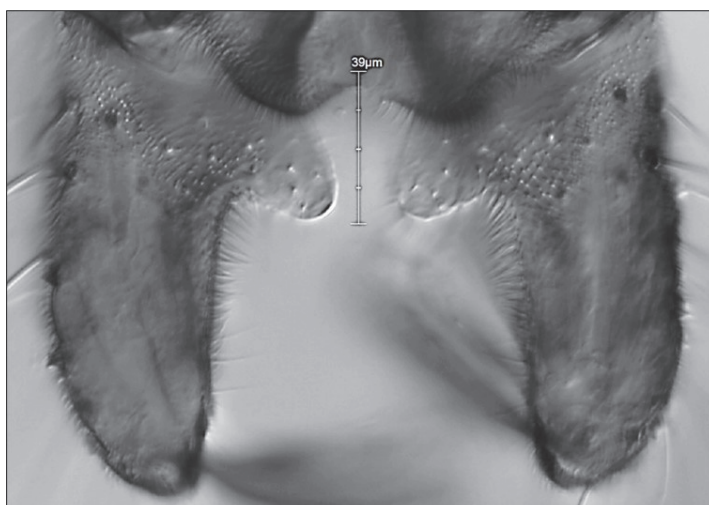


Photo 5: Male genitalia, with focus on the dorsal plane of the inferior volsella

Diagnostic characters

The absence of a virga and tip of anal point with normal setae and microtrichia is characteristic for the *irritus* group. The C-extension ends not proximal to apex M3+4 is a unique character within the *irritus* group. The antennal ratio of over 1.3 is much higher than in all other known males of *Paraphaenocladus* (Saether and Wang (1995)).

Within the *irritus* group, *P. voltheus* shares the high number of acrostychals, dorsocentrals and setae on R3+4 with *P. irritus* as well as the setae in m proximal to RM. As can be deduced from the drawings of Saether and Wang (1995), *P. voltheus* seems the only species in the *irritus* group missing microtrichia on the dorsal surface of the inferior volsella. Further *P. voltheus* has an exceptionally large apex of the crista dorsalis and usually more than 10 (7-18) squama setae.

Description female imago

Total length 1.9-2.3 mm (3). Wing length 1.4-1.9 mm (2).



Photo 6: Female. Habitus

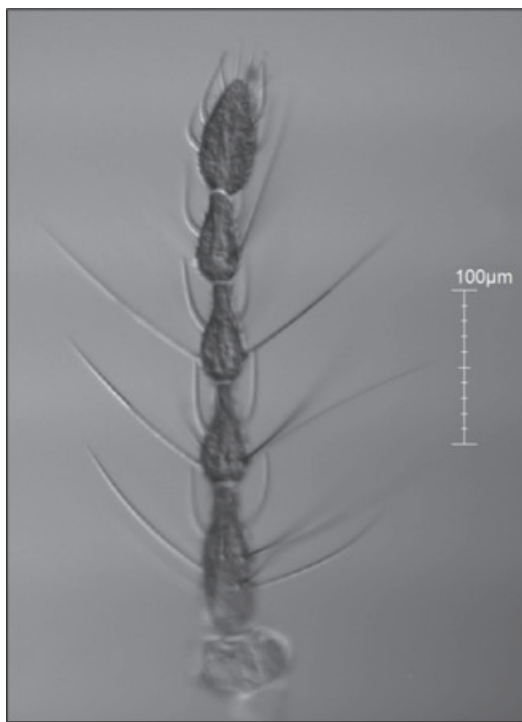


Photo 7: Female antenna

Head. Eyes bare and with a dorso-medial extension; antennal ratio 0.26-0.29 (2); antennal segment length in μm : 84-95, 60-64, 60-63, 57-60 and the flagellum 72-76 (all 2). Each of the 4 basal segment is vase-shaped and contains 2 sensillae. The flagellum is spindle-shaped and beset with 4 sensillae (all 1). All sensillae are bent towards the antenna apex; Maxillary palp with 5 segments. Length (μm) 32, 40, 88, 100, 132 (all 1); stipes 160 μm long (1). Temporals 12 in a continuous arc and not in separate groups (2); postorbitals 4 (1).

Thorax (n = 3). Anteprenotals lateral 6; acrostychals 18-23 in 3 groups; dorsocentrals 23-28 in 1-3 rows; prealars 7-8 and scutellars 8-11.

Wing (photo 8). (n = 3 unless otherwise stated): Length 1.4-1.9 mm; C-extension 88-112 μm (4) clearly distal from M3+4 (photo 3) with 4-7 non-marginal setae; Cu1 with a sinuous apex Sc, RM, devoid of setae. R with 27-37 setae; R1 with 25-51; R4+5 with 46-75 setae; M with 4-13; M1+2 with 87-98; M3+4 with 51-57 setae. Cu with 26-36; Cu1 with slight sinuate apex and with 20-32 setae; Pcu with 50-70 setae; An with 18-45 setae (all 3). Cell m proximal to RM with (14)-20-35-(40) (10); r3+4 appr. 300; m1+2 appr. 300, m3+4 with appr. 100-250; cu+an with appr. 200-300 setae. Squama with 8-15 (8) setae.

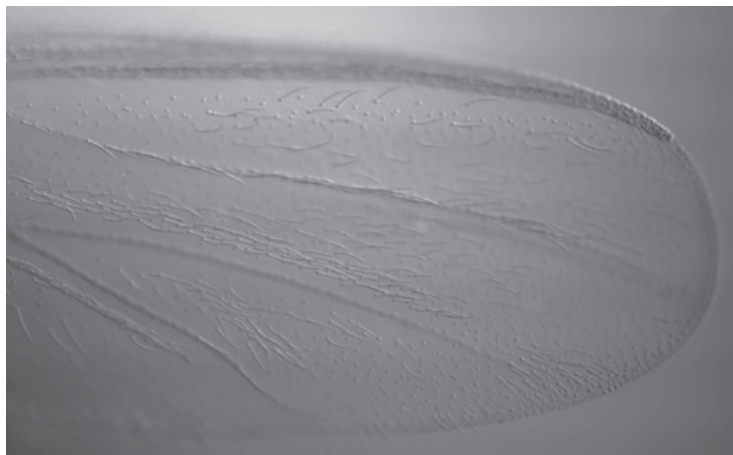


Photo 8: Female wing distal part

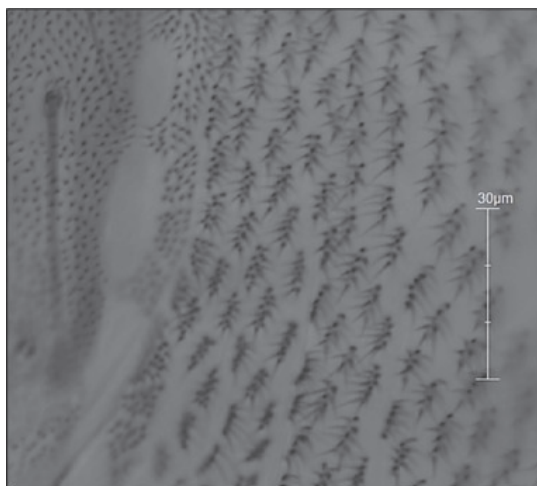


Photo 9: "Combs" on female pleurites

Abdomen (n = 2). The females also bare only a field of large setae in the medial part of the tergites and on the dorsal edge of the pleurites. On the sternites a third group of setae is present between the median and lateral groups. The pleurites are densely set with little combs composed of diverging spinules (photo 9), which are absent on the male pleurites (photo 3).

Legs. Pulvilli are vestigial.

Genitalia. Tergite 8 has a prominent lateral notch in the distal third (horizontal arrows photo 10) and tergite 9 is entire and curved inwards at the apex (vertical arrow photo 10). Seminal capsules 86 μm long and 46 μm broad (1). Spermathecal duct with 2 sharp loops. Cercus 80 μm long. Gonocoxite 9 with about 21 normal setae of which 5 are longer than the cercus. Gonapophyses 8 consist of an upper rectangular plate and a rounded one beneath. The gonocoxapodeme is a sharply pointed ridge (Photo 11).

**Tab. 2: Setation on the male abdominal segments
n = 1**

♂♂	medial	medio-lateral	lateral
T1	42	0	6
T2	45	0	18
T3	32		18
T4	28	0	20
T5	32	0	18
T6	26	0	14
T7	27	0	12
T8	33	0	10
T9	0	0	18
S1	0	0	0
S2	2	2	14
S3	3	6	16
S4	3	10	18
S5	10	8	16
S6	14	6-8	14
S7	19	4	12
S8	46	0	8
S9	14	0	0

**Tab. 4: Setation on the abdominal segments
n = 2 unless stated otherwise**

♀♀	medial	medio-lateral	lateral
T1	24(1)	0	0
T2	24-36	0	6-8
T3	25-28	0	6-8
T4	16-24	0	6-8
T5	26-28	0	6
T6	18-32	0	8
T7	16-20	0	6
T8	32-38	0	0
T9	25-38	0	0
S1	0	0	0
S2	0-1	2-4	0
S3	2-4	6-8	0
S4	4-5	8-14	8
S5	7-8	10-14	10
S6	9	10-14	8
S7	8	6-12	8
S8	0	22-24	4
S9	16	0	0

Tab. 3: Length (μm) of leg segments

♂♂	P1	P2	P3
Femur	688-840	680(1)	752-840
Tibia	776-848	656-800	800-920
Tars 1	560-624	336-384	528-608
Tars 2	312-344	176-200	256-280
Tars 3	224-240	120-144	170-184
Tars 4	144-152	88-96	96-112
Tars 5	96-104	88-96	88-112

Leg ratio 0.70-0.74 0.48-0.51 0.63-0.67

Tab. 5: Length of segments (μm , n = 3)

♀♀	P1	P2	P3
Femur	512-560	552-616	608-752
Tibia	528-624	520-584	632-704
Tars 1	344-408	240-272	384-440
Tars 2	184-232	112-160	192-216
Tars 3	112-160	96-104	128-144
Tars 4	80-104	64-72	80-88
Tars 5	80-88	56-80	80-88

Leg ratio 0.63-0.65 0.46-0.48 0.60-0.65

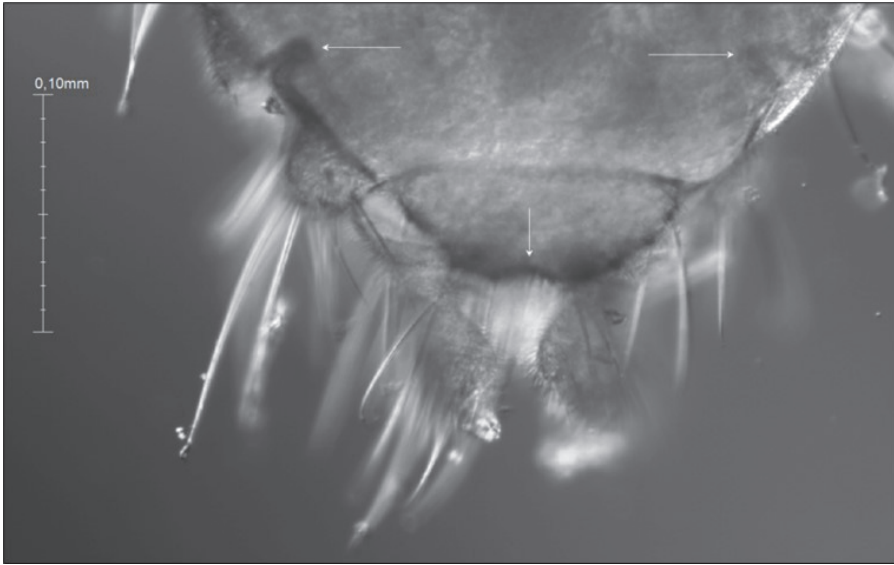


Photo 10: Female genitalia in dorsal view

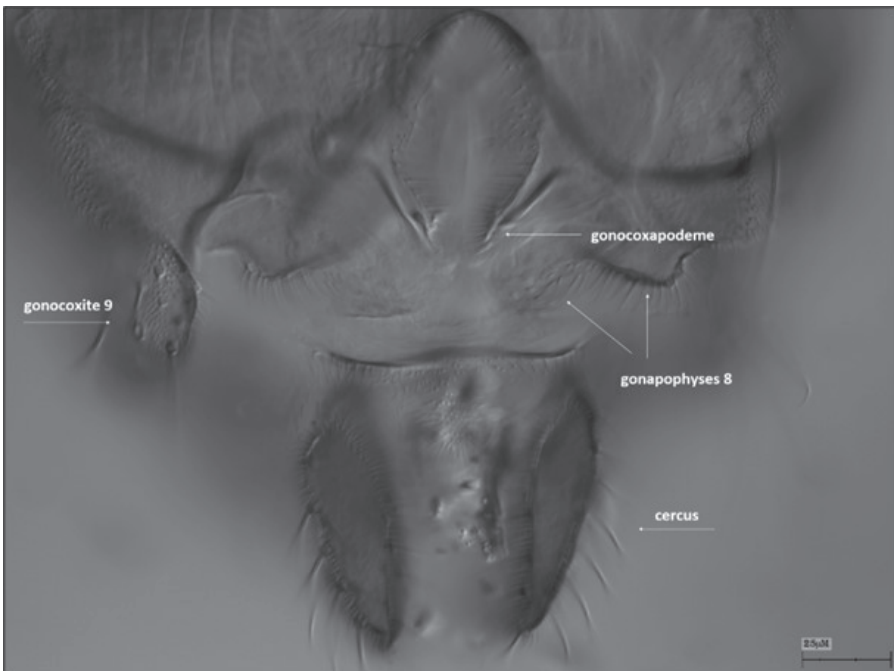


Photo 11: Female genitalia in ventral view

Diagnostic characters: There are far less consistent measurements done on females. Presumably the incurved apex and undivided tergite 9 are diagnostic. Also the combs on the pleurites and the incision lateral on tergite 8 seem outstanding. The C-extension ending distal to M3+4 is not mentioned for any female in Saether and Wang (1995).

Pupa

The pupa of *P. voltheus* is unknown.

Larva

The larva is a typical *Paraphaenocladius* with the preanal segment curved over the remaining segments and complex ventromental plates. It resembles the larva of *P. pseudirritus* (see Strenzke 1950: 220-224) with any combs on the anterior parapods (photo 12 arrow) and the ventromental plates partly duplicated with turned “wings”. For differences with *P. pseudirritus* see the key below.

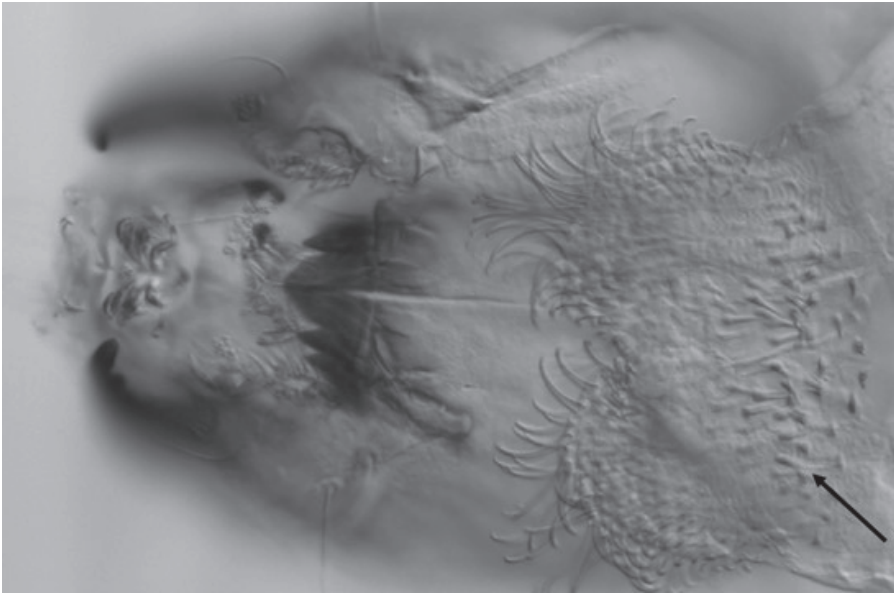


Photo 12: Larva, head ventrally with single median tooth of mentum and small combs on the base of the anterior parapod

3 Keys to males, females and larva of the *irritus* group in *Paraphaenocladus*

3.1 Key to the known males of the *Paraphaenocladus irritus* group based on Seather & Wang (1995)

In italics the adaptations made in their key

- 1 Anal point bluntly or sharply triangular or rounded, with setae and microtrichia to apex; cell m proximal to RM usually bare, at most with 3-16 setae. Virga absent
irritus-group 2
- When anal point triangular or rounded, apex microtrichia-free and virga present. Cell m proximal of RM with or without setae
other species
- 2 Antennal ratio >1.3; dorsocentrals >22. Crista dorsalis sharply triangular and as high as the megaseta. *Cu1 strongly sinuate. C-extension not proximal to apex M3+4. Inferior volsella devoid of microtrichia on dorsal plane* *P. voltheus*
- Antenna ratio <1.1; dorsocentralis ≤22. Crista dorsalis much less pronounced. *Cu 1 slightly sinuate. C-extension proximal to apex M3+4. Inferior volsella with microtrichia on the dorsal plane* 3
- 3 Cell m proximal to RM with 4-16 setae. *Cu with 9-26 setae. R1 with ≥15 setae. Cu1 with ≥28 setae* *P. irritus*
- Cell m proximal to RM bare. *Cu with 0-6 setae. R1 with <10 setae. Cu1 with ≤18 setae*
- 4 Antennal ratio 0.5-0.7. R with ≥10 setae. *M1+2 ≥30 setae. Cu1 with setae* *P. pseudirritus*
- Antennal ratio 0.9-1.1. R with <10 setae; *M1+2 <25 setae. Cu1 bare* *P. intercedens*

3.2 Key to the known females of *Paraphaenocladus irritus* group

Female of *P. irritus* unknown and only 1 damaged female pupa of *P. pseudirritus* without genitals (Seather & Wang, 1995)

- 1 Sternite 3 without setae, ultimate palpomere <80 μm *P. intercedens*
- Sternite 3 with setae; ultimate palpomere >120 μm 2
- 2 Cell m proximal to RM with 25-33 setae. C-extension distal to apex M3+4 *P. voltheus*
- Cell m proximal to RM bare. C-extension proximal to apex M3+4 *P. pseudirritus*

3.3 Key to the known larvae in fourth instar of *Paraphaenocladus irritus* group

Only the larvae of *P. pseudirritus* and *voltheus* are known.

- 1 Small eye placed oblique before the much bigger second eye (photo 13), the small eye roundish, at most with a small gap. First antennal segment 2½-3 times longer than second segment, 3½-4 times as long as wide. Head length 0.30-0.35 mm *P. voltheus*
- Small eye larger and strikingly sickle-shaped, placed below the large dorsal eye. First antennal segment 2 times longer than second segment, 3 times as long as wide. Head length 0.25-0.28 mm *P. pseudirritus*



Photo 13: Larva, apical eye very small and oblique under the large eye

4 Life cycle

Adults were caught in nature only in April. From samples taken in early spring and reared indoors in mini-emergence traps adults emerged in March and April. In summer we found never adults or larvae of this species, although we have taken many samples in summer, also at three localities where the species had been found in spring. Larvae were found from November.

From a sample taken in Agelerbroek April, 23, 2019 no adults emerged that year, only one female in March 2020. This sample was kept moist during the whole year.

These data indicate a true summer diapause, because obviously adults do not emerge in summer, independent of conditions except for daylength. A summer diapause is not exceptional in terrestrial living chironomids for instance in *Geothocladius* and *Gymnometriocnemus*. Within the genus *Paraphaenocladus* a summer diapause was not known: *P. pseudirritus*, *P. penerasus* and *P. impensus* have (in The Netherlands) two up to four generations a year.

5 Autecology

In bottom samples of 4 dm² taken in March and April we got as a maximum 57 up to 75 adults emerging i.e. 14-19/dm².

The species has been found in moist to wet woodland. However in summer the upper layers of the bottom can be almost completely dry, for instance in Voltherbroek, Agelerbroek and Berkenputten. In Voltherbroek and Agelerbroek we found no *P. impensus* or *pseudirritus*, in Berkenputten *P. impensus* was very scarce. Very probably the summer diapause is an adaptation to summer drought.

P. voltheus was found mainly in alder carr. Only at two localities the landscape was semi-open without alder carr.

In general *Paraphaenocladus* species are not found in acid conditions. However we found *P. voltheus* sometimes in bottoms with pH between 5 and 6. It is possible that this

species can live in more or less acid environment, but more probably these measurements are based up changing acidity under the influence of rain.

The gut content of two larvae contained inert organic material and fungal remains. No plant tissue or algal remains have been encountered.

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Addresses of the authors:

Alexander Klink, Hydrobiologisch adviesburo Klink, Boterstraat 28, 6701 CW Wageningen, Nederland. Email: agklink@klinkhydrobiology.com

Henk Moller Pillot, Leyparkweg 37, 5022 AA Tilburg, Nederland. Email: henkmollerpillot@hetnet.nl

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