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A Review of the Genus Polypedilum Kieffer
The cytotaxonomy of Polypedilum aberrans Tshernovskji

(Diptera, Chironomidae)

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Abstract

The male, female and pupa of *Polypedilum aberrans* Tshernovskji are described for the first time. The species is characterised cytotaxonomically. The banding patterns of the polytene chromosomes of *P. aberrans* and *P. nubifer* are compared. Analysis of the banding pattern indicates that the relationships of these species are close. Sequences in arms A, E, and F are apparently quite ancient, probably ancestral, because of their occurence in *P. nubifer* (A_1/A_1; E_3/E_3; F_1/F_1) *P. nubeculosum*, and *P. aberrans*. The species is female heterogametic. The females always being heterozygous for the differential heterochromatic end on chromosome IV. The two species: *P. aberrans* and *P. nubifer* are very similar but are readily distinguishable by the hypopygium of the males and by their cytology.

Introduction

Polypedilum aberrans Tshernovskji is a species known through a larva described by Tshernovskyi (1949). Pinder & Reiss (1983) considered this species as a synonym of *Polypedilum nubifer* (Skuse). The two species have alternate Lauterborn organs on the larval antenna.

The imago (♂) of *P. nubifer* has been reviewed by Freeman (1961), and the cytology has been described by Porter & Martin (1977). *P. nubifer* has been recorded from North Africa, Iraq, Sri Lanka and Australia (Freeman, 1961).

A description of the imago and pupa of *P. aberrans* does not exist. Marker features of the chromosomes of this species have been given by Michailova (in press). Pankratova (1983) reviewed the larva of this species and presented its known distribution: USSR, Bulgaria and Hungary.

The purpose of the present paper is to describe the adult and pupa stages of *P. aberrans* from material reared in the laboratory; to characterise this species cytotaxonomically and to show that the immature stages and cytology serve to separate *P. aberrans* and *P. nubifer*. They are readily recognisable species. The karyotypic relationships between these species is also indicated.

Material and methods

Larval material of *P. aberrans* was collected in lake Durankulak, VII. 1979. Specimens of this species from Hungary, collected in Kondor-tö VII. 1983 and VIII. 1986, were also studied. In the laboratory larvae grow to maturity in about 3–4 weeks. Laboratory reared adults were used for keeping a stock of this species. The crossing of adult midges was made by a method described earlier (Michailova, 1985).

18 specimens (10♂♂ and 8♀♀), 5 pupae and 35 larvae have been examined. Adults were fixed in 70% alcohol and the preparations made according to Schlee (1966). Larvae were fixed in 3:1 ethanolacetic acid and stored in a deep freeze. The general terminology follows Saether (1980).

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Karyological preparations of salivary glands and gonads of IVth instar larvae (35 specimens: 5 from Durankulak and 30 from Kondor-tö) were prepared applying the well known acetorcein method. After analysis the slides were made permanent by freezing in liquid nitrogen, to enable removal of the cover glass, and mounting in euparal.

The chromosomes of *P. nubifer* have been proposed as a standard for the comparative cytotaxonomy of the genus *Polypedilum* (PORTER & MARTIN, 1977).

Following the homology with the chromosomes of this standard, the chromosomes of *P. aberrans* were designated as: Ist(AB), IInd (CD), IIIrd(EF) and IVth(G). Every chromosome has been divided into sections, beginning from 1 in the left arm of every chromosome. This division is not the same as that of *P. nubifer*, done by PORTER & MARTIN (1977) (a photo map of each arm of *P. nubifer* was not given). The common sections of the chromosomes between these species have been shown in outline in the text.

External morphology of Polypedilum aberrans
(Figs. 1, 2)

Imago: ♂

Colour: Thorax and abdomen dark brown, legs, light brown.

Thorax: Antepronotal and dorsocentral lobes well formed, dark brown, about 35 dorsocentral setae in 2 rows. Scutellum dark brown with 20 setae in two rows. Postnotum brown.

Legs: With middle and hind tibial combs, each with one spur (Fig. 1 A). Legs proportions: (μm, n = 5).

	F	T	T_{a_1}	T_{a_2}	T_{a_3}	T_{a_4}	T_{a_5}
P_1	485	425	480	275	255	180	105
P_2	550	440	300	160	130	85	75
P_3	600	515	400	135	190	115	90

Hypopygium: (Fig. 1 B). Gonocoxite consists of two parts, distal part is as long as wide. Gonostylus 2× as long as wide. Volsella apically curved. Anal point is straight from the very beginning. Anal field oval. Coxpodeme well developed. Phallapodeme rod-like.

Imago: ♀

Colour: light brown

Genitalia. (Fig. 1 C). Gonosternite oval, with long setae and darkly coloured edges. Gonapophysis IX well formed, reaches almost the middle of SV III. Coxosternapodeme dark, dense and arched.

Pupa:

Exuvia light brown. 2. segment in a lower part with a row of setae. 3.–7. segments with a group of setae in an upper and lower part. Posterolateral spurs of segment 8. with 8 or 9 spines (Fig. 1 D).

Larva: (Fig. 2 A, B, C).

Antenna, labrum, mandible and premandible as in TSHERNOVSKJI (1949, fig. 47) and PANKRATOVA (1983, fig. 201).

Fig. 1. *Polypedilum aberrans*. A, Hind leg with comb and spur. B, Hypopygium; Ca – coxapodeme; Pha – phallapodeme; AnP – anal point; Gc – gonocoxite; Gs – gonostylus. C, Genitalia (♀); Gca – Gonocoxapodeme; Va – vaginal apodeme; Ce – cercus. D, Pupa: 8. segment, posterolateral spur with 8 spines.

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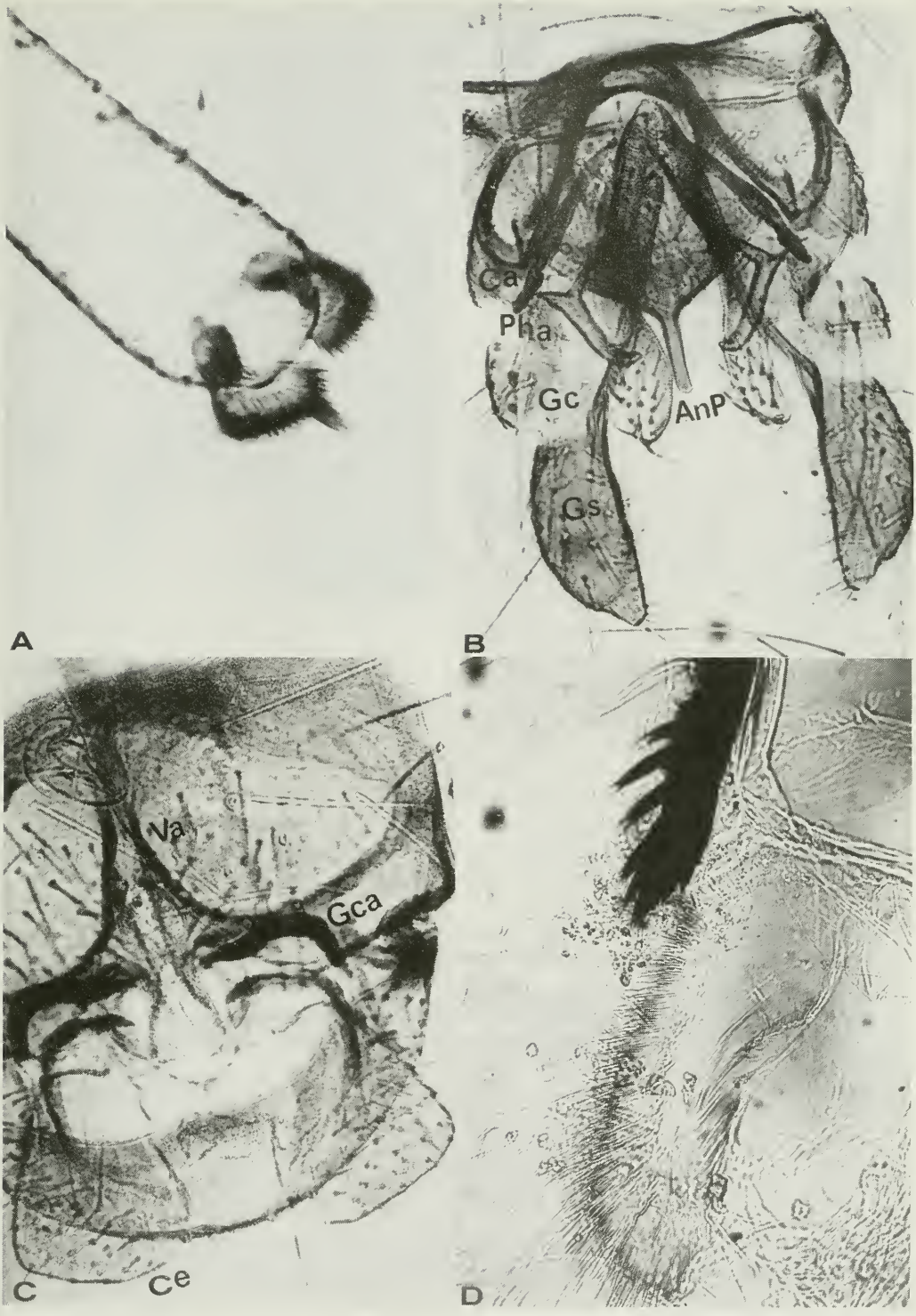

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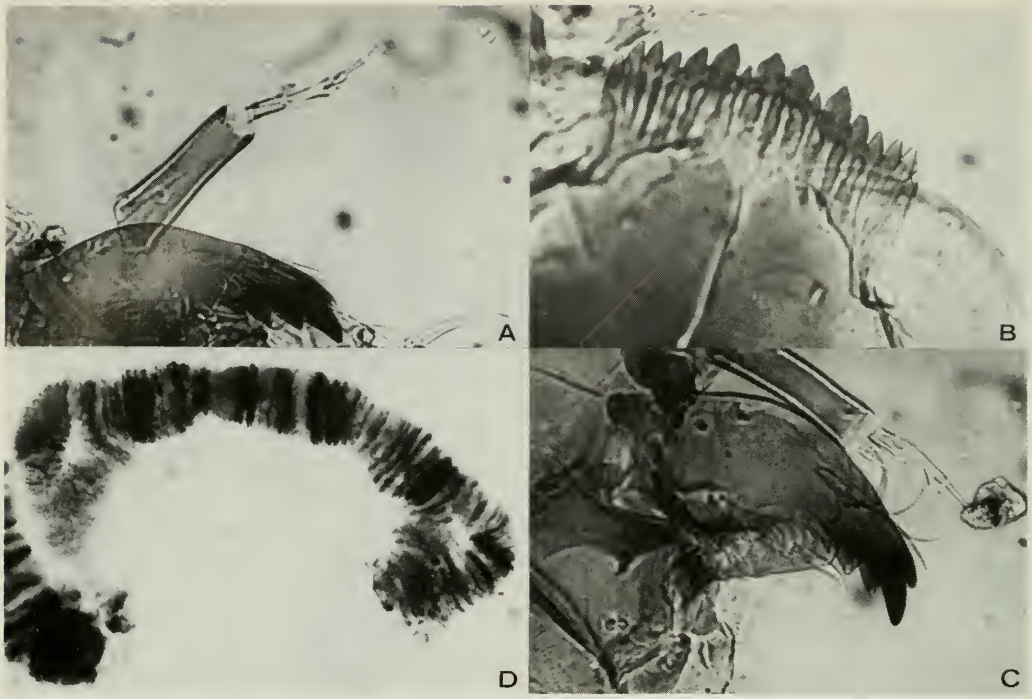


Fig. 2. *Polypedilum aberrans*. A, Antenna. B, Labrum. C, Mandible. D, Heterozygous inversion in arm B.

The karyotype of Polypedilum aberrans
(Fig. 3 A, B, C, D)

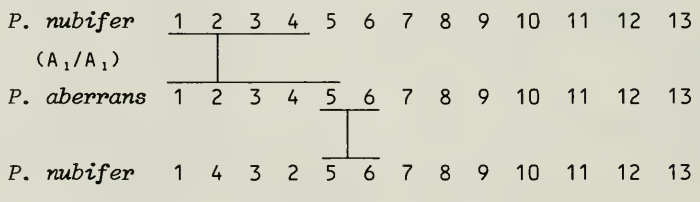
2n = 8. Chromosome I (AB), and II (CD), Ist (AB), IInd (CD) chromosomes – metacentric; Chromosome III (EF) – submetacentric and IV (G) – acrocentric. Every chromosome with a dark heterochromatin band in the centromere region. Sex chromosomes were not found. The species is female heterogametic. The females always being heterozygous for the heterochromatic end on the chromosome IV.

Chromosome polymorphism has been observed only in arm B of Ist chromosome.

The chromosome markers have been described by MICHAILOVA (in press).

Chromosome I with arms AB similar to its counterpart in *P. nubifer*. PORTER & MARTIN (1977) reported five rearrangements in arm A. Only two of them are in homozygous condition. *P. aberrans* has only one type of band sequence. Part of which coincides with that of *P. nubifer* (A_1/A_1) and part – with A_2/A_2.

Chromosome I



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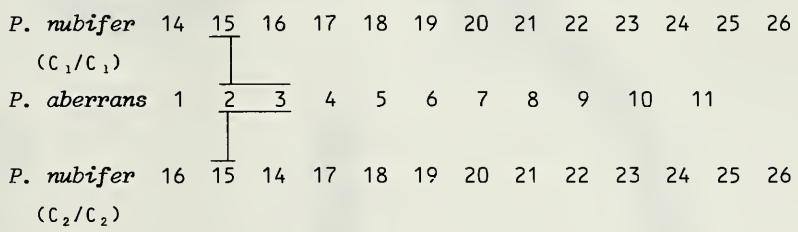
Arm B of *P. nubifer* has four rearrangements in heterozygous condition (Porter & Martin, 1977) on both populations of *P. aberrans,* we found only one type of heterozygous inversion with a very low frequency (Fig. 2D).

Chromosome II, with arms CD showing considerable change from the sequences seen in *P. nubifer.* Three rearrangements have been recognized in arm C of *P. nubifer* (Porter & Martin, 1977). In arm C of *P. aberrans* only one type of band sequence was found. The two groups of dark bands in section 2–3 of *P. aberrans* exist in *P. nubifer,* described by Porter & Martin (1977) as C_1/C_1 and C_2/C_2. The other sequences of *P. aberrans* must have changed considerably in appearance compared with the standard. Arm C shows a sequence not previously reported.

Chromosome II

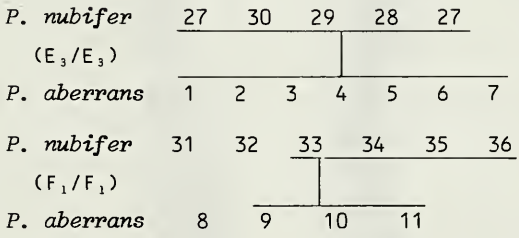


Arm D is considerably changed in *P. aberrans.*

Chromosome III, with arms EF. There are four rearrangements in arm E of *P. nubifer* (Porter & Martin, 1977). All have been seen in the homozygous condition. In *P. aberrans* only one type of band sequence was seen in which the banding pattern on section 1–6 is very similar to that of E_3/E_3 of *P. nubifer.* The bands after this sequence appear to differ from that of P. nubifer.

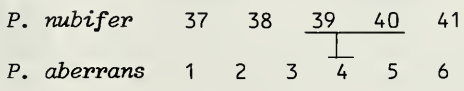
There were four rearrangements in arm F of *P. nubifer* (Porter & Martin, 1977). Two of them have been seen in the homozygous conditions. Arm F of *P. aberrans* has only one type of sequence. Section 9–11 of *P. aberrans* is identical to F_1/F_1 described by Porter & Martin (1977) for *P. nubifer.*

Chromosome III



Chromosome IV, or arm G, appears similar to *P. nubifer.* When a detailed analysis of the chromosome is attempted the bands in section 4 of *P. aberrans* can be positively correlated with those in the middle of *P. nubifer.*

Chromosome IV



Porter & Martin (1977) reported six rearrangements in arm G of *P. nubifer.* In *P. aberrans* there are only two types of sequences which are in one and the same salivary gland. These differ in their functional activity.

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P. aberrans is female heterozygous for a heterochromatic end (Fig. 3 D, G_2), while males are homozygous for this. The female rearrangements are similar to G_2/G_2 of *P. nubifer*.

Fig. 3. *Polypedilum aberrans*. A, Chromosome Ist (AB). B, Chromosome IInd (CD). C, Chromosome IIIrd (EF). D, Chromosome IVth (G_1 or G_2); N – nucleolus; BR – Balbiani ring; c – centromere region.

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Discussion

Detailed study of adults of *P. nubifer* and *P. aberrans* indicated that there are two different species. These species are very similar but are readily distinguishable by the male hypopygium. The coxite of *P. nubifer* consists of two parts. The second part is strongly prolongated (Freeman, 1961). The second part of coxite of *P. aberrans* is as long as wide. Between these parts in *P. aberrans* there is a well formed concavity (Fig. 1 B). Such a concavity is not seen in *P. nubifer* (Freeman, 1961). The volsella of *P. aberrans* is terminally recurved (Fig. 1 B). The hypopygium of *P. aberrans* differs from that of *P. nubifer* by having broader gonostyli while the gonostyli of *P. nubifer* are more slender (Freeman, 1961). The two species differ in the shape of the anal point. That of *P. nubifer* is narrower basally while the anal point of *P. aberrans* is straight from base to apex (Fig. 1 B).

Cytogenetical investigations of material which keys out as *P. nubifer* (Porter & Martin, 1977) and *P. aberrans* revealed the existence of two readly recognisable species with four chromosomes in the salivery gland. The banding patterns of *P. aberrans* and *P. nubifer* also indicate a close relationship. The salivary gland chromosomes of *P. aberrans* show sufficient similarity in banding pattern to those of *P. nubifer*, mainly in arms A, E, and F. In the genus *Polypedilum* as in the genus *Chironomus* arms A, E, and F seem to have been more stable in evolution. Arms B, C, D, and G as we have seen show more differences between the species. However within the arms it is not yet possible to suggest homology of more than a few of the most conspicious band groups. Every species has marker features on each chromosome. Some sequences differ between these species by a simple inversion.

There is no cytological evidence and external morphological data to support the suggestion that *P. aberrans* should be considered as a synonym of *P. nubifer*.

In the present case a few species of *Polypedilum* have been studied cytologically: *P. nubifer* (Porter & Martin, 1977), *P. aberrans*, *P. nubeculosum* and *Polypedilum* sp. (Chironominae genuinae N3 Lipina) (Michailova, in press). This allows determination of the relative age of some sequences. Phylogenetically central sequences with wide distribution are more ancient than sequences with restricted distribution. With the exception of the G chromosome, the other chromosomes of *P. aberrans* and *P. nubeculosum* have only one type of band sequence. Porter & Martin (1977) reported few rearrangements for arms A, C, E, F, G of *P. nubifer*. One of them is common for *P. nubifer*, *P. aberrans* and *P. nubeculosum*. The banding pattern of *P. aberrans* arm A, section 2–5 and the banding pattern of the same arm of *P. nubifer*, section 2–4 a 9 (A_1/A_1) are common for the species. In arm C there are also common patterns: bands in section 2–3 of *P. aberrans* and 15 (C_1/C_1 or C_2/C_2) of *P. nubifer*. The banding pattern of arm E of *P. aberrans*, section 2–6 corresponds with that of *P. nubifer*, section 27–30–27al (E_3/E_3). Arm F: the banding pattern, section 9–11 *(P. aberrans)* and section 33 c 9–36 b 12 *(P. nubifer)* is common for both species. The banding pattern ($_4$) of arm G *(P. aberrans)* and that in the middle of arm G *(P. nubifer)* are common.

These band sequences have been found in *P. nubeculosum* also (Michailova, in press). These patterns could be considered as "basic patterns" of genus *Polypedilum* in sense of Wülker (1980). Perhaps these common patterns existed in a hypothetical stem species. Starting from the hypothetical species these patterns have been retained in one species but in an other *(P. nubifer)* have undergone different mutations. On that way *P. nubifer* displays a high frequency of chromosomal rearrangements. There is an other explanations also: the common ancestor was polymorphic for a number of sequences which still occur as polymorphism only on *P. nubifer*.

Porter & Martin (1977) reported a female heterogamety of *P. nubifer*. They have also found heterozygous males. A more likely explanations is that the species is still in the process of changing from the normal male heterogamety to female heterogamety (Porter & Martin, 1977). In *P. aberrans* this process is going forward. Only females always carry the large heterochromatinized differential segment.

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